Le rôle de la consommation de viandes, charcuteries et poissons dans l'étiologie de cancer du côlon et du rectum : résultats de l'Etude Prospective Européenne sur la Nutrition et le Cancer (EPIC)

Teresa Norat-Soto

INSTITUT NATIONAL AGRONOMIQUE PARIS-GRIGNON

Ecole Doctorale ABIES

THÈSE

pour obtenir le grade de

DOCTEUR DE L'INSTITUT NATIONAL AGRONOMIQUE PARIS-GRIGNON

Discipline : Sciences Biologiques appliquées à l'alimentation (Nutrition Humaine)

présentée et soutenue publiquement par

Teresa NORAT SOTO

le 6 Septembre, 2007

LE ROLE DE LA CONSOMMATION DE VIANDES, CHARCUTERIES ET POISSONS DANS L'ETIOLOGIE DE CANCER DU COLON ET DU RECTUM : RESULTATS DE L'ETUDE PROSPECTIVE EUROPEENNE SUR LA NUTRITION ET LE CANCER (EPIC)

Read meat, processed meat and fish and colorectal cancer : the European Prospective Investigation into Nutrition and Cancer (EPIC).

Laboratoire d'accueil: Unité de Nutrition, Centre International de Recherche sur le Cancer, 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France

Thèse dirigée par M. le Professeur Robert BENAMOUZIG

Jury :

M. le Professeur Daniel TOME (Président du Jury)

M. le Professeur Serge BRIANÇON (Rapporteur)

M. le Professeur Jean FAIVRE (Rapporteur)

M. le Professeur Serge HERCBERG (Rapporteur)

M. le Professeur Robert BENAMOUZIG (Directeur de Thèse)

Remerciements

Je remercie le Professeur Serge Briançon, le Professeur Jean Faivre et le Professeur Serge Hercberg qui m'ont fait l'honneur d'accepter la charge de rapporteur de cette thèse.

Je remercie le Professeur Robert Benamouzig, directeur de cette thèse, pour les précieux conseils qu'il m'a prodigués, pour l'intérêt qu'il a porté à mon travail et sa constante disponibilité.

Je remercie le Professeur Daniel Tomé d'avoir suivi et soutenu mon projet de recherche, et d'avoir accepté de présider cette thèse.

Je remercie le Professeur Elio Riboli pour son accueil au sein de son équipe au Centre International de Recherche sur le Cancer à Lyon. Il m'a donné l'opportunité de travailler dans la passionnante étude EPIC et m'a fait profiter de son expérience.

Merci aux chefs de projet de l'étude EPIC ainsi que leurs équipes dans chacun de 23 centres participants dans l'étude, et tout particulièrement au Docteur Sheila Bingham, Directrice du centre de Nutrition et Cancer à l'Université de Cambridge, Royaume Uni, pour sa précieuse collaboration.

Je remercie le Docteur Rashmi Sinha, Vice-directrice du Département de Nutrition au Centre National du Cancer (National Cancer Institute), Etats Unis, pour m'avoir donné la possibilité de collaborer dans son projet de recherche.

Merci à Livio Riboli-Sasco et Oscar Punal-Norat, pour leur aide à la rédaction en Français.

Je tiens à remercier mes professeurs ainsi que mes anciens collègues de travail à La Havane, Cuba, pour leurs encouragements.

Enfin, un grand merci à ma famille, pour leur soutien permanent.

Résumé

Nous avons analysé la relation entre le risque de cancer colorectal et la consommation de viande et de poisson dans la population qui participe dans l'Etude Prospective Européenne sur le Cancer et la Nutrition. Il s'agit d'une étude sur plus de 400 000 sujets volontaires de dix pays européens. Le risque de cancer colorectal apparaît lié à un niveau de consommation élevé de viande rouge. Le *hazard ratio* associé à une consommation supérieure à 160 grammes par jour par rapport à une consommation inférieure à 20 grammes par jour est de 1.35 (95% IC=0.96-1.88). La consommation élevée de poisson semble diminuer le risque de cancer colorectal. Le *hazard ratio* pour une consommation de poisson supérieure à 80 grammes par jour par rapport à celle de moins de 10 grammes par jour est de 0.69 (IC=0.54-0.88). La consommation de volailles n'a pas d'incidence sur le risque de cancer colorectal.

Nous avons intégré par méta-analyse nos résultats avec ceux des études prospectives publiées entre 1990 et juin 2006. D'après 14 études, le risque relatif moyen associé aux niveaux de consommation de viande rouge les plus hauts par rapport aux niveaux les plus bas est de 1.34 (95% CI=1.09-1.21). En ce qui concerne les niveaux de consommation de poisson les plus hauts par rapport aux niveaux les plus bas (treize études), le risque relatif moyen est de 0.87 (95%CI=0.78-0.97).

Nos résultats montrent qu'une diminution des apports de viande rouge parmi les gros consommateurs devrait conduire à une diminution du risque de cancer colorectal pour cette population. La consommation de fibre alimentaire et de poisson en grosses quantités semble diminuer le risque, mais cette relation doit être confirmée par d'autres études.

Mots clés: cancer, colon-rectum, épidémiologie, alimentation, viande rouge, poisson

Abstract

Colorectal cancer is one of the main causes of cancer incidence and mortality worldwide. Compelling experimental and epidemiological evidence has been gained to indicate that the diet is an important factor in the modulation of this disease.

We investigated the relationship between red meat and fish with colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC), a multicentre study including about half a million volunteer subjects from 10 European countries. In this population, colorectal cancer risk is positively associated to red and processed meat intake.

The *hazard ratio* associated to more than 160 grams per day compared to 20 grams per day of red meat is 1.35 (95% IC=0.96-1.88). Colorectal cancer risk is inversely related to fish intake. The *hazard ratio* is 0.69 (IC=0.54-0.88) for more than 80 grams per day of fish intake compared to 10 grams per day. White meat intake does not appear to be related to colorectal cancer risk.

We summarized by meta-analysis the results of prospective studies published between 1990 and June 2006 with our own results. Overall, high read meat consumption is significantly associated with colorectal cancer risk. The average relative risk for the highest *vs*. the lowest intake level of intake as reported in 14 studies is 1.34 (95% CI=1.09-1.21). The average relative risk for the highest *vs*. the lowest fish intake level is 0.87 (95%CI=0.78-0.97).

Our results support that the reduction of red meat consumption in individuals with high intake will contribute to the reduction of colorectal cancer risk in the population. High intake of dietary fibre and of fish should decrease the risk, but these results need to be confirmed in further studies.

Key words: cancer, colorectal, epidemiology, diet, red meat, fish

Table de matières

Liste de tableaux.

Liste de figures

LISTE DES ANNEXES

II. INTRODUCTION

3. Situation épidémiologique du cancer colorectal.

4. Rappel des caractéristiques anatomiques et fonctionnelles du côlon-rectum et de la cancérogenèse colorectal.

4.1 Anatomie fonctionnelle du côlon-rectum

4.2 Cancérogenèse colorectal.

5. Principaux facteurs de risque du cancer colorectal.

3.1 Facteurs génétiques

3.2 Inactivité physique et obésité

3.3 Alcool

3.4 Tabac

3.5 Statut inflammatoire

3.6 Thérapie hormonale substitutive

3.7 Alimentation et cancer colorectal

3.7.1 Les apports caloriques.

3.7.2 Les lipides alimentaires.

3.7.3 Les fibres alimentaires.

3.7.4 Les fruits et les légumes.

3.7.5 Le folate.

3.7.6 Le calcium.

3.7.7 La viande et le poisson.

6. Objectifs du travail.

1. Situation épidémiologique du cancer colorectal.

Le cancer représente un problème majeur de santé publique. Chaque année, près de 10 millions de cas de cancer sont diagnostiqués dans le monde et plus de 6 millions de personnes disparaissent des suites d'un cancer (1).

Les données dont on dispose actuellement indiquent une grosse disparité géographique des taux d'incidence et de mortalité par cancer dans le monde aussi bien du point de vue de son fardeau global que par type ou localisation des cancers (2). Les pays développés de l'Europe, l'Amérique du Nord, l'Asie et l'Australie présentent des taux relativement élevés des cancers où les facteurs liés au métabolisme hormonal et nutritionnel peuvent jouer un rôle plus important, tels que le cancer du sein chez les femmes et de la prostate chez l'homme ainsi que de cancer du côlon et de rectum. Les populations des pays en voie de développement d'Afrique, d'Amérique Latine et d'Asie sont affectées d'une façon disproportionnée par les cancers dans lesquels les agents infectieux sont causals. A certaines exceptions près, ces pays ont des taux élevés de cancer de l'estomac, du foie et du col de l'utérus. Ils présentent également des taux relativement élevés des cancers de voies aérodigestives supérieures (bouche, pharynx, larynx et œsophage). En même temps, les cancers du sein, de la prostate et du côlon-rectum deviennent de plus en plus nombreux dans les zones urbaines.

En France, les tumeurs malignes représentent la première cause de mortalité chez l'homme (29 % de l'ensemble des décès) et la seconde chez la femme, après les maladies cardiovasculaires (23 % de l'ensemble des décès)(3). Les localisations de cancer les plus fréquentes sont le sein, le côlon et le rectum, la prostate et les voies aérodigestives supérieures. Avec 278 000 nouveaux cas de cancer dans l'année 2000 contre 160 000 en 1980, la France connaît une augmentation considérable de l'incidence des cancers. 115 000 personnes meurent d'un cancer chaque année, 800 000 personnes vivent avec un cancer et 2

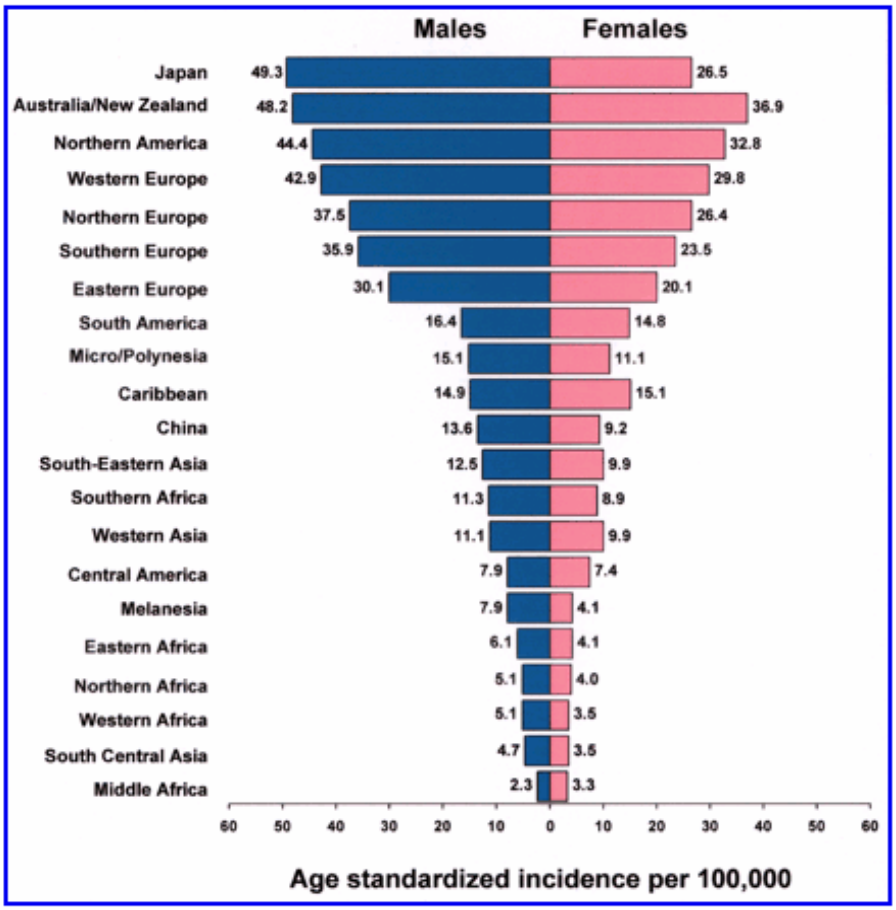
millions de personnes ont eu un cancer. La France affiche la plus mauvaise mortalité prématurée d'Europe liée au cancer, en raison des comportements à risque (tabac, alcool, maladies professionnelles) et de la faiblesse de la prévention.

Le cancer colorectal se trouve parmi les cancers avec taux d'incidence et de mortalité plus élevées dans le monde. Ce cancer se place au troisième rang de la pathologie cancéreuse au monde, avec environ 1 million de nouveaux cas par an en 2002 (9,4% du nombre total des cancers) et au quatrième rang de causes de mortalité, avec presque un demi-million de décès pour les deux sexes confondus dans la même année (4). Par nombre de cas prévalent, ce cancer se place au deuxième rang des cancers, à continuation du cancer de sein, avec approximativement 2,8 millions de personnes vivantes 5 ans après le diagnostic de cancer colorectal. La survie à 5 ans est estimée en 65% de cas en Amérique du Nord, 54% en Europe de l'ouest, 34% en Europe de l'est, et 30% en Inde (chez les hommes) (4).

L'occurrence de cancer du côlon est environ 20 fois plus élevée dans certaines régions du monde que dans d'autres (Figure 1) (5). Les taux d'incidence les plus élevés, supérieurs à 40 nouveaux cas pour 100 000 habitants par an, apparaissent en Amérique du Nord, l'Australie, l'Europe de l'ouest et chez les hommes au Japon. Par contre, le cancer colorectal est rare en Asie et surtout en Afrique. Les taux sont intermédiaires dans les régions méridionales de l'Amérique du Sud.

Le nombre de nouveaux cas de cancer colorectal en France est estimé à 19 229 chez les hommes et à 15718 chez les femmes pour l'année 2002 (12,7% et 13,4% respectivement de tous les cancers confondus excepté les cancers cutanés non mélanique) ce qui correspond à des taux de cancer standardisés de 40.8 nouveaux cas de cancer pour 100 000 habitants chez les hommes et de 25.9 nouveaux cas pour les femmes par an (5).

Figure I : Taux d'incidence du cancer colorectal standardisés sur l'âge selon le sexe dans différentes régions géographiques (taux pour 100 000 habitants)



Source : J. FERLAY, F. BRAY, P. PISANI and D.M. PARKIN.
GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide
IARC CancerBase No. 5. version 2.0, IARCPress, Lyon, 2004

Dans son ensemble, le cancer de côlon apparaît avec la même fréquence approximativement chez les hommes et chez les femmes mais la localisation au niveau du côlon droit est plus fréquente chez la femme (6). Néanmoins, dans des régions géographiques caractérisées par une haute incidence de cancer du côlon, telle que l'Amérique du Nord et l'Australie, ainsi

qu'au Japon et en Italie où les taux d'incidence ont augmenté rapidement, les taux de cancer du côlon chez les hommes excèdent maintenant ceux des femmes de 20% ou plus.

Le cancer du rectum, y compris les cancers situés au niveau de la jonction recto sigmoïdienne, représentent environ 40 % des cancers colorectaux, soit près de 6 % de tous les cancers. Le cancer rectal est jusqu'au deux fois plus fréquent chez les hommes que chez les femmes. La disparité géographique pour le cancer du rectum est semblable à celle décrite pour le cancer du côlon, mais avec des variations des taux inférieures à celles de cancer du côlon (5). Parmi les populations à haut risque, le cancer de côlon est deux fois plus fréquent que le cancer du rectum. Dans les pays à faible risque, les risques de cancer du côlon et du rectum sont généralement de la même grandeur. De manière générale, les taux d'incidence du cancer colorectal sont en train d'augmenter rapidement dans les pays où le risque était bas, tout particulièrement au Japon mais aussi dans d'autres pays d'Asie, tandis que dans des zones géographiques à risque plus élevé, les taux sont plutôt stables (nord et l'ouest de l'Europe) ou ont tendance à diminuer (Amérique du Nord), ceci notamment dans les groupes d'âges les plus jeunes (7).

Des études sur des populations migrantes ont montré que les populations provenant des régions géographiques à faible incidence de cancer colorectal et qui émigrent vers des régions caractérisées par une incidence plus élevée ont tendance à acquérir des risques comparables à ceux des pays hôtes dans une période de temps relativement courte, parfois dans la même génération (2). Par exemple, les taux d'incidence chez les populations d'origine japonaise au Hawaï (51.2 par 100.000 habitants chez l'homme et 30.8 chez la femme) et à Los Angeles (48.0 par 100.000 chez l'homme, 32.8 chez la femme) sont parmi les taux plus élevés dans le monde (8).

Les variations du risque de cancer colorectal avec le temps, l'augmentation des taux d'incidence dans certaines régions du monde à mesure que ces pays deviennent de plus en plus industrialisés et que leurs habitants adoptent un mode de vie plus proche de celui des pays occidentaux développés, et les études sur des populations migrantes, indiquent que des facteurs environnementaux et en particulier le mode de vie, interviennent dans l'étiologie du cancer colorectal (9;10). La variabilité dans le temps et la disparité géographique des taux d'incidence ne peuvent pas être expliqués par des différences génétiques. En effet, le risque de développer un cancer de la même localisation pour deux jumeaux identiques est inférieur à 10% (11).

2. Rappel de caractéristiques anatomiques et fonctionnelles du côlon-rectum et de la cancérogenèse colorectal.

2.1 Anatomie fonctionnelle du côlon-rectum.

Le côlon est un organe musculaire d'environ 125 cm de longueur *in vivo* qui s'étend de la valvule iléo-cæcale jusqu'au rectum. Quatre segments sont distingués dans le côlon : le côlon ascendant dont la partie inférieure se termine au niveau du cæcum, le côlon transverse, le côlon gauche ou descendant et le côlon sigmoïde relié au rectum par la jonction recto-sigmoïdienne. Sur le plan anatomique, le rectum, qui mesure environ 15 cm est la partie terminale du tube digestif compris entre la jonction recto-sigmoïdienne et le sphincter anal.

La paroi du côlon comprend quatre couches fondamentales présentes dans les autres organes creux du tube digestif : la muqueuse interne, recouverte par l'épithélium colorectal, la sous-muqueuse, la couche musculaire circulaire et la couche musculaire longitudinale. La muqueuse est parsemée de nombreuses glandes qui s'ouvrent à sa surface et sécrètent du mucus dans la lumière. Les types cellulaires présents à la surface ainsi que les glandes ressemblent à ceux de l'intestin grêle mais le nombre de cellules caliciformes y est plus élevé. On trouve parfois à l'état normal des filaments glaireux dans les selles. La musculeuse longitudinale ou externe est composée de trois bandes qui s'étendent du cæcum au rectum où elles fusionnent pour former une couche musculaire externe uniforme. La deuxième couche de la paroi rectale correspond à l'ensemble sous-séreuse-séreuse. Dans les zones rectales sans péritoine, la séreuse représente le tissu périrectal.

La fonction du côlon est la digestion et l'absorption des aliments non digérés et absorbés dans l'intestin grêle, ainsi que la concentration des matières fécales par absorption d'eau et d'électrolytes. La muqueuse interne est le siège d'échanges entre la lumière intestinale et l'intestin. La couche musculaire présente une suite de dilatations sacculaires et de

resserrements : les haustra. Une onde de contraction ou haustration, permet le cheminement des matières fécales. Le côlon droit joue un rôle majeur dans l'absorption de l'eau et des électrolytes, de même que dans la fermentation des sucres non digérés; le côlon gauche et le rectum interviennent surtout dans l'entreposage et l'évacuation des selles. Le rectum est exposé de manière plus directe et concentré à la matière fécale que le côlon.

Le côlon humain est siège d'un nombre élevé de micro-organismes qui consomment, stockent, et redistribuent l'énergie, et négocient des transformations chimiques physiologiquement importantes. La taille de la population bactérienne, environ 10^{11} bactéries/ml de contenu colique, dépasse de loin la taille de toutes les autres communautés microbiennes en relation avec la surface de notre corps (12). La flore de l'intestin se compose de différentes lignées de bactéries anaérobiques strictes avec la capacité de communiquer entre elles et avec l'hôte et de maintenir la population par autoréplication. Leur substrats sont à la fois d'origine alimentaire (résidus de la digestion-absorption en amont), endogène (mucus, sécrétions digestives et cellules de desquamation) et bactérienne. Le côlon est le premier organe exposé à la résultante des interactions aliment-flore intestinale. Toutefois, les métabolites bactériens générés dans le côlon peuvent être réabsorbés au niveau du tractus intestinal et exercer des effets sur d'autre cibles.

La majeure partie des résidus de la digestion arrivant dans le côlon sont des fibres alimentaires. Les sucres complexes contenus dans les fibres alimentaires y sont dégradés par activité bactérienne en acides gras à chaîne courte, en butyrate, en propionate et en acétate, qui sont acheminés par transport passif et actif à l'intérieur des cellules de l'épithélium colique où ils constituent une importante source d'énergie.

L'émulsion et l'absorption des lipides a lieu dans l'intestin grêle. Des acides biliaires sont sécrétés dans l'intestin grêle pour permettre l'émulsion des lipides. Les acides biliaires qui

arrivent dans le côlon sont métabolisés en acides biliaires secondaires par une enzyme de la flore colique, la 7 alpha -déhydroxylase. Chez l'homme, la quantité de lipides totaux qui entrent dans le côlon en conditions physiologiques a été évaluée entre 5 et 8 g/j, ce chiffre pouvant être considérablement augmenté en situations pathologiques -insuffisance pancréatique, mucoviscidose, cholestase, résections intestinales- (13). Dans le côlon on trouve aussi des lipides bactériens et des lipides provenant de la desquamation des colonocytes. Les acides gras libres sont cytotoxiques, de même que les acides biliaires (14).

Les produits de la digestion des protéines alimentaires sont absorbés dans l'intestin grêle. Les molécules azotées arrivant dans le côlon, principalement des acides aminés, sont métabolisés par la flore colique en ammonium qui peut être réabsorbée et convertie en urée dans le foie, utilisée dans la synthèse protéique bactérienne, ou retenue dans le contenu colique. A part l'azote alimentaire non absorbé, l'azote contenu dans le côlon est constitué de l'azote endogène issu des sécrétions digestives et de l'urée provenant du recyclage hépatique (15).

Le côlon se caractérise par la présence des cryptes. Dans l'intestin grêle, les villosités et les cryptes contribuent à l'élargissement de la superficie d'absorption. En revanche, il n'y a aucun besoin d'une grande superficie d'absorption dans le côlon. La persistance évolutive des cryptes du côlon s'explique probablement par la protection que cette microarchitecture confère aux cellules souches contre l'environnement mutagène du lumen intestinal.

2.2 Cancérogenèse colique.

Le terme « cancérogenèse » recouvre l'ensemble des mécanismes responsables du développement des cancers, incluant toutes les formes de cancers. On attribue aux cancers une origine clonale, à partir d'une seule cellule somatique dont les mécanismes régulateurs de la croissance, de la différentiation et de la prolifération vont être gravement perturbés. Les altérations génétiques sont de différents types selon qu'il s'agit de mutations génétiques,

d'aberrations et remaniements chromosomiques ou des dommages primaires de l'ADN, c'est-à-dire affectant directement la structure physicochimique de l'ADN. Cette théorie implique que l'événement initiateur de la cancérogenèse a lieu au niveau du génome de la cellule. Les caractères nouveaux acquis par la cellule devront être transmis aux cellules filles.

Le développement d'un cancer est un phénomène prolongé dans le temps pouvant comporter plusieurs décennies. Le développement de la recherche expérimentale depuis plusieurs dizaines d'années a permis d'élaborer des modèles de cancérogenèse qui intègrent une succession d'événements génétiques et épigénétiques que l'on peut résumer en 4 phases principales : *initiation*, *promotion*, *progression* et *invasion* de la tumeur cancéreuse.

Approximativement 95% des tumeurs colorectales malignes sont des adénocarcinomes, dont la plupart se transforment à partir des anomalies de la muqueuse colique vers des adénomes et puis des carcinomes. Le modèle de la séquence adénome-carcinome comporte trois éléments fondamentaux: 1) les tumeurs colorectales sont le résultat de l'activation des oncogènes par mutation; 2) plusieurs mutations génétiques sont nécessaires pour la malignisation cellulaire; 3) c'est l'accumulation des altérations génétiques plutôt que leur ordre chronologique qui détermine les caractéristiques de la tumeur colorectale (16).

Dans la séquence adénome-carcinome de progression tumorale, la première lésion identifiable est le foyer de cryptes aberrantes, une lésion dysplasique de l'épithélium colique. Les foyers de cryptes aberrantes subissent expansion pour former des polypes adénomateux, macroscopiquement visibles. La formation d'un microadénome en réplication, exposé aux substances génotoxiques du contenu luminale rendrait à son tour plus probable l'occurrence d'un deuxième événement cancérogène. Les adénomes peuvent évoluer vers des carcinomes in situ, mais la vaste majorité des adénomes ne donne pas origine à un carcinome. Approximativement, 25% des adénomes de plus de 1 cm se transformeront en cancer du

vivant des sujets (17). Les mécanismes conduisant un carcinome in situ par étapes successives jusqu'à l'invasion régionale et la métastase ne sont pas bien maîtrisés (18).

Il est largement accepté que les tumeurs colorectales trouvent leur origine dans des cellules souches situées au pôle basal de la crypte intestinale. Pour qu'il y ait une population des cellules mutées qui donne lieu plus tard à un adénome, une cellule souche de la crypte (ou une des cellules-filles) doit subir l'événement initiateur. En raison de la microarchitecture du côlon, l'événement initiateur devrait être d'origine systémique plutôt que intraluminale. Or, la structure de la crypte et la dynamique de la réplication cellulaire garantissent que l'interaction entre le contenu du côlon et les cellules en réplication est essentiellement inexistante (19). La présence des cellules dysplasiques dans le pôle apical des la cryptes dont les cellules du pôle basal n'ont pas subi de mutations a été constatée récemment. Cette constatation a donné origine à l'hypothèse de la morphogénèse du pôle apical vers le pôle basal de la crypte (*top-down*), coexistant avec la réplication des cellules souches du pôle basal de la crypte. Selon cette hypothèse, les cellules génétiquement modifiées se déplaceraient latéralement pour donner origine aux nouvelles cryptes qui remplaceraient éventuellement les cryptes normales (20).

L'identification de caractères génétiques héréditaires spécifiques du cancer colorectal, de modifications génétiques des proto-oncogènes, gènes suppresseurs de tumeurs et gènes du système de réparation de dommages de l'ADN (MMR ou *mismatch repair genes*) ont permis la formulation des plusieurs voies moléculaires de cancérogenèse colorectale (16).

La première voie moléculaire (APC-β caténine-Tcf MYC) est caractéristique de la séquence adénome-carcinome. L'événement clé des modifications génétiques est la mutation germinale ou somatique du gène APC -de polypose adénomateuse colique- (21). Une mutation constitutionnelle délétère du gène *APC* est identifiée dans environ 90 % des polyposes

adénomateuses familiales (FAP ou *familial adenomatous polyposis*) (22). La présence des mutations dans le gène APC a été identifiée dans les tumeurs colorectales qui n'ont pas de contexte héréditaire évident ou sporadiques, qu'elles soient bénignes ou malignes, suggérant que la mutation de l'*APC* est un événement précoce de la tumorogenèse colorectal (23) . La mutation de l'APC origine les conditions - anomalies d'adhérence, de migration, et de réplication- pour la formation d'un polype (24) et pour la transition de la muqueuse colique normal au carcinome métastatique (25).

La deuxième voie moléculaire est la voie du cancer colorectal héréditaire sans polyadénomes (HNPCC ou *hereditary non polyposis colon cancer).* Le syndrome HNPCC est dû à des mutations des gènes impliqués dans la réparation des dommages primaires de l'ADN (22). Au moins six gènes responsables des prédispositions au HNPCC ont été identifiés. Les plus fréquents sont localisés sur les chromosomes 2, 3 et 7 : *hMSH2, hMLH1, hPMS1* et *hPMS2* et sont impliqués respectivement dans 31 %, 33 %, 2 % et 4 % des cas (26)

La troisième voie moléculaire est par l'intermédiaire des colites ulcératives. Cette maladie est un contribuant mineur au fardeau global du cancer colorectal dans la population, mais les individus avec des colites ulcératives ont un risque 20 fois plus élevée de cancer du côlon (27). La voie colites ulcérative-cancer colorectal implique une séquence dysplasie-carcinome qui n'est pas associée avec la formation de polypes. Les changements moléculaires somatiques sont peu définis, mais il est connu que les mutations de l'APC sont rares (moins de 10% de cas) et que la mutation du gène p53 est un événement tôt (28).

Finalement, la quatrième voie décrite est par l'inactivation du gène du récepteur d'estrogène. La formulation de cette voie moléculaire repose sur certains évidences montrant que presque tous les cancers de côlon résultent des cellules dont le gène du récepteur d'œstrogène a été inactivé (29).

3. Principaux facteurs de risque de cancer du côlon et du rectum.

Des différences au niveau de l'embryologie, la physiologie et la morphologie entre la région proximale (droite) ou distale (gauche) à la flexure splénique de l'intestin font supposer que les voies de développement de tumeurs colorectales sont différentes selon le site d'origine de la tumeur. Les tumeurs localisées sur le côlon distal et le rectum ont leur origine à partir des polypes adénomateux plus fréquemment que les tumeurs localisées sur le côlon proximal, pour lesquels un parcours de novo est souvent invoqué. Les tumeurs colorectales chez les sujets atteints de polypose adénomateuse familiale se localisent en générale sur la partie droite du côlon autant que dans le cancer colorectal héréditaire sans polyadénomes, les tumeurs se localisent fondamentalement dans la partie gauche. La prévalence de mutations K-ras et le profil des mutations du gène p53 varient selon la région de l'intestin où se trouve le cancer (30).

Compte tenu de la carence de données suffisantes sur les facteurs de risque potentiels impliqués dans l'étiologie du cancer selon localisation anatomique à l'intérieur de l'intestin, nous traiterons dans ce rappel les facteurs de risque de cancer colorectal pris dans leur ensemble. Lorsque cela sera possible, nous ferons référence aux facteurs de risque selon la localisation de la tumeur.

3.1 Facteurs génétiques.

Seulement 5 à 10 % des cancers colorectaux sont des cancers héréditaires à transmission autosomique dominante. Les cancers dits sporadiques - qui n'ont pas de contexte héréditaire évident - représentent la majorité des néoplasies colorectales (31).

Les cancers héréditaires sont eux-mêmes subdivisés en deux groupes, exemplifiés par les deux syndromes plus importants. Le premier réunit les cancers caractérisés par la présence de

polyadénomes multiples : c'est la polypose adénomateuse familiale. Dans le deuxième groupe figure le cancer colorectal héréditaire sans polyadénomes.

La polypose adénomateuse familiale, qui est à l' origine de moins de 1 % des cancers colorectaux, est caractérisée par le développement, parfois dès l'enfance, des multiples adénomes sur le côlon et le rectum, qui peuvent être accompagnés par des manifestations extra coliques. Faute d'ablation chirurgicale, les individus affectés développent un carcinome vers l'âge de 40 ans, c'est à dire, approximativement 20 ans plutôt que les mêmes cancers parmi les individus non affectés.

Le cancer colorectal héréditaire sans polyadénomes ne montre pas de tendance à la polyposie étendue. Les traits caractéristiques de ce syndrome sont les antécédents familiaux, la révélation du cancer relativement tôt dans la vie adulte et l'association élevée avec d'autres tumeurs, en particulier ceux qui impliquent l'endométrium, l'appareil urinaire, l'estomac, et le système biliaire.

Les facteurs mis en cause dans la survenue des formes non héréditaires de cancer colorectal impliquent aussi bien des facteurs épigénétiques, notamment environnementaux et nutritionnels, que des facteurs génétiques (32). Les facteurs épigénétiques interagissent probablement avec le « fond génétique » des individus et particulièrement avec certains gènes de susceptibilité, agissant de manière additionnelle et constituant l'assise de prédispositions génétiques mineures. Les études épidémiologiques révèlent un risque de cancer du côlon multiplié par 2 chez les sujets possédant un membre de la famille au premier degré atteint de ce cancer. Le risque est multiplié par 1,5 si le membre de la famille est âgé de plus de 60 ans au moment du diagnostic ou par 3 si le diagnostic a eu lieu avant l'âge de 60 ans. Si les deux parents sont atteints, le risque est multiplié par 4 quel que soit l'âge de diagnostic (33).

3.2 Inactivité physique et obésité.

Pris dans son ensemble, les données scientifiques indiquent de manière assez convaincante que le bilan énergétique, déterminé par un équilibre entre les apports et les dépenses énergétiques, joue un rôle protecteur contre le cancer du côlon (34).

L'inactivité physique émerge en tant qu'un des facteurs de mode de vie le plus fortement lié au risque de cancer du côlon et des adénomes colorectaux. L'effet protecteur de l'activité physique semble être plus importante pour les adénomes de grande taille que pour ceux de petite taille (35). L'association de l'activité physique avec le risque de cancer est plus forte pour les tumeurs situées dans la région distale du côlon (36). L'activité physique n'apparaît pas comme un facteur de protection contre le cancer du rectum (37).

La réduction moyenne de risque de cancer du côlon parmi les participants le plus physiquement actifs par rapport aux sujets moins actifs observée dans les études épidémiologiques est de 40-50% (34). La proportion du risque de cancer du côlon attribuable à l'inactivité physique a été estimé en 13-14% (38).

Les mécanismes biologiques pour expliquer l'effet protecteur de l'activité physique dans l'étiologie de cancer du côlon restent très controversés. Les mécanismes invoquent principalement l'effet de l'activité physique sur l'hyperinsulinémie, l'obésité, la diminution du temps de transit intestinal, ainsi que d'autres effets probables tels que la diminution de la sécrétion des acides biliaires et la modification de la flore intestinale et des effets sur le système immunitaire (39;40). En outre, l'activité physique pourrait affecter le risque de cancer indirectement à travers son influence sur le comportement alimentaire, le tabagisme et la consommation d'alcool (41).

En dépit d'un certain manque de concordance, l'index de masse corporelle (IMC) s'est avéré d'être lié au risque de cancer du côlon dans la majorité des études épidémiologiques (34). En

général, les études rapportent un risque de cancer deux fois plus élevé chez les sujets avec index de masse corporelle égal ou supérieur à 30 kg/m2 (sujets obèses), comparés à ceux qui ont un index au-dessous de 23 kg/m2 (sujets minces). L'obésité est aussi associée au risque des adénomes colorectaux (34;35). L'index de masse corporel est plus étroitement associé à la survenue des adénomes de grande taille qu'à celle des adénomes de plus petite taille, ce qui fait penser que les mécanismes en rapport avec l'excès de poids agissent à une étape ultérieure, peut-être contribuant à la promotion et à la progression des adénomes vers le cancer (34). Les études ne montrent pas en général, l'existence de relation entre le poids corporel et le cancer du rectum.

La relation entre le poids corporel et le cancer du côlon a été observée de manière plus concordante chez les hommes que chez les femmes. La raison de cette différence est peu claire, mais une explication possible serait un effet de protection de l'hyper-œstrogénémie chez les femmes post-ménopausées avec excès de poids (42).

Il a été proposé que les composants du syndrome métabolique, en particulier la résistance à l'insuline et le hyperinsulinémie résultante, puissent être le lien fondamental entre le bilan énergétique et le cancer du côlon (43). L'inactivité physique, l'index de masse corporelle élevé et l'adiposité centrale sont liés au risque élevé de cancer colorectal et à l'hyperinsulinémie. L'insuline a un effet mitogénique sur des cellules coliques in vitro (44;45) et elle s'est avéré carcinogénique chez les rats (46;47).

D'autre part, les niveaux élevés d'insuline peuvent augmenter la biodisponibilité du facteur de croissance similaire à l'insuline (IGF)-1 plasmatique par l'inhibition de la synthèse de protéines de liaison (IGFBPs) de l'IGF-1. Plusieurs études chez l'homme ont trouvé une association, en générale de faible magnitude entre les niveaux élevés de l'IGF-1 et le risque élevé de cancer du côlon (48). Malgré la plausibilité biologique, le nombre limité d'études conduites jusqu'au présent et le manque de standardisation des méthodes de laboratoires pour

déterminer les niveaux d'IGF-1 plasmatique et de ses protéines de liaison ne permettent pas arriver aux conclusions définitives sur cette association.

3.3 Alcool.

Dans plusieurs études épidémiologiques, les hommes et les femmes qui consomment plus d'alcool se sont avérés soumis à un plus grand risque de cancer colorectal ou de son précurseur adénomateux. Une méta-analyse récente qui a synthetisé quantitativement ces résultats montre que le risque de cancer du côlon et du rectum augmente de 15% pour chaque 100 grammes d'augmentation des apports d'alcool dans les boissons (49). Les données existantes ne soutiennent pas qu'une source d'alcool pourrait être plus fortement associée au risque de cancer colorectal que l'alcool dans l'ensemble (50-52). Il a été proposé que le risque attribué à l'alcool serait en partie lié à son effet anti-folique ou plus spécifiquement à ses effets sur la méthylation de l'ADN (53;54).

3.4 Tabac.

Le tabac est le facteur de risque de cancer le mieux établi et le plus important, aussi bien par la force de l'association entre tabagisme et risque de cancer, que pour le nombre de cancers dont l'étiologie est influencée par le tabac. Le tabac augmente le risque de cancer du poumon, du larynx, du pharynx, de l'œsophage, du pancréas, de la vessie, de l'estomac et du rein (55). De manière moins convaincante, des études ont montré que le tabac peut aussi augmenter le risque de leucémie myeloïde, de cancer du foie et du col utérin. D'autres types de cancers, tels que le cancer du sein, de l'ovaire et de la prostate ne semblent pas liés au tabagisme.

Le tabac n'est pas clairement associé au risque de cancer colorectal dans les études épidémiologiques (56). Cependant, plusieurs études ont démontré que les gros fumeurs ont un risque d'adénomes colorectaux - un précurseur établi de cancer colorectal- de deux à trois fois

plus élevé que les individus non-fumeurs (57). Ces résultats sont en faveur d'une relation entre le tabac et le cancer colorectal. Pour expliquer ces résultats apparemment incohérents Giovannucci et collaborateurs en 1994 ont suggéré que le tabac est un initiateur de la cancérogenèse (57). Une longue période d'induction est nécessaire avant que les altérations moléculaires conduisent à la formation d'un adénome et puis d'un cancer. L'association tabac-cancer colorectal ne serait donc pas observable dans des études qui ne prennent pas en compte le temps d'induction dans l'analyse et l'interprétation des données.
Toutefois, la relation tabac-cancer colorectal reste encore à vérifier par des études prospectives avec un suivi suffisamment long.

3.5 Statut inflammatoire.

Dans des nombreuses études épidémiologiques, la prise fréquente de l'aspirine a été trouvée en association avec un risque diminué d'adénomes et de cancer colorectal (50). Des études d'intervention randomisées récentes ont montré que chez des patients présentant une histoire d'adénome colorectal ou de cancer, l'aspirine réduit le risque de récurrence d'adénome dans un délai de 1 à 3 ans (58). La dose et la durée nécessaires de l'utilisation de l'aspirine pour la prévention du cancer colorectal ne sont pas bien connues. La chimio prévention optimale peut exiger des doses d'aspirine élevées et malheureusement, les effets adverses de l'aspirine, y compris le saignement gastro-intestinal, sont dépendants de la dose (59).

Il n'est pas certain que les drogues anti-inflammatoires non stéroïdiennes (NSAIDs), qui partagent plusieurs des mécanismes d'action de l'aspirine, exercent un effet antinéoplasique semblable (60) .

3.6 Thérapie hormonale substitutive.

Des études épidémiologiques d'observation et d'intervention ont suggéré un effet protecteur de la thérapie hormonale substitutive sur le risque de cancer colorectal (61). Dans une méta-analyse de 18 études observationnelles, la diminution du risque de cancer du côlon a été estimée à 20% et pour le cancer de rectum à 19%, chez les femmes qui ont utilisé la thérapie d'œstrogènes ou la thérapie combinée d'œstrogène et progestine (61). Plus récemment, l'étude d'intervention américaine WHI (*Woman Health Initiative*) (62) et l'étude HERS II (*Heart and Estrogen/progestin Replacement Study*) (63) ont montré l'existence d'une relation inverse entre le risque de cancer du côlon et l'utilisation de la thérapie combinée œstrogène-progestine mais l'absence d'effet de la thérapie d'œstrogène.

Le nombre de cas de cancer du côlon qui pourrait être prévenu avec la thérapie combinée œstrogène-progestine est estimé en 6 cas pour chaque 10 000 femmes (62). Plusieurs études concordent sur l'augmentation du risque de maladies cardiovasculaires, cancer du sein et d'autres maladies avec la thérapie combinée œstrogène-progestine (62;64). L'excès de risque est probablement faible chez les utilisatrices (e.g. 8 femmes pour chaque 10 000 utilisatrices auraient cancer du sein attribuable au traitement hormonal) mais supérieur aux bénéfices estimés. Le bilan entre les risques et les avantages de la thérapie hormonale substitutive est tel, que son utilisation répandue pour la prévention du cancer du côlon n'est pas recommandée (64).

3.7 Alimentation et risque de cancer colorectal.

L'idée que l'alimentation et les cancers puissent avoir un rapport remonte dans le temps jusqu'à la dynastie Song en Chine (960-1279 après JC), où le constat de la relation causale entre nutrition déficiente et cancer de l'œsophage était déjà avancé (65). Plus proche de nous,

les études épidémiologiques dites écologiques, qui comparent la consommation alimentaire et la mortalité par cancers de différentes régions ou pays et prennent en compte l'effet des migrations, ont renforcé l'hypothèse de la relation alimentation/cancer. Ces études ont montré l'existence de corrélations internationales fortes entre le risque de cancers de l'intestin gros et les apports *per capita* de viande et lipides alimentaires (66), spécifiquement d'origine animale (67) ainsi qu'une relation inverse avec les apports de fibres alimentaires (68). Des études en laboratoire, chez les animaux et des études épidémiologiques observationnelles dites analytiques, car elles apportent des éléments permettant d'établir une relation de cause à effet entre aliments et risque de cancers ainsi que des études d'intervention sur les individus ont permis d'améliorer nos connaissances sur l'effet que certains aliments exercent sur le risque de certains cancers.

Les hypothèses actuelles sur le mode d'action des facteurs alimentaires sur la progression tumorale sont multiples. Certains facteurs alimentaires, comme des vitamines, minéraux ou oligo-élements interviendraient dans la modulation de systèmes enzymatiques impliqués dans le métabolisme de cancérogènes. L'alimentation interviendrait également dans la protection de l'ADN contre les lésions oxydatives et les réparations fautives de l'ADN, ainsi que dans la restauration des communications intercellulaires. Certains facteurs alimentaires interviendraient aussi dans l'activation et/ou l'inactivation d'oncogènes ou de gènes suppresseurs de tumeurs et dans la régulation des mécanismes de prolifération et différenciation cellulaire responsables de la promotion tumorale de cellules initiées. Ces multiples mécanismes qui peuvent interagir ou avoir des effets antagonistes sur la cancérogenèse en fonction du type, de la localisation du cancer et d'une série d'autres cofacteurs potentiels, reflètent la complexité de l'alimentation comme facteur d'exposition dans l'étude de son association avec le cancer (50;65).

Les données des études en laboratoire, chez les animaux et des études épidémiologiques sur l'alimentation et le cancer ont été évalués par trois comités d'experts internationaux: le groupe d'experts du Centre national d'Études et de Recommandations sur la Nutrition et l'Alimentation (CNERNA) en France, 1996 (65), le groupe de travail sur l'alimentation et le cancer du *Committee on Medical Aspects of Food and Nutrition Policy (COMA)* au Royaume UNI, 1998 (69), et le groupe d'experts du *Word Cancer Research Fund/American Institute for Cancer Research* (WCRF/AICR) en 1997 (50). Dans ce rappel, nous ferons notamment référence aux conclusions des trois comités d'experts ainsi qu'aux résultats des études de cohorte publiées plus récemment qui ont apportés des nouvelles preuves de la relation entre l'alimentation et le cancer.

3.7.1 Les apports caloriques.

Nous avons déjà discuté l'hypothèse (Chapitre I, Section 3.2) selon laquelle l'hyperinsulinémie serait le facteur commun dans la relation entre le risque de cancer du côlon et les apports énergétiques excessifs, l'inactivité physique ainsi que l'excès de poids corporel. En ce qui concerne l'alimentation, les apports caloriques excessifs ont un rôle majeur sur l'hyperinsulinémie et certains facteurs alimentaires pourraient aussi avoir une contribution, tel que suggéré par certaines études. Les apports élevés de sucrose et d'aliments riches en hydrates de carbone ont été associé avec le cancer colorectal, en particulier chez des individus avec style de vie sédentaire (70;71). Les régimes alimentaires caractérisés par un index glycémique élevé semblent augmenter le risque de cancer colorectal (72), mais toutes les études ne s'accordent pas sur cette association (73;74).

3.7.2 Les lipides alimentaires.

Outre les études écologiques qui ont montré l'existence d'une association entre les apports élevées de lipides alimentaires et d'acides gras saturés et le risque de cancer du côlon,

plusieurs études ont montré une incidence plus élevée de tumeurs colorectales chez des rats nourris avec des régimes de contenu lipidique similaire aux régimes alimentaires des humains, avec un effet de promotion pour les acides gras saturés et insaturés (75) ce qui a fait supposer que c'est la composition en lipides et non la quantité totale qui serait impliquée dans l'étiologie du cancer du côlon.

Bien que les comparaisons écologiques (66;76) et les études chez les animaux (77) soutiennent une association positive entre la prise des lipides et le risque du cancer colorectal, les résultats des études épidémiologiques analytiques ont été peu concluants (50).

Une méta-analyse qui a mis en commun les résultats de 13 études de type cas-témoins n'a trouvé aucune association entre les lipides de l'alimentation et le risque colorectal (78). Parmi six études de cohorte (79-82), aucune évidence d'association n'a été observée dans quatre études (70). Une association positive avec les acides gras saturés a été reportée dans quelques études (83;84) mais non dans d'autres (70;81;82). Dans deux études, une association positive entre les acides gras polyinsaturés et le risque du cancer colorectal a été observée (85;86), alors que dans d'autres études aucune association n'a été observée (80;81;87;88). Très peu d'études ont examiné l'association avec des acides gras diététiques spécifiques (89). Finalement, les études d'intervention n'ont pas montré que les régimes faibles en lipides diminuent la récurrence de polypes (90) ou de cancer du côlon (91).

Certaines études cas-témoins qui trouvent une association positive entre le risque de cancer du côlon et les apports en lipides, trouvent aussi une relation positive entre le risque de cancer et les apports énergétiques, ce qui n'est pas surprenant, étant donné la corrélation positive entre la prise calorique et les apports lipidiques. Les lipides sont les nutriments les plus riches en calories par unité de poids et de ce fait sont majoritairement invoqués par les nutritionnistes dans le développement de l'obésité, bien que leur rôle dans l'obésité soit contesté. Les

associations observées entre les lipides alimentaires et le cancer colorectal pourraient donc être statistiquement « confondues » par les apports caloriques.

3.7.3 Les fibres alimentaires.

Burkitt et Trowell (92) ont rendu populaire l'hypothèse selon laquelle les fibres alimentaires- définies comme les composantes des cellules végétales qui sont résistantes à la digestion par les sécrétions du tractus gastro-intestinal humain- ont un rôle critique dans la cancérogenèse colorectale. Cette hypothèse est concordante avec les résultats de plusieurs études cas-témoins (50;65;69), mais elle n'a pas été confirmée dans toutes les études de cohorte. Ainsi, dans la grande étude européenne EPIC, une association inverse significative entre les fibres alimentaires et le cancer de côlon a été observée (93;94), alors que les données du *Pooling Project* des études de cohorte ne confirment pas cette association (95). Le manque de concordance des études a été expliqué en raison de la nature hétérogène de la fibre et de la manière dont la fibre est définie ou mesurée dans les aliments et d'autre part, par le manque d'hétérogénéité des apports des populations étudiées et par des erreurs de mesures intrinsèques aux questionnaires alimentaires utilisés (93;94).

3.7.4 Les fruits et les légumes.

Les groupes d'experts (50;65;69) sont globalement en accord sur la possible diminution du risque de cancer colorectal liée à la consommation élevée de légumes Les données sur les fruits ont été beaucoup moins convaincantes par rapport à un possible effet protecteur. Parmi les différents mécanismes biologiques qui ont été évoqués pour expliquer la relation avec les légumes, le plus important est probablement celui lié aux effets des fibres alimentaires sur la fonction, la biologie et l'environnement microbiologique du côlon, même si d'autres hypothèses relatives à l'effet protecteur des microconstituants des fruits et des légumes restent d'actualité. Parmi les microconstituants de fibres et des légumes qui peuvent

avoir un effet protecteur on trouve le folate, les carotenoïdes, les vitamines C et E, le sélénium, les dithiolthiones, les glucosinolates et les indoles, les isothiocyanates, les flavonoïdes, les phénols, les stérols, les inhibiteurs de protéase et le limonène. Ces agents peuvent agir par le même mécanisme ou par voie complémentaire, y compris l'induction d'enzymes de désintoxication, l'inhibition de la formation de nitrosamines, l'approvisionnement des substrats pour la formation d'agents antinéoplasiques, la dilution des cancérogènes dans le tractus digestif, la modification du métabolisme hormonal, des effets antioxydants, entre autres (96;97).

Pourtant, dans les études de cohorte plus récentes, l'absence d'association entre la consommation élevée de légumes et le risque de cancer du côlon a été presque unanimement observée (98). Malgré les résultats négatifs des études, le rôle protecteur des légumes n'a pas été écarté par les épidémiologistes parce que, bien que sans atteindre la signification statistique, la plupart des risques relatifs observés sont inférieures à l'unité. Selon Willett, (99) la protection des micronutriments des fruits et des légumes a pu être masquée par la prise de vitamines et minéraux sous forme de compléments alimentaires et dans des aliments fortifiés, ce qui est fréquents actuellement dans certaines populations économiquement aisées . D'autre part, des données suggèrent l'existence d'un seuil de consommation au-dessus duquel il n'y a pas de relation dose-réponse (moins de 2,5 portions de fruits et de légumes par jour)(100). Dans ce cas, la relation entre le risque de cancer colorectal et les apports en fruits et légumes ne serait observé que dans des études avec un nombre suffisant de sujets avec consommation des fruits et légumes inferieure au seuil de l'exposition.

Une explication d'ordre plutôt méthodologique est le manque de puissance statistique pour détecter une association faible en présence d'erreurs de mesure importantes. Il faut noter que la mesure de la portion alimentaire des légumes pose de difficultés, particulièrement en ce qui

concerne les apports en légumes cuits ou en plats élaborés (101). L'effet de l'erreur de mesure serait d'affaiblir l'estimation du risque relatif vers l'absence d'association (102).

3.7.5 Le folate.

Le folate, l'une des vitamines du groupe B, se trouve principalement dans les fruits et les légumes, en particulier dans les légumes à feuilles vertes. Des études en laboratoire, chez les animaux et des études épidémiologiques d'observation (97), ainsi que des études d'intervention (103) avec supplémentation en folate ont montré un rôle protecteur du folate sur le cancer colorectal. Dans quelques études prospectives (104-106) les apports en folate étaient positivement associés à une diminution de risque de cancer du côlon proximal, mais non associés au cancer du côlon distal et du rectum, tandis que d'autres études prospectives n'ont pas donné de preuve d'association (107;108). Jusqu'à présent, le manque de concordance des résultats des études épidémiologiques ne permet pas d'élaborer une conclusion définitive sur cette relation.

Freudenhein et colaborateurs ont été les premiers à proposer l'hypothèse folate-cancer colorectal (53). L'hypothèse repose sur la fonction du folate en tant que donneur de monocarbones pour la synthèse de nucléotides et la méthylation de l'ADN et sur l'observation que l'hypométhylation de l'ADN est un trait fréquent des tumeurs (109). Toutefois, l'importance du folate dans les mécanismes de méthylation de l'ADN n'est pas encore bien connue. La participation du folate dans la méthylation de l'ADN est partiellement régulée par l'enzyme méthylène tétrahydrofolate réductase (MTHFR). L'étude des polymorphismes du gène de l'enzyme MTHFR a donné des résultats divergents (110).

3.7.6 Le calcium.

L'hypothèse que le calcium et les produits laitiers peuvent jouer un rôle protecteur sur le cancer colorectal a été mise en évidence dans plusieurs études prospectives épidémiologiques de grande taille et dans des études d'intervention (111-115). Ces résultats sont concordants

avec des expériences chez des rongeurs qui montrent que l'administration de suppléments de calcium conduit à la diminution de la prolifération cellulaire induite par les sels biliaires ou la diméthylhydrazine (116), ainsi qu'à une réduction de l'incidence et la multiplicité de tumeurs colorectales (117). On a émis l'hypothèse que le calcium aurait un effet protecteur sur le cancer colique en formant des savons insolubles avec les acides biliaires et les acides gras, neutralisant ainsi leurs effets stimulants sur la prolifération de la muqueuse intestinale. Cette hypothèse, bien que soutenue par un certain nombre d'études expérimentales sur l'animal montre cependant des résultats contradictoires dans des études cliniques menées chez l'homme (118-122). Une autre hypothèse basée sur des études *in vitro* sur des cellules épithéliales humaines (116;123) évoque une action intra-cellulaire du calcium qui inhiberait la prolifération des cellules épithéliales coliques en induisant leur différenciation. Cette hypothèse repose sur l'absorption de calcium par la cellule par l'intervention indirecte de la vitamine D.

3.7.7 La viande et le poisson.

Les trois comités d'experts (50;65;69) ont conclu que les données scientifiques indiquent que la consommation élevée de viande rouge et surtout celle de viande traitée (charcuterie) augmentent probablement le risque de cancer colorectal Cependant, les deux groupes d'experts ont convenu que les données épidémiologiques n'étaient pas concordantes. Quelques études montrent une relation inverse avec la consommation élevée de poisson, ou une relation positive avec le ratio viandes rouges / viandes blanches, qui a été expliqué par la substitution de viande rouge par viande blanche (volailles et poisson) dans l'alimentation (79;80). Nous discuterons au cours de ce rapport les données épidémiologiques en faveur de la relation entre la viande rouge et le cancer colorectal.ainsi que les principales hypothèses qui pourraient expliquer cette association.

Finalement, le profil alimentaire riche en viandes rouges et viandes traitées, en acides gras saturées et *trans*-insaturées, en hydrates de carbones et en sucres raffinés a été trouvé en rapport avec le risque de cancer colorectal (124).

4. Objectifs du travail.

Notre objectif consiste à étudier la relation entre la consommation de viandes et de poissons et le risque de cancer colorectal à travers l'Etude Prospective Européenne sur le Cancer et la Nutrition (*EPIC : European Prospective Investigation into Cancer and Nutrition*), une étude multicentrique prospective incluant plus de 400 000 sujets volontaires de dix pays européens, établie pour étudier la relation entre des facteurs nutritionnels, métaboliques, génétiques et de mode de vie, avec le risque de cancer.

Nous avons tout d'abord réalisé une synthèse descriptive et méta-analytique des résultats des études épidémiologiques de type cas-témoins et de cohorte étudiant la relation entre le risque de cancer colorectal et la consommation de viande rouge (Chapitre II). Cette revue systématique de la littérature a été complétée par une mise a jour des connaissances sur la relation des hydrocarbures polycycliques aromatisés et les amines hétérocycliques qui se forment pendant la cuisson de la viande, et le risque de cancer colorectal.

Le chapitre III présente une étude descriptive des profils de consommation de viande rouge dans les différentes cohortes participantes à EPIC. Nous donnons aussi une description du projet EPIC en ce qui concerne les caractéristiques des sujets participants, les méthodes de collection des données alimentaires et non-alimentaires, les procédures pour recueillir les mesures anthropométriques et les échantillons de sang, ainsi que les méthodes de suivi pour le statut vital, la mortalité et l'incidence de cancer. Nous avons ensuite estimé les apports moyens journaliers de viande rouge et des types divers de viande rouge, à partir des rappels de 24 heures recueillis dans des sous-échantillons de chaque cohorte. Enfin, nous avons estimé les apports de viande rouge et du poisson selon les principales méthodes de cuisson de la viande et du poisson dans chaque sous-cohorte.

Les résultats de notre étude sur la relation entre le risque de cancer colorectal et la viande rouge, la charcuterie, les volailles et le poisson sont présentés dans le chapitre IV. Nous avons aussi examiné si les fibres alimentaires (facteur de protection contre le cancer colorectal dans la population de l'étude EPIC) pouvaient modifier la relation entre la viande rouge et le cancer colorectal.

CHAPITRE II. VIANDE ROUGE ET CANCER COLORECTAL. SYNTHESE DES RESULTATS DES ETUDES EPIDEMIOLOGIQUES.

1. **Consommation de viande et risque de cancer colorectal : méta-analyse de la relation dose-réponse observée dans des études épidémiologiques.** *Meat consumption and colorectal cancer risk: dose-response meta-analysis of epidemiological studies. Int J Cancer. 2002; 98 : 241-56.*

2. **Méthodes de cuisson de la viande et risque de cancer**. *Meat cooking and cancer risk In: Nutrition and Lifestyle: Opportunities for Cancer Prevention pp 181-6. Edited by E. Riboli and R. Lambert. IARC Scientific Publications No 156 IARC Press Lyon 2002*

1. Consommation de viande et risque de cancer colorectal : méta-analyse de la relation dose-réponse observée dans des études épidémiologiques.

Basé sur:

Meat consumption and colorectal cancer risk: dose-response meta-analysis of epidemiological studies. Norat T, Lukanova A, Ferrari P et Riboli E. Int J Cancer. 2002; 98 : 241-56.

Le groupe du Centre National d'Etudes et de Recommandations sur la Nutrition et l'Alimentation (CNERNA-1996) en France (65), le groupe du *World Cancer Research Fund/American Institute for Cancer Research* (1997) (50) et le *Working Group on Diet and Cancer of the Committee on Medical Aspects of Food and Nutrition Policy* (COMA-1997) (69) ont conclu que la consommation élevée de viande rouge pourrait être liée a l'augmentation de risque de cancer colorectal et ont recommandé la consommation de volailles et de poisson de préférence à celle de viande rouge. Ces conclusions se basent sur des résultats non entièrement concordants, mais qui montraient en général soit une association positive, soit l'absence d'association.

Dans notre étude, nous avons approfondi l'évaluation faite par les groupes d'experts avec l'utilisation d'une approche méta-analytique qui avait pour objectifs :

- faire un bilan qualitatif et quantitatif des données épidémiologiques sur la relation viande rouge-cancer colorectal apportées au travers d'études cas-témoins et de cohorte publiées dans des journaux scientifiques de langue anglaise entre 1973 et 1999,
- estimer la fraction du risque de cancer colorectal attribuable à la consommation de viande rouge ainsi que la fraction de risque qui pourrait être prévenue avec des modifications hypothétiques des apports de viande rouge dans des populations de différentes régions géographiques.

Nous avons inclus dans la méta-analyse 34 études de type cas-témoins et 14 études de cohorte cités dans la base de données MEDLINE (*National Library of Medicine*, Washington, DC). Deux mesures de synthèse de l'association ont été calculées. D'abord, nous avons extraits les risques relatifs associés aux niveau de consommation de viande rouge plus haut par rapport au niveau plus bas reporté dans chaque étude et ensuite, nous avons calculé la moyenne pondérée des risques relatifs (RR) utilisant comme coefficient de pondération l'inverse de la variance du risque relatif dans chaque étude (125). Ensuite, nous avons modélisé la relation «dose – réponse » entre les apports en viande rouge et le risque de cancer colorectal à l'aide d'un modèle log-linéaire ($lnRR = bX$, où la consommation de viande rouge était traitée comme « facteur de exposition X » et le risque de cancer colorectal comme « variable de réponse ». Nous avons utilisé la méthode proposée par Greenland et Longnecker (126) qui permet de tenir compte de la corrélation entre risques relatifs calculés avec la même catégorie de référence. La mesure de l'effet a été la moyenne pondérée des coefficients *b* des modèles de régression. Des modèles d'effets aléatoires ont été utilisés lorsque nous avions des preuves statistiques d'hétérogénéité d'effets.

La consommation de viande rouge est associée positivement au risque de cancer colorectal (RR=1.35 ; 95% intervalle de confiance [IC]= 1.21-1.51, comparaison du niveau de consommation de viande rouge le plus haut par rapport au niveau de consommation le plus bas) [Tableau I]. Avec le modèle linéaire de la relation « dose-réponse », nous avons estimé à 24% l'augmentation du risque de cancer colorectal pour chaque augmentation de la consommation de viande rouge de 120 grammes, et à 36% l'augmentation de risque pour une augmentation de 30 grammes de viande traitée (charcuterie) [Tableau II]. Ces résultats suggèrent que l'association entre le risque de cancer colorectal et la viande traitée est plus forte que l'association avec la viande rouge qui n'a pas été traitée. Néanmoins, cette

observation n'est, à ce stade, qu'une hypothèse de travail pour des analyses futures, puisqu'il s'agit d'une analyse de sous-groupe *a posteriori*.

La fraction du risque de cancer colorectal dans la population attribuable à la consommation de viande rouge a été calculée en utilisant le coefficient de régression linéaire et les apports de viande rouge *per capita* pour plusieurs régions géographiques. Pour estimer les apports *per capita* de viande rouge, nous avons utilisé les données des bilans alimentaires de population de l'Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO-UN ; http://faostat.fao.org). Ces données sont basées sur la disponibilité des aliments. Nous avons donc partiellement corrigés la surestimation de la consommation réelle avec un facteur de correction que nous avons déduit à l'aide des enquêtes alimentaires réalisées dans les mêmes populations ou dans des populations similaires. Nous avons utilisé le même coefficient b de régression pour toutes les régions géographiques, compte tenu du nombre limité d'études.

Nous avons estimé la part du risque attribuable à la consommation de viande rouge dans les trois régions géographiques suivantes : Australie/Nouvelle Zélande, Argentine/Uruguay/Paraguay et l'Amérique du Nord, à 25.6%, 19.6% et 13.9% chez les hommes et à 19.2%, 13.6% et 9.5% chez les femmes respectivement. Les parts de risque estimées pour les pays situées au nord, sud et l'est de l'Europe sont respectivement de 5.9%, 7.7% et 5.6% pour les hommes et de 5.3%, 3.9% et 2.4% pour les femmes. La fraction du risque qui pourrait être prévenue par une diminution de la consommation moyenne jusqu'à 10 grammes par jour et par habitant varie entre 7% et 19% selon la région géographique. Enfin, cette fraction est négligeable dans les pays à très faible consommation moyenne de viande rouge et de charcuterie (Tableau III).

Pour une interprétation correcte de ces résultats, il faut considérer que la méthode utilisée suppose que la relation entre le risque de cancer colorectal et les apports de viande rouge est de type causal et que l'estimation de la force de l'association (le coefficient beta du modèle de

régression) n'est pas soumise aux biais. D'autre part, nous avons combiné des études qui diffèrent dans les populations étudiées, les définitions de groupes alimentaires, les questionnaires alimentaires et les facteurs de confusions pris en compte. Les populations étudiées montrent également des différences dans la préparation et la préservation des produits alimentaires. Les inexactitudes des données disponibles des apports alimentaires habituels (FAO-UN) jouent également sur l'exactitude des résultats.

Nos estimations indiquent l'effet potentiel de la modification d'un facteur individuel - la diminution de la consommation de viande rouge chez les gros consommateurs dans cette étude- sur l'incidence du cancer colorectal dans la population en utilisant les données disponibles avec des modèles statistiques. L'approche suivie, bien qu'objective pour la méthode, constitue au même temps une simplification du problème qui ne prend pas en compte d'autres facteurs potentiellement associés au risque de cancer colorectal de manière directe ou indirecte et qui pourraient se modifier suite à la modification des apports de viande rouge, tels que, par exemple, la consommation de fruits et de légumes ou les apports caloriques totaux.

Les conclusions de cette étude n'impliquent pas l'élimination de la viande rouge de l'alimentation humaine mais ils suggèrent que la réduction de la consommation de viande rouge et de charcuterie chez les gros consommateurs pourrait contribuer à la prévention primaire du cancer colorectal.

Int. J. Cancer: **98,** 241–256 (2002)

DOI 10.1002/ijc.10126

Publication of the International Union Against Cancer

MEAT CONSUMPTION AND COLORECTAL CANCER RISK: DOSE-RESPONSE META-ANALYSIS OF EPIDEMIOLOGICAL STUDIES

Teresa NORAT, Annekatrin LUKANOVA, Pietro FERRARI and Elio RIBOLI*

Unit of Nutrition and Cancer, International Agency for Research on Cancer, Lyon, France

The hypothesis that consumption of red and processed meat increases colorectal cancer risk is reassessed in a meta-analysis of articles published during 1973–99. The mean relative risk (RR) for the highest quantile of intake *vs.* the lowest was calculated and the RR per gram of intake was computed through log-linear models. Attributable fractions and preventable fractions for hypothetical reductions in red meat consumption in different geographical areas were derived using the RR log-linear estimates and prevalence of red meat consumption from FAO data and national dietary surveys. High intake of red meat, and particularly of processed meat, was associated with a moderate but significant increase in colorectal cancer risk. Average RRs and 95% confidence intervals (CI) for the highest quantile of consumption of red meat were 1.35 (CI: 1.21–1.51) and of processed meat, 1.31 (CI: 1.13–1.51). The RRs estimated by log-linear dose-response analysis were 1.24 (CI: 1.08–1.41) for an increase of 120 g/day of red meat and 1.36 (CI: 1.15–1.61) for 30 g/day of processed meat. Total meat consumption was not significantly associated with colorectal cancer risk. The risk fraction attributable to current levels of red meat intake was in the range of 10–25% in regions where red meat intake is high. If average red meat intake is reduced to 70 g/week in these regions, colorectal cancer risk would hypothetically decrease by 7–24%.

Key words: *meat; colorectal cancer; attributable risk; preventable fraction*

Experimental and epidemiological studies have shown that food and nutrition modify colorectal cancer risk. The scientific evidence has been evaluated and summarised in recommendations by different expert groups that conclude that red meat consumption is likely to be related to increased risk of colorectal cancer. In 1996, the Colon Cancer Panel of the World Health Organisation-consensus conference on Nutrition in Prevention and Therapy on Cancer[1] concluded that consumption of red meat and processed meat was probably associated with increased risk for colorectal cancer and recommended that consumption of fish and poultry should be preferred to red meat. In the same year, the Centre national d'Ètudes et de Recommandations sur la Nutrition et l'Alimentation (CNERNA) in France published an evaluation of the scientific data on nutrition and cancer, in which the experts concluded that a diet poor in vegetables and rich in meat or fat of animal origin (excepting fish) is usually associated with an increased risk of colon cancer.[2] More recently, 2 major reports by the World Cancer Research Fund (WCRF)/American Institute for Cancer Research Report (AICR)[3] and the Working Group on Diet and Cancer of the Committee on Medical Aspects of Food and Nutrition Policy (COMA)[4] of the United Kingdom, recommended that western populations should decrease their consumption of red meat and increase consumption of vegetables in order to reduce colorectal cancer risk. Both panels agreed that the epidemiological results on meat were not consistent, but recognised that the studies conducted so far found either increased colorectal cancer risk or no association with risk, while no study has found a reduction in risk associated with high meat consumption.

Several hypotheses have been developed to explain the association between colorectal cancer risk and red meat.[5] The fat content of red meat could influence colon cancer risk by increasing the excretion of bile acids, whose products may act as tumour promoters by a non-specific irritant effect that increases cell proliferation in the colonic mucosa.[6,7] Other products of fat digestion, such as diacylglycerides, could selectively induce mitogenesis of adenomas and some carcinoma cells.[8] Fat could act by increasing saturated fatty acid content, or decreasing polyunsaturated fatty acid content in cell membranes leading to a reduction of the number and activity of insulin receptors.[9,10] Hyperinsulinemia could act as a growth factor and tumor promoter[11,12] and recent epidemiological evidence supports the association of insulin resistance with colon cancer risk.[13] The meat fat-hypothesis is consistent with the finding that lean beef did not promote colon carcinogenesis in rats[14] and that high consumption of beef could increase the concentration of secondary faecal bile acids.[15,16] Nevertheless, epidemiological studies have failed to show a consistent relationship between fat intake and colorectal cancer.[5,17]

During digestion, dietary protein is broken down into amino acids that are further degraded to ammonia, which may be carcinogenic to the colon.[18] There is, however, very limited evidence that protein per se increases colorectal cancer risk and some epidemiological studies have even reported a protective association between dietary protein and colon cancer. A possible explanation for this unexpected finding is that low intake of methionine may contribute to DNA methylation abnormalities, which might appear to be important in the initiation and progression of colon cancer.[19] Meat can be a major source of protein, but there is no evidence of an effect of meat protein on colorectal cancer risk.

Red meat has a higher iron content than white meat. Dietary iron enhances lipid peroxidation in the mouse colon and augments dimethylhydrazine-induced colorectal tumours in mice and rats[20] but the results of epidemiological studies are still insufficient.[21,22]

Red meat intake[23] enhances the production of endogenous promoters and possible carcinogens[24,25] such as *N*-nitroso compounds (NOC), which have been shown to induce the formation of DNA adducts in human colonocytes.[26] The same effect has not been observed with white meat.[23,27] NOC are also formed endogenously because the amines and amides produced primarily by bacterial decarboxylation of amino acids can be *N*-nitrosated in the presence of a nitrosating agent.[28–30] Nitrosamines have been detected in foods with added nitrates or nitrites, including salt-preserved fish and meat and in food processed by smoking or direct-fire dry-

Abbreviations: 95% CI, 95% confidence intervals; AP, attributable proportion; EPIC, European Prospective Investigation into Cancer and Nutrition; FAO, Food and Agricultural Organisation; FFQ, food frequency questionnaire; HCA, heterocyclic amines; NOC, *N*-nitroso compounds; PAH, polycyclic aromatic hydrocarbons; PP, preventable proportion; RR, relative risk.

Grant sponsor: World Cancer Research Fund.

*Correspondence to: Unit of Nutrition and Cancer, IARC, 150, Cours Albert Thomas, 69372 Lyon Cedex 08, France. Fax: +33-472-73-83-61. E-mail: Riboli@iarc.fr

Received 30 May 2001; Revised 3 September 2001; Accepted 21 September 2001

ing.[31,32] Supplements of nitrate have been shown to elevate faecal NOC levels.[27]

A mechanism that has attracted particular attention is the formation of heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH) in meat when it is cooked at high temperature for a long time or over an open flame. HCA and particularly the 2-amino-1-methyl-6-phenylimidazo (4,5-b) pyridine (PhIP) and the 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) are powerful mutagens and carcinogenic in mice, rats and non-human primates in a wide variety of organs, mainly the liver, but also skin, lung, colon and mammary gland.[23,33] The carcinogenic potential of heterocyclic amines in humans has not been established. PAHs are widely believed to make a substantial contribution to the overall burden of cancer in humans via tobacco smoking, occupational and environmental exposures. The major dietary sources of PAHs are cereals and vegetables rather than meat due to environmental contamination, except where there is high consumption of meat cooked over an open flame, as when barbecuing.[34,35] Information on dietary practices, such as cooking methods (frying, broiling, smoking and barbecuing), meat doneness and surface browning has been used to evaluate the potential relationship of dietary exposure to HCAs and PAHs with colorectal cancer or colorectal adenoma risk,[36–50] but the epidemiological evidence is still limited and many methodological issues need to be solved. The fact that the metabolism of heterocyclic amines can be more or less efficient depending on the genetic variability of at least three enzymes involved in *N*-acetylation (NAT1, NAT2 and CYP1a2) makes the problem more complex and data from epidemiological studies[45,51–57] on acetylation status and colorectal cancer risk are sparse and somewhat conflicting.

In this article, the epidemiological literature on meat and colorectal cancer is reviewed and the results quantitatively summarized with two purposes. The first is to reassess the status of the meat/colorectal cancer hypothesis based on the global epidemiological evidence. The second aim is to provide estimates of the proportion of colorectal cancer attributable to current red meat consumption, as well as estimates of the effect that hypothetical changes in red meat consumption could have on colorectal cancer incidence in different geographical areas of the world, assuming that the association is causal and that the simulated change in meat consumption levels could be achieved.

MATERIAL AND METHODS

Search methods

The criteria for inclusion of epidemiological studies were: case-control or cohort studies evaluating the relationship between total meat, red meat or processed meat and colon, rectal or colorectal cancer risk; in males, females or in both sexes combined; with incidence or mortality as the endpoint; providing the information required for the statistical analysis; published in English between 1973 and 1999 and referenced in the Medline database (National Library of Medicine, Washington, DC). Besides the MEDLINE search, we systematically examined the list of references in the identified articles.

The definition of exposure varied between studies. In most of the articles, total meat (sometimes simply called meat) included white and red meat from all sources while in others, fresh meat only was considered. Red meat was sometimes defined as the intake of beef, veal, pork, mutton and lamb consumed fresh, whereas in others, processed red meat was also included as part of the red meat group. Processed meat was defined in our article as the group including any of the following foods: ham, raw ham, cured or smoked bacon, sausage, cured or smoked lunch meat, salami, nitrite-treated meats and meat-products. "Charcuterie" and "delicatessen" were also considered equivalent to "processed meat."

Statistical methods

The overall effect-size statistics estimated were the average of the logarithm of the observed relative risks (estimated as the odds ratio in most of the studies) associated to the highest versus the lowest level of consumption, as reported in the papers. The RR was weighted by the inverse of its variance. A random effect meta-analysis was performed in situations where heterogeneity was present[58] to incorporate the between-study component of variance in the weight.[59] Only studies reporting RR estimates with confidence intervals or quantitative information allowing their computation were included in the meta-analysis.

For the dose-response analysis, the method proposed by Greenland and Longnecker[60] was used, that accounts for the correlation between risk estimates for separate exposure levels depending on the same reference group. The summary estimate was the pooled coefficient b in the linear-logistic regression model $\ln RR = bX$, where X is the difference of meat intake between each category and the reference category. The individual slopes of each study were combined by weighted average, using the inverse of their variances as weights. Random effect models were assumed when there was evidence of heterogeneity. 95% confidence intervals (CI) were calculated for the common regression slopes. An SAS macro was written for this purpose.

We extracted from the studies the risk estimates that reflected the greatest degree of controlling for confounders (*i.e.*, risk factors or energy). The method required that the number of case subjects, the number of control subjects, the adjusted logarithm of the RR and its variance estimates for three or more exposure levels were known. Some extra-computation was performed to complete the required data, provided that the paper gave the information to do so. If this was not possible, the paper was not included in the dose-response analysis. The log-rank test of Begg and Mazumdar[61] were used to explore publication bias.

Interstudy variation was analyzed by performing subgroup identification[62] and meta-regression analysis[60] using the Genmod procedure in SAS. The main sources of heterogeneity examined were design (case-control or cohort), site (colon, rectum or colorectal), geographical area (USA, Europe or other), gender (males, females or both genders combined) and meat definition (fresh meat and fresh plus processed meat together).

Rescaling of exposure

For the dose-response analysis the intake was rescaled to grams per day. If the highest category was open-ended, the open-ended boundary was calculated using as interval length the width of the closest interval. When the lowest category was open-ended, the lowest boundary was considered as zero. The value of X of each category was then calculated as the mid-point of the logarithm of the boundaries, retransformed to grams per day.

When the exposures were expressed on a qualitative scale (*e.g.*, high, medium, low), we used the mean consumption and the variance given in the article to estimate midpercentiles of each category assuming lognormal distribution. When exposure was expressed as the frequency of consumption, we used 120 g as the approximate average "portion size" of meat and of red meat and 50 g as "serving size." The portion size of processed meat was 50 g as well. We based our decision on the results of the Continuing Survey of Food Intakes by Individuals 1989–91 of the United States[63] and preliminary results of the Dietary Survey of the European Prospective Investigation into Cancer and Nutrition (EPIC) (Riboli, unpublished data).[64]

Fraction of colorectal cancer risk attributable to red meat consumption

We obtained estimates of the proportion of risk attributable to red meat consumption (AP) using the relative risks estimated with the dose-response curve associated to quartiles of consumption of red meat using non-consumption as reference category. The formula provided by Miettinen was applied.[65]

As estimates of the prevalence of red meat consumption by geographical area, we used per caput intakes provided in Food Balance Sheets by the Food and Agricultural Organisation (FAO, http://apps.fao.org), corrected for overestimation with data published from 18 national dietary surveys.[66,67] The correction factor was computed as the ratio between the per caput calorie intake estimated in a dietary survey in a given country and the per caput calorie intake published by the FAO for that country in the same year as the survey. Caloric intake was chosen to deduce an overall "correction factor," even if its overestimation is not exactly the same as for red meat, because energy values were available in all the surveys. For geographical areas for which we were not able to find dietary surveys, the correction factor of the closest region was applied (Appendix 1). A ratio of male/female consumption was computed in the surveys providing the information and its average applied for those regions for which this information was not available.

Quartiles of consumption were calculated assuming a lognormal distribution. To do that, we applied the total coefficient of variation of red meat consumption by gender estimated in the EPIC cohort study, that is, 83% for women and 85% for men.

Finally, the exercise included an estimation of the proportion of cancer cases that could potentially be prevented assuming a hypothetical reduction in red meat consumption in each population to an average of 70 g/week, *i.e.*, a small portion of red meat once a week. The preventable proportion (PP) was estimated as proposed by Miettinen.[65]

Attributable risk could not be estimated for processed meat consumption because we could not find estimates of processed meat consumption worldwide.

RESULTS

Characteristics of studies

Thirty-four case-control studies[37,39,41,43,47,54,68–95] and 14 cohort studies[19,40,42,44,48,50,57,96–102] were identified in our search. The main characteristics of the studies are presented in Appendix 2. Fourteen case-control studies were carried out in Europe, 11 in the USA (including 2 in Hawaii), 3 in Japan, 2 in Australia and 1 each in Canada, China, Singapore and Argentina. Nine out of the 14 cohort studies were conducted in USA, 2 of which were based on Adventist Populations. Four cohorts were European and 1 was Japanese. Twenty-two of the case-control studies reported results on colon cancer risk, but only 16 provided also results on rectal cancer risk. Twelve studies reported the results for the 2 sites combined and not separately for colon and rectum. Ten case-control studies gave the results separately for men and women and 2 case-control studies were carried out only in men. The remaining reported odds ratios for both sexes combined. Seven of the cohorts reported results for colorectal cancer, only 1 analysed colon and rectal cancer separately and 6 focused only on colon cancer. Four cohort studies were carried out in men; 3 in women and 3 cohort studies reported the results separately for both men and women.

Total meat was defined as fresh plus processed meat in 19 studies, whereas only fresh meat was evaluated in 8 studies. Fish was reported together with meat in 4 case-control studies and eggs in 2. Red meat was defined as fresh beef, pork and lamb consumption in 13 studies whereas processed red meat was also included in this category in 11 studies.

Total meat

Twenty-one case-control and 6 cohort studies investigated total meat consumption and colorectal cancer risk, of which 3 case-control and one cohort study found a significant positive association. Only 1 study found a significantly reduced colorectal cancer risk for meat consumption.

Average relative risk

All cohort studies were included in the estimation of the average RR. Three case-control studies were excluded for the following reasons: odds ratios reported only when they were significant,[84] no confidence intervals,[80] or no odds ratios provided.[37] The excluded RR were not significant with the exception of a study reporting a significant risk decrease for cancer of the rectum, but not of the colon[80] and the significant values found in another study for 2 of the 8 odds ratios reported.[84]

The pooled estimate of the average RR was 1.14 (95% CI 0.99–1.31) (Fig. 1). There was evidence of lack of homogeneity when all studies were considered together. The estimates by subgroups together with the results of the heterogeneity tests are given in Table I. Only one cohort study on an Adventist population[42] found a significant association. In this study, both red meat and white meat contributed independently to a risk increase of 85% in subjects consuming meat once a week or more often, compared with non-consumers. Studies in which meat was defined as fresh meat have a lower average relative risk (RR: 1.01; 95% CI: 0.64–1.60) than studies defining meat as fresh plus processed meat (1.16; 95% CI: 1.01–1.34). The subgroups of cohort studies, the subgroups of males, females and of cancer of the rectum were the only subgroups not heterogeneous.

Dose-response meta-analysis

Eighteen studies (5 cohort and 13 case-control) were included in the dose-response meta-analysis and 9 were excluded, of which 2[84,93] found a significant risk increase associated with high consumption. In addition to 3 case-control studies that were excluded from the previous analysis,[37,80,84] 7 more studies were excluded because the exposure was classified in 2 categories[69,71,74] or because the distribution of cases and control subjects by exposure level[78,87,93,97] was not provided.

Among the 18 studies included in the meta-analysis, only 4 case-control and 1 cohort study reported exposure in grams per day. For case-control studies the inter-quantile mean range of intake was 126 g/day for studies reporting consumption in g/day

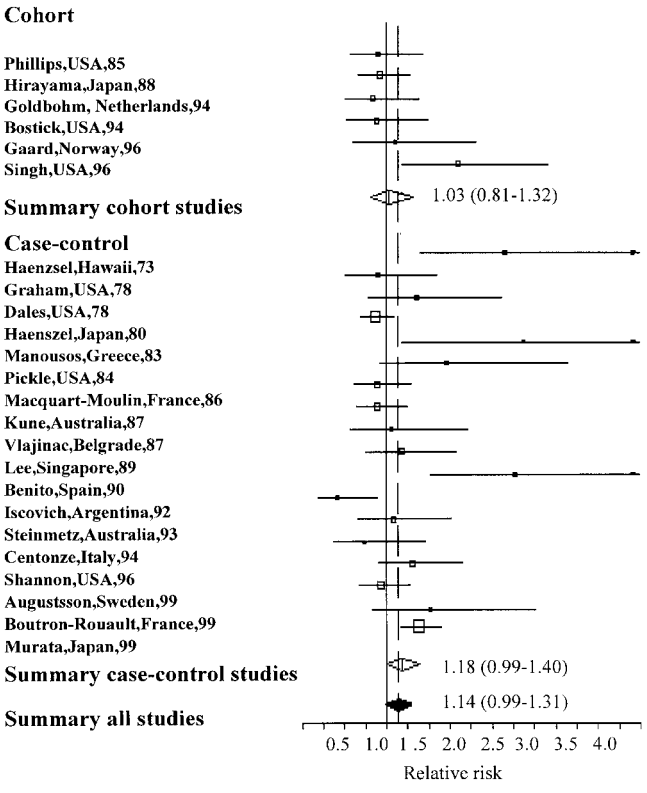


FIGURE 1 – Relative risks (highest vs. lowest category) for case-control and cohort studies (meat).

TABLE I – AVERAGE RELATIVE RISK FOR HIGHEST *VERSUS* LOWEST LEVEL OF INTAKE OF TOTAL MEAT, RED MEAT AND PROCESSED MEAT[1]

Sub-groups	Total meat			Red meat			Processed meat		
	RR (95% CI)	*n*	*p* Het.	RR (95% CI)	*n*	*p* Het.	RR (95% CI)	*n*	*p* Het.
All studies	1.14 (0.99–1.31)	24	<0.001	1.35 (1.21–1.51)	23	<0.001	1.31 (1.13–1.51)	23	<0.001
Case-control	1.18 (0.99–1.40)	18	<0.001	1.36 (1.17–1.59)	14	<0.001	1.29 (1.09–1.52)	16	<0.001
Cohort	1.03 (0.81–1.32)	6	0.14	1.27 (1.11–1.45)	9	0.45	1.39 (1.09–1.76)	7	0.85
Colon	1.09 (0.90–1.33)	15	0.01	1.32 (1.18–1.48)	19	<0.001	1.22 (1.06–1.39)	15	<0.001
Rectum	1.31 (1.00–1.73)	5	0.24	1.36 (1.17–1.57)	7	0.23	1.21 (0.98–1.50)	5	0.14
Males	1.05 (0.85–1.30)	7	0.64	1.40 (1.20–1.64)	9	0.64	1.57 (1.27–1.93)	7	0.22
Females	1.01 (0.81–1.25)	7	0.32	1.13 (0.85–1.50)	8	0.03	1.17 (0.95–1.44)	7	0.85
Europe	1.20 (0.88–1.63)	8	<0.001	1.46 (1.22–1.75)	7	0.03	1.39 (1.12–1.74)	10	0.001
USA	1.32 (1.03–1.70)	8	0.05	1.30 (1.12–1.52)	13	0.002	1.38 (1.10–1.73)	10	<0.001
Fresh meat only	1.01 (0.64–1.60)	6	0.001	1.28 (1.11–1.47)	13	0.003			
Fresh and processed meat	1.16 (1.08–1.34)	18	0.004	1.49 (1.26–1.77)	11	0.02			

[1]*n*, number of studies. *p* Het., *p* heterogeneity test.

TABLE II – DOSE-RESPONSE ANALYSIS[1]

	Total meat			Red meat			Processed meat		
	RR (95% CI)	*n*	*p* Het.	RR (95% CI)	*n*	*p* Het.	RR (95% CI)	*n*	*p* Het.
All studies	1.12 (0.98–1.30)	18	<0.001	1.24 (1.08–1.41)	17	<0.001	1.36 (1.15–1.61)	16	<0.001
Case-control	1.10 (0.94–1.29)	13	<0.001	1.26 (1.02–1.55)	8	<0.001	1.37 (1.13–1.66)	9	0.002
Cohort	0.99 (0.71–1.39)	5	0.18	1.22 (1.05–1.41)	9	0.17	1.54 (1.10–2.17)	7	0.001
Colon	1.10 (0.83–1.45)	14	0.02	1.23 (1.04–1.46)	14	0.01	1.32 (1.02–1.70)	8	0.10
Rectum	1.89 (1.02–3.51)	5	0.01	1.64 (0.64–4.21)	2	0.11		—	
Males	1.07 (0.85–1.34)	6	0.25	1.36 (1.18–1.55)	9	0.12	1.48 (1.08–2.04)	6	<0.001
Females	0.87 (0.72–1.09)	6	0.47	1.11 (0.78–1.56)	8	0.03	1.44 (1.10–1.89)	4	0.69
Europe	1.26 (1.05–1.51)	9	0.14	1.56 (1.07–2.26)	5	0.01	1.39 (1.09–1.77)	8	<0.001
USA	1.04 (0.75–1.45)	5	0.01	1.22 (1.05–1.41)	10	<0.001	1.54 (1.32–1.78)	6	0.63
Fresh meat only	1.01 (0.71–2.19)	6	0.03	1.19 (0.91–1.55)	8	<0.001			
Fresh and processed meat	1.15 (0.99–1.35)	12	0.001	1.28 (1.11–1.48)	9	0.01			

Relative risks for a consumption of 120 g/day (meat and red meat) or 30 g/day (processed meat) *vs.* no consumption.–[1]*n*, number of studies. *p* Het.: *p* heterogeneity test.

and slightly lower, 114 g/day for studies where the rescaling was applied. In cohort studies the mean ranges were 100 g/day and 94 g/day respectively.

The results for all studies combined and for subgroups are given in Table II. The RR estimated from the beta pooling that is associated with a consumption of 120 g/day of meat compared to no consumption is RR: 1.12 (95% CI: 0.98–1.30). On average, the epidemiological studies included in the analysis found no increase in colorectal cancer risk associated with this level of meat intake. The publication bias test was not statistically significant ($p = 0.58$).

There is heterogeneity between all studies, but homogeneity is not rejected for cohort studies. Meta-regressions using the beta estimates for each study as the dependent variable, and the design, geographical area, cancer site, sex and meat definition as explanatory variables were tested in different models using as weight the inverse of the variance of the beta estimate. The only significant predictor of beta was geographical area, with North American studies finding lower slopes than studies from other geographical areas. When the two variables, geographical area and meat definition were included together in the model, the difference between American studies and the other geographical areas disappeared. The slope for studies on fresh meat was lower than for fresh plus processed meat, but they were not significantly different.

Red meat

Fifteen case-control and 9 cohort studies investigated red meat. Six case-control studies reported a significant risk increase or significant trend associated with higher levels of red meat intake. In 2 of them the association was significant for cancer of the rectum but not for the colon. In 1 study there was a significant trend in females but not in males or in both sexes combined. Two out of 9 cohort studies reported relative risks significantly higher than 1.

Average relative risk

Only 1 case-control study,[80] which did not provide confidence intervals, was excluded from the analysis (Fig. 2). In contrast with the results for total meat, the estimated averaged RR for red meat was significantly higher than one (RR: 1.35; 95% CI: 1.21–1.51). As for total meat, homogeneity was not rejected for cohort studies, while case-control studies were heterogeneous and, on average, provided higher RR estimates than cohort studies (Table I). The subgroups of European and North American studies are internally heterogeneous, with Europeans having a larger average relative risk. Within North American studies, cohort studies have a larger average relative risk (RR: 1.45; 95% CI: 1.20–1.76) than case-control studies (RR: 1.28; 95% CI: 0.87–1.89). Studies on males and on cancer of the rectum were homogeneous. For the remaining subgroups homogeneity was rejected. When considering the definition of exposure, a higher average RR was obtained from studies that included processed meat in the red meat group (RR: 1.49; 95%CI: 1.26–1.77), compared to studies that did not (RR: 1.28; 95% CI: 1.11–1.47).

Dose-response meta-analysis

Eighteen studies (9 case-control and 9 cohort) were included in the dose-response meta-analysis whereas 5 studies had to be excluded. The reasons for exclusion are: no confidence interval,[80] only 2 levels of exposure,[74,90] no distribution of cases and control subjects by exposure level[87] and RR not reported.[39] One of the excluded studies found a significant risk increase associated with high consumption[90] and the others found no significant risk increase. The inter-quantile mean ranges of intake were 116 g/day for 3 cohort studies that reported intake in g/day and 101 g/day for the remaining cohort studies for which we estimated intake by

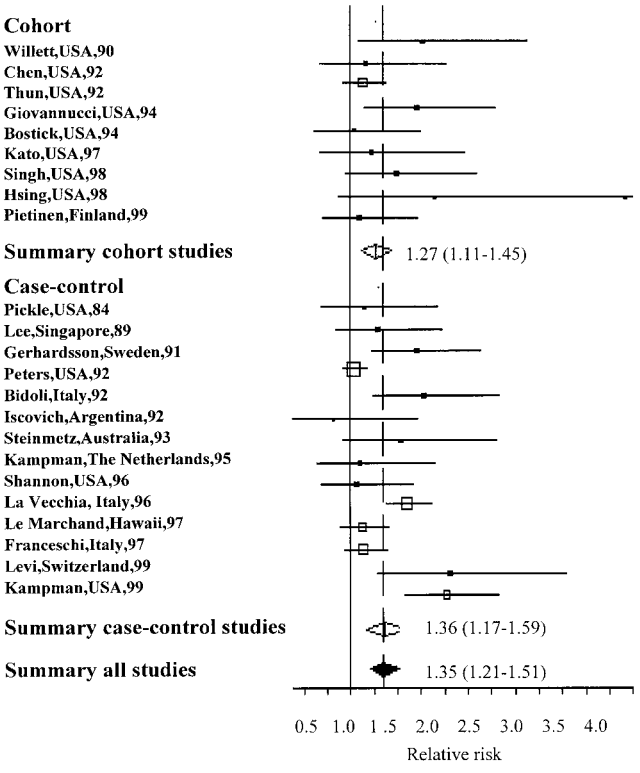


FIGURE 2 – Relative risks (highest *vs.* lowest category) for case-control and cohort studies (red meat).

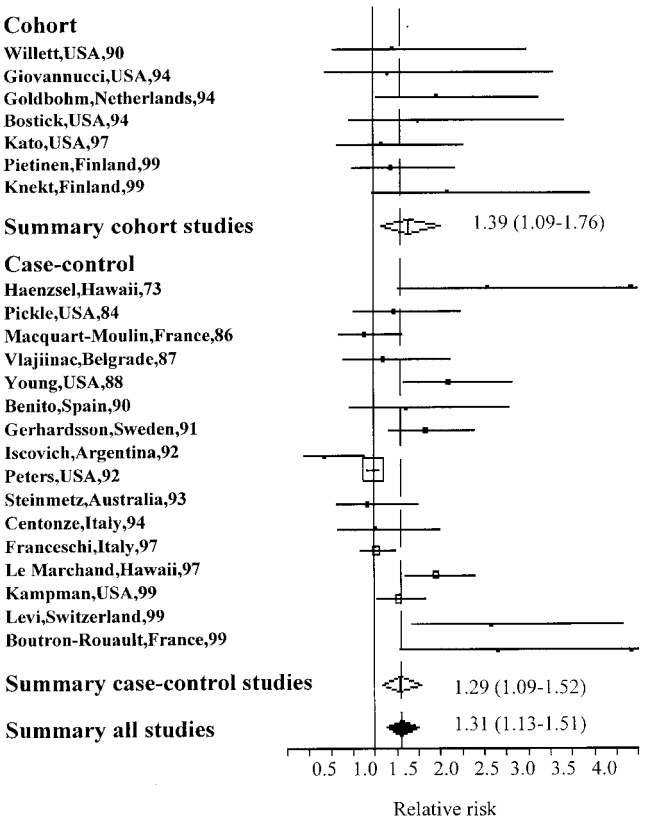


FIGURE 3 – Relative risks (highest *vs.* lowest category) for case-control and cohort studies (processed meat).

rescaling. The three case-control studies that reported intake in g/day had the same inter-quantile mean range as the remaining case-control studies after rescaling (93 g/day).

The results are presented in Table II. The estimated risk associated with consumption of 120 g/day of red meat compared to no consumption was 1.24 (95% CI: 1.08–1.41). Based on the studies included in the meta-analysis, there was no evidence of publication bias ($p = 0.52$). There was heterogeneity between all studies together but homogeneity was not rejected for cohort studies (p-heterogeneity = 0.17). In the meta-regression analysis, only the model with geographical area as predictor produced statistically significant estimates: the estimate of relative risk was higher for European than for North American studies. The significance of geographical area disappeared when meat definition was included in the model.

The dose-response was stronger and statistically significant for studies that included processed meat in the red meat group (RR: 1.28; 95%CI: 1.11–1.48) compared to studies that investigated only fresh red meat, for which the estimated risk was not significant (RR: 1.19 95% CI: 0.91–1.55). If the American studies are considered separately, studies evaluating only fresh meat reported lower risks on average (RR: 1.05; 95%CI: 0.55–2.00) than studies where the red meat category included processed meats (RR: 1.24; 95%CI: 1.07–1.43) but the homogeneity was rejected for both groups. The results are similar for European studies, where the RR estimated for studies on fresh red meat is lower (RR: 1.41; 95%CI: 0.91–2.20) than for studies on fresh red meat plus processed meat (RR: 2.07; 95% CI: 1.25–3.42).

Processed meat

Processed meat was evaluated in 29 studies, 22 case-controls and 7 cohorts. Two cohort studies found a significant trend, one cohort found a significantly increased risk for consumption between 2 and 4 times/week compared to no consumption and 12 case-control studies reported odds ratios significantly higher than 1.

Average relative risks

Six studies were not included in the estimation of the average relative risks because either they did not provide confidence intervals,[69,75,80] each type of processed meat was evaluated separately[79,85] or the number of subjects was very small.[82] The average RR for the 23 studies included in the analysis was 1.31 (95% CI: 1.13–1.51) (Fig. 3). The results were heterogeneous and, similarly to what was found for total meat and red meat, homogeneity was not rejected within cohort studies. Subgroup analysis within case-control studies showed that homogeneity was not rejected for the subgroups of males, females and for rectal cancer (Table I).

Dose-response meta-analysis

Sixteen studies (9 case-control and 7 cohort) were included in the dose-response meta-analysis and 13 case-control studies were excluded, of which 6 studies had also been excluded from the previous average RR estimation. The reasons for exclusion were: only 2 categories of exposure,[74,78,88] RR estimated only for the highest level of consumption[37,41] and distribution of case and control subjects not provided.[39,87] Five of the excluded studies reported a significant risk increase associated with increased consumption, 1 a non-significant decrease and the remainder found non-significant risk increases. There is no evidence of publication bias ($p = 0.75$). The mean range of intake for case-control studies was 39 g/day for the 3 studies that reported intake quantitatively and 34 g/day when rescaled; for cohort studies the mean range was 60 g/day (2 cohorts) and 30 g/day respectively.

The association estimated with the pooled dose-response meta-analysis was stronger for processed meat than for any other meat type considered in this study (Fig. 4). The relative risk estimated for a consumption of 30 g/day compared with no consumption was 1.36 (95% CI: 1.15–1.61) (Table II). The same relative risk would be associated with a consumption of 170 g/day of red meat,

according to the results of the dose-response meta-analysis on red meat. Overall, the studies are not homogenous (p-heterogeneity $<$ 0.001), but heterogeneity was not rejected for cohort studies. None of the variables evaluated in the meta-regression analysis explained the heterogeneity.

Estimation of the fraction prevented by current consumption of red meat worldwide

The per caput intake of red meat by geographical area estimated for 1995 is presented in Table III. The regions with the lowest correction factor, *i.e.*, with the highest discrepancy between FAO data and current consumption, are Europe, United States and High Income Asia (correction factors of 0.69, 0.60 and 0.70 respectively). These discrepancies can be explained in part because food waste in these countries is high and possibly because the surveys from which the correction factors were deduced are of better quality. The correction factor for Middle East Asia was similar to the value for Europe and America, but was based only on a survey in Turkey. FAO per caput intakes were lower than the mean consumption reported in dietary surveys for India and for males in Low Income Asia, China, India, South America, Caribbean, North Africa and Sub-Saharan Africa.

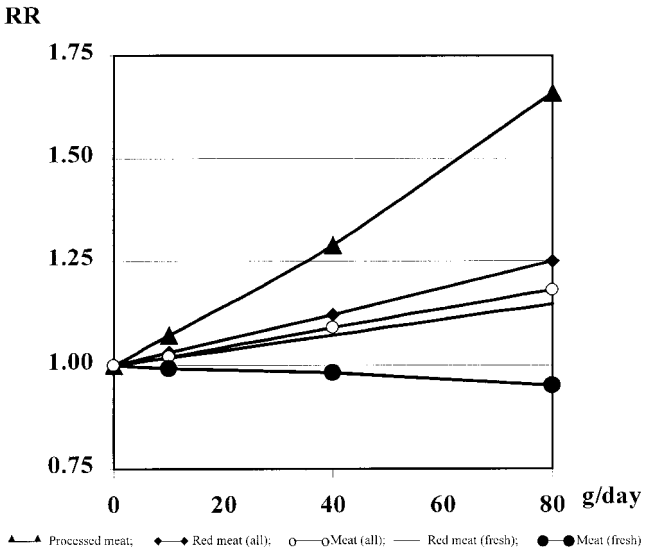


FIGURE 4 – Dose-response analysis of relative risk of colorectal cancer for meat consumption.

The proportion of colorectal cancer incidence attributable to current levels of red meat intake was computed using the beta-pooled estimates in the dose-response analysis. The same slope was used for all geographical areas and for both sexes for two main reasons: first the overall estimate had the advantage of being based on a larger number of studies and second, the subgroups defined by geographical area and by sex were not homogeneous.

The proportion of cancer risk attributable to current red meat consumption compared to non-consumption, as well as the preventable proportion simulating a shift of average consumption to 70 g/week are presented in Table III. The attributable proportion ranges from almost 25% for men in some countries of South America, followed by Australia and New Zealand (19.6%) and North America (13.9%) where consumption of red meat is high, to 2–3% in Chinese and Indian women, who eat very little red meat. When a hypothetical reduction to an average consumption of 70 g/week is simulated, the proportion of preventable risk ranges from 25–11.9% in men and from 17.2–7.5% in women in countries where the consumption is very high. In countries where the contribution of red meat to the diet is very low, as in India, Africa and some regions of Asia, less than 5% of the incidence could be potentially prevented.

DISCUSSION

The quantitative summary of the published literature on the risk of colorectal cancer and meat consumption suggests that high intakes of red meat and of processed meat are associated with increased risk of colorectal cancer. No significant association was found for total meat consumption and colorectal cancer risk. These results are consistent for case-control and cohort studies, for American, European and Asian studies (with the exception of one Argentinean study), for studies on males, females and both genders combined, and for studies on colon, rectal and colorectal cancer.

The use and interpretation of meta-analysis in epidemiology has raised methodological debates and controversial opinions. The most obvious limitation is that results are combined from studies conducted with different methods in different populations, resulting in heterogeneity. In our meat-analyses, heterogeneity was more often present within case-control than within cohort studies, which

TABLE III – PROPORTION OF COLORECTAL CANCER RISK ATTRIBUTABLE TO CURRENT RED MEAT CONSUMPTION AND PROPORTION PREVENTABLE BY REDUCING PER CAPUT RED MEAT CONSUMPTION TO 10 GRAMS PER DAY[1]

World regions	Males			Females		
	Red meat per caput g/day	AP %	PP %	Red meat per caput g/day	AP %	PP %
North America	85.9	13.9	11.9	57.7	9.5	7.5
Central America	41.5	11.1	9.1	30.2	5.1	3.1
Caribbean	26.0	4.2	2.4	18.9	3.2	1.2
Argentina, Uruguay, Paraguay	168.1	25.6	23.7	122	19.2	17.2
Rest of South America	70.3	11.5	9.5	51	8.4	6.5
North and Central Europe	47.3	7.8	5.9	35.0	5.8	3.9
Southern Europe	59.0	9.7	7.7	43.7	7.3	5.3
Eastern Europe	45.3	7.5	5.6	34.8	5.8	3.9
ex-URSS Asia	33.8	5.6	3.7	26.0	4.4	2.4
Middle east Asia	21.6	3.6	1.7	15.7	2.7	0.7
High income Asia	26.6	0.4	2.5	19.3	3.2	1.3
Middle income Asia	14.3	2.4	0.5	10.4	1.7	NC
Low income Asia	26.9	4.5	2.6	19.5	3.5	1.3
China	12.8	2.2	0.2	9.3	1.6	NC
India	15.1	2.6	0.6	11.0	1.9	0.0
North Africa	30.0	5.0	3.1	21.7	3.7	1.7
Subsaharan Africa	20.7	3.5	1.5	15.0	2.5	0.6
Australia, New Zealand	125.7	19.6	17.7	84.1	13.6	11.6
Oceania	41.0	6.8	4.9	29.7	5.0	3.0

[1]NC, not computed because per caput consumption is 10 g/day or less; AP, proportion attributable; PP, proportion preventable.

could be explained to some extent by the fact that most of the cohorts are North-American and used similar methodologies for dietary assessment. Case-control studies from North America and also from Europe remained heterogeneous, however, when studies in the two geographical areas were analyzed separately. Homogeneity was not always rejected when composing subgroups by sex and by cancer site. It is not clear how much of it could be explained by publication bias, because it may be that results are reported separately by sex or cancer site only when they correspond to a certain expectation.

Even though the effect-size estimates differed slightly between case-control and cohort studies, recall bias is very unlikely to account for the positive association we found between red and processed meat and colorectal cancer risk because the directionality of the summary measure of association was the same for both types of studies. Differences between the 2 study designs can partially explain the differences. The time interval between the period covered by the dietary assessment and diagnosis of the disease is usually 1 year (recent diet) in case-control studies although it can be as large as 10–20 years (current diet at the time of subject recruitment) in cohort studies.

Additional methodological issues concern the dietary measurement methods and their validation. We did not attempt to stratify studies by type of questionnaire or by results of their validity studies, because the information given in the papers was very often insufficient to do so. The imprecision of dietary assessment methods causes random measurement errors, which lead to underestimation of the magnitude of the relationship between dietary intake and cancer risk. It has been estimated that, for typical degrees of measurement error, the underestimation is roughly 2-fold ,[103] but this may be larger if dietary intake was not assessed during the period of exposure most relevant to cancer etiology, which is not known with any precision. We decided not to apply formal corrections for measurement error, which would have increased the pooled relative risk estimates because, with very few exceptions, no data from dietary questionnaire validation studies were available for the different types of questionnaire used and for the specific underlying study population.

There is the theoretical possibility that the association between red meat and processed meat and colorectal cancer risk could be due to uncontrolled confounding factors. Known or suspected risk factors were controlled for in many of the studies. It is the opinion of the authors that the diversity of the populations where the studies were carried out argues against the hypothesis that unknown confounders can entirely explain the association.

We found that relative risks for total and red meat were more elevated in studies that included processed meat in the definition of these 2 meat groups than in studies that evaluated fresh meat and fresh red meat (Fig. 4), that could be a support for an increased effect of processed meat. These results should be taken with caution for different reasons: these subgroups were set up *a posteriori*, after the data had been seen, and the finding could be spurious; besides, the definition of meat groups is not always clear in the publications. Nevertheless, this finding is in agreement with the summary relative risk per gram of intake estimated from the dose-response relationship, which was higher for processed meat than for red meat consumption.

The calculation of population attributable risks for diet has specific methodological limitations, particularly due to the fact that the population distribution by exposure level is not precisely known and the association with cancer risk is measured with some approximations. We estimated the prevalence of red meat consumption using data that do not refer to individuals, but to populations. In order to estimate the attributable risk fraction, we used the overall slope estimated in the dose-response analysis instead of slopes estimated for subgroups of different geographical areas, sex or cancer sub-sites. Our decision was mainly due to the fact that most of the studies were carried out in the USA and in Western Europe, and there were not enough studies to obtain meaningful estimates for specific geographical areas of the world. The overall slope had the advantage of being the result of the largest number of available studies. The coefficient of variation applied for the estimation of quartile distribution of red meat intake was the value found in the preliminary analysis of EPIC data. The application of a lower coefficient of variation will not change the estimates substantially, but if the variability is much higher than the hypothetical value used, our estimates of attributable risk and preventable proportion would be an overestimation of the real unknown corresponding values. For North America, for example, if a coefficient of variation of an extreme value such as 200% is applied, instead of 85% as we did, the attributable risk fraction in men will be 9% instead of 14% and the preventable fraction 8% instead of 12 %. The decrease is more important if the average intake level is high than if it is low.

Estimates of cancer risks attributable to diet have been published in the past. Doll and Peto, in their widely quoted 1981 paper[104] estimated that 35% of all US cancer deaths and even 90% of colon cancer deaths were attributable to diet. These figures now appear questionable because epidemiological evidence suggests quite strongly that physical activity accounts for an important percentage of avoidable colon cancer. More recently, Willett[105] estimated that 50–80% of colorectal cancer deaths could be avoidable by dietary change. In the Health Professionals Follow-Up Study,[106] it was estimated that about a third to a half of colon cancer risk might be avoidable if exposure to 6 risk factors (overweight, physical activity, supplementation with folic acid, alcohol consumption, smoking and red meat intake) were modified to become equal to that of the men in the approximate bottom 20% or bottom 5% of a risk score distribution. In a case-control study, La Vecchia *et al.*[107] estimated that 56% of colon cancer risk would have been avoided if all subjects were moved to the lowest exposure levels of 6 risk factors considered together. The attributable risk for individual factors was 12% for high education, 14% for low physical activity, 14% for high energy intake, 22% for low vegetable intake, 7% for high eating frequency, and 8% for a family history of colorectal cancer. In a case-control study in Northern Italy,[90] the proportion of risk of colorectal cancer attributable to red meat consumption was estimated as 16% for males and 17% for females. In our study, the estimates of colorectal cancer risk attributable to current red meat consumption were 9.7% and 7.3% for Southern European men and women. The highest estimates of the attributable fraction correspond to the areas of highest per caput red meat consumption, Argentina, Uruguay and Paraguay, followed by Australia and New Zealand and by North America.

We computed the reduction in cancer risk that could potentially be achieved with a hypothetical dietary reduction of average red meat consumption from current levels to an average of 70 g/week. In simulating a change, we chose as goal the intake of this small portion size once a week because at this level there is no evidence of excess risk compared to no consumption. Therefore, this assumption does not require complete avoidance of red meat. Such a reduction could potentially lead to a decrease in colorectal cancer risk in men as high as 17.9% in Australia and 12.1% in North America. According to the estimated preventable proportions, approximately 22,000 incident cases could be avoided in North America, 21,000 in Europe, 7,000 in Asia and 6,000 in South America.

In calculating attributable and preventable fractions, we assumed that the association between red meat consumption and colorectal cancer is causal and free from bias. Our estimates refer only to a single risk factor, but individual dietary factors may not contribute independently. Other dietary and non-dietary factors, such as vegetable and fruit intake, smoking habits, reproductive history, physical activity and infectious agents, may also contribute to risk differences. The isolated change of a single dietary factor represents a simplification and it may well be that interventions addressing the totality of diet-related risk factors could re-

move a larger proportion of excess risk. Based on the available data, it is not possible to determine to what extent reducing exposure to modifiable risk factors at various ages, after exposure at varying levels for varying duration, will prevent colorectal cancer. Neither is it possible to estimate the latency between a reduction in average red meat consumption occurring in a given population and the expected reduction in colorectal cancer incidence.

Our results do not imply that meat consumption should be completely avoided as part of a balanced diet. Nevertheless, they support previous recommendations[3] to adopt a diet characterized by low intake of red and processed meat.

REFERENCES

1. Scheppach W, Bingham S, Boutron-Ruault MC, et al. WHO consensus statement on the role of nutrition in colorectal cancer. Eur J Cancer Prev 1999;8:57–62.
2. CNERNA-CNRS. Alimentation et Cancer. Evaluation des données scientifiques. Riboli E, Decloitre F, Collet-Ribbing C, Coordinators. Paris: Lavoisie 1996.
3. World Cancer Research Fund. Food, nutrition and the prevention of cancer: a global perspective. Washington, DC: Am Inst Cancer Res, 1997.
4. COMA. Report of the Working Group on diet and cancer. Nutritional aspects of the development of cancer. London: The Stationery Office, 1998.
5. Potter JD. Colorectal cancer: molecules and populations. J Natl Cancer Inst 1998;91:916–32.
6. Narisawa T, Magadia NE, Weisburger JH, et al. Promoting effect of bile acids on colon carcinogenesis after intrarectal instillation of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine in rats. J Natl Cancer Inst 1974;53:1093–7.
7. Chomchai C, Bhadrachari N, Nigro ND. The effect of bile on the induction of experimental intestinal tumors in rats. Dis Colon Rectum 1974;17:310–2.
8. Friedman E, Isaksson P, Rafter J, et al. Fecal diglycerides as selective endogenous mitogens for premalignant and malignant human colonic epithelial cells. Cancer Res 1989;49:544–8.
9. Field CJ, Ryan EA, Thomson AB, et al. Diet fat composition alters membrane phospholipid composition, insulin binding, and glucose metabolism in adipocytes from control and diabetic animals. J Biol Chem 1990;265:11143–50.
10. Yorek M, Leeney E, Dunlap J, et al. Effect of fatty acid composition on insulin and IGF-I binding in retinoblastoma cells. Invest Ophthalmol Vis Sci 1989;30:2087–92.
11. McKeown-Eyssen G. Epidemiology of colorectal cancer revisited: are serum triglycerides or plasma glucose associated with risk? Cancer Epidemiol Biomarkers Prev 1994;3:687–95.
12. Giovannucci E. Insulin and colon cancer. Cancer Causes Control 1995;6:164–79.
13. Bruce WR, Giacca A, Medline A. Possible mechanisms relating diet and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 2000; 9:1271–9.
14. Pence BC, Butler MJ, Dunn DM, et al. Non-promoting effects of lean beef in the rat colon carcinogenesis model. Carcinogenesis 1995;16: 1157–60.
15. Mastromarino A, Reddy BS, Wynder EL. Metabolic epidemiology of colon cancer: enzymic activity of fecal flora. Am J Clin Nutr 1976; 29:1455–60.
16. Reddy BS, Hanson D, Mangat S, et al. Effect of high-fat, high-beef diet and of mode of cooking of beef in the diet on fecal bacterial enzymes and fecal bile acids and neutral sterols. J Nutr 1980;110: 1880–7.
17. Giovannucci E, Goldin B. The role of fat, fatty acids, and total energy intake in the etiology of human colon cancer. Am J Clin Nutr 1997;66:1564S–71S.
18. West DW, Slattery ML, Robison LM, et al. Dietary intake and colon cancer: sex- and anatomic site-specific associations. Am J Epidemiol 1989;130:883–94.
19. Giovannucci E, Rimm EB, Stampfer MJ, et al. Intake of fat, meat, and fiber in relation to risk of colon cancer in men. Cancer Res 1994;54: 2390–7.
20. Babbs CF. Free radicals and the etiology of colon cancer. Free Radic Biol Med 1990;8:191–200.
21. Deneo-Pellegrini H, De Stefani E, Boffetta P, et al. Dietary iron and cancer of the rectum: a case-control study in Uruguay. Eur J Cancer Prev 1999;8:501–8.
22. Kato I, Dnistrian AM, Schwartz M, et al. Iron intake, body iron stores and colorectal cancer risk in women: a nested case-control study. Int J Cancer 1999;80:693–8.
23. Bingham SA. High-meat diets and cancer risk. Proc Nutr Soc 1999; 58:243–8.
24. Clinton SK, Bostwick DG, Olson LM, et al. Effects of ammonium acetate and sodium cholate on *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine-induced colon carcinogenesis of rats. Cancer Res 1988;48:3035–9.
25. Bingham SA, Pignatelli B, Pollock JR, et al. Does increased endogenous formation of *N*-nitroso compounds in the human colon explain the association between red meat and colon cancer? Carcinogenesis 1996;17:515–23.
26. Autrup H, Harris CC, Trump BF. Metabolism of acyclic and cyclic *N*-nitrosamines by cultured human colon. Proc Soc Exp Biol Med 1978;159:111–5.
27. Rowland IR, Granli T, Bockman OC, et al. Endogenous *N*-nitrosation in man assessed by measurement of apparent total *N*-nitroso compounds in faeces. Carcinogenesis 1991;12:1395–401.
28. Forman D. Dietary exposure to *N*-nitroso compounds and the risk of human cancer. Cancer Surv 1987;6:719–38.
29. Mirvish SS. Role of *N*-nitroso compounds (NOC) and *N*-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC [published erratum appears in Cancer Lett 1995;97:271]. Cancer Lett 1995;93: 17–48.
30. Bartsch H, Spiegelhalder B. Environmental exposure to *N*-nitroso compounds (NNOC) and precursors: an overview. Eur J Cancer Prev 1996;5(Suppl):11–7.
31. Scanlan RA. Formation and occurrence of nitrosamines in food. Cancer Res 1983;43:2435S–40S.
32. Hotchkiss JH. Preformed *N*-nitroso compounds in foods and beverages. Cancer Surv 1989;8:295–321.
33. Sugimura T. Nutrition and dietary carcinogens. Carcinogenesis 2000; 21:387–95.
34. Nagao M, Honda M, Seino Y, et al. Mutagenicities of smoke condensates and the charred surface of fish and meat. Cancer Lett 1977; 2:221–6.
35. Phillips DH. Polycyclic aromatic hydrocarbons in the diet. Mutat Res 1999;443:139–47.
36. Norat T, Riboli E. Meat consumption and colorectal cancer: a review of epidemiologic evidence. Nutr Rev 2001;59:37–47.
37. Young TB, Wolf DA. Case-control study of proximal and distal colon cancer and diet in Wisconsin. Int J Cancer 1988;42:167–75.
38. Lyon JL, Mahoney AW. Fried foods and the risk of colon cancer [see comments]. Am J Epidemiol 1988;128:1000–6.
39. Peters RK, Garabrant DH, Yu MC, et al. A case-control study of occupational and dietary factors in colorectal cancer in young men by subsite. Cancer Res 1989;49:5459–68.
40. Goldbohm RA, van den Brandt PA, Van 't Veer P, et al. A prospective cohort study on the relation between meat consumption and the risk of colon cancer. Cancer Res 1994;54:718–23.
41. Gerhardsson de Verdier M, Hagman U, Peters RK, et al. Meat, cooking methods and colorectal cancer: a case-referent study in Stockholm. Int J Cancer 1991;49:520–5.
42. Singh PN, Fraser GE. Dietary risk factors for colon cancer in a low-risk population . Am J Epidemiol 1998;148:761–74.
43. Augustsson K, Skog K, Jagerstad M, et al. Dietary heterocyclic amines and cancer of the colon, rectum, bladder, and kidney: a population-based study. Lancet 1999;353:703–7.
44. Hsing AW, McLaughlin JK, Chow WH, et al. Risk factors for colorectal cancer in a prospective study among U.S. white men. Int J Cancer 1998;77:549–53.
45. Welfare MR, Cooper J, Bassendine MF, et al. Relationship between acetylator status, smoking, and diet and colorectal cancer risk in the north-east of England. Carcinogenesis 1997;18:1351–4.
46. Sinha R, Chow WH, Kulldorff M, et al. Well-done, grilled red meat increases the risk of colorectal adenomas. Cancer Res 1999;59: 4320–4.
47. Kampman E, Slattery ML, Bigler J, et al. Meat consumption, genetic susceptibility, and colon cancer risk: a United States multicenter case-control study. Cancer Epidemiol Biomarkers Prev 1999;8:15–24.
48. Gaard M, Tretli S, Loken EB. Dietary factors and risk of colon cancer: a prospective study of 50,535 young Norwegian men and women. Eur J Cancer Prev 1996;5:445–54.
49. Knekt P, Steineck G, Jarvinen R, et al. Intake of fried meat and risk of cancer: a follow-up study in Finland. Int J Cancer 1994;59:756–60.
50. Pietinen P, Malila N, Virtanen M, et al. Diet and risk of colorectal cancer in a cohort of Finnish men. Cancer Causes Control 1999;10: 387–96.
51. Ilett KF, David BM, Detchon P, et al. Acetylation phenotype in colorectal carcinoma. Cancer Res 1987;47:1466–9.

52. Lang NP, Butler MA, Massengill J, et al. Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increase the risk for colorectal cancer or polyps. Cancer Epidemiol Biomarkers Prev 1994;3:675–82.
53. Roberts-Thomson IC, Butler WJ, Ryan P. Meat, metabolic genotypes and risk for colorectal cancer. Eur J Cancer Prev 1999;8:207–11.
54. Le Marchand L. Combined influence of genetic and dietary factors on colorectal cancer incidence in Japanese Americans. J Natl Cancer Inst Monogr 1999;101–5.
55. Bell DA, Stephens EA, Castranio T, et al. Polyadenylation polymorphism in the acetyltransferase 1 gene (NAT1) increases risk of colorectal cancer. Cancer Res 1995;55:3537–42.
56. Probst-Hensch NM, Sinha R, Longnecker MP, et al. Meat preparation and colorectal adenomas in a large sigmoidoscopy-based case-control study in California (United States). Cancer Causes Control 1997;8: 175–83.
57. Chen J, Stampfer MJ, Hough HL, et al. A prospective study of N-acetyltransferase genotype, red meat intake, and risk of colorectal cancer. Cancer Res 1998;58:3307–11.
58. Hardy RJ, Thompson SG. Detecting and describing heterogeneity in meta-analysis. Stat Med 1998;17:841–56.
59. Dersimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–88.
60. Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. Am J Epidemiol 1992;135:1301–9.
61. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 1994;50:1088–101.
62. Colditz GA, Burdick E, Mosteller F. Heterogeneity in meta-analysis of data from epidemiologic studies: a commentary. Am J Epidemiol 1995;142:371–82.
63. Krebs-Smith SM, Cleveland LE, Ballard-Barbash R, et al. Characterizing food intake patterns of American adults. Am J Clin Nutr 1997; 65(Suppl 4):S1264–8.
64. Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26:Suppl 1:S6–14.
65. Miettinen OS. Proportion of disease caused or prevented by a given exposure, trait or intervention. Am J Epidemiol 1974;99:325–32.
66. Food and Agriculture Organization of the United Nations, Statistical Division. Compendium of food consumption statistics from household surveys in developing countries. Volume 1: Asia. Rome: FAO. 1994.
67. Food and Agriculture Organization of the United Nations, Statistical Division. Compendium of food consumption statistics from household surveys in developing countries. Volume 2: Africa, Latin America and Oceania. Rome: FAO. 1994.
68. Haenszel W, Berg JW, Segi M, et al. Large-bowel cancer in Hawaiian Japanese. J Natl Cancer Inst 1973;51:1765–79.
69. Dales LG, Friedman GD, Ury HK, et al. A case-control study of relationships of diet and other traits to colorectal cancer in American blacks. Am J Epidemiol 1979;109:132–44.
70. Graham S, Dayal H, Swanson M, et al. Diet in the epidemiology of cancer of the colon and rectum. J Natl Cancer Inst 1978;61:709–14.
71. Haenszel W, Locke FB, Segi M. A case-control study of large bowel cancer in Japan. J Natl Cancer Inst 1980;64:17–22.
72. Manousos O, Day NE, Trichopoulos D, et al. Diet and colorectal cancer: a case-control study in Greece. Int J Cancer 1983;32:1–5.
73. Miller AB, Howe GR, Jain M, et al. Food items and food groups as risk factors in a case-control study of diet and colo-rectal cancer. Int J Cancer 1983;32:155–61.
74. Pickle LW, Greene MH, Ziegler RG, et al. Colorectal cancer in rural Nebraska. Cancer Res 1984;44:363–9.
75. Tajima K, Tominaga S. Dietary habits and gastro-intestinal cancers: a comparative case- control study of stomach and large intestinal cancers in Nagoya, Japan. Jpn J Cancer Res 1985;76:705–16.
76. Macquart-Moulin G, Riboli E, Cornee J, et al. Case-control study on colorectal cancer and diet in Marseilles. Int J Cancer 1986;38:183–91.
77. Kune S, Kune GA, Watson LF. Case-control study of dietary etiological factors: the Melbourne Colorectal Cancer Study. Nutr Cancer 1987;9:21–42.
78. Vlajinac H, Adanja B, Jarebinski M. Case-control study of the relationship of diet and colon cancer. Arch Geschwulstforsch 1987;57: 493–8.
79. La Vecchia C, Negri E, Decarli A, et al. A case-control study of diet and colo-rectal cancer in northern Italy. Int J Cancer 1988;41:492–8.
80. Tuyns AJ, Kaaks R, Haelterman M. Colorectal cancer and the consumption of foods: a case-control study in Belgium. Nutr Cancer 1988;11:189–204.
81. Lee HP, Gourley L, Duffy SW, et al. Colorectal cancer and diet in an Asian population: a case-control study among Singapore Chinese. Int J Cancer 1989;43:1007–16.
82. Wohlleb JC, Hunter CF, Blass B, et al. Aromatic amine acetyltransferase as a marker for colorectal cancer: environmental and demographic associations. Int J Cancer 1990;46:22–30.
83. Benito E, Obrador A, Stiggelbout, et al. A population-based case-control study of colorectal cancer in Majorca. I. Dietary factors. Int J Cancer 1990;45:69–76.
84. Hu JF, Liu YY, Yu YK, et al. Diet and cancer of the colon and rectum: a case-control study in China. Int J Epidemiol 1991;20:362–7.
85. Bidoli E, Franceschi S, Talamini R, et al. Food consumption and cancer of the colon and rectum in north-eastern Italy. Int J Cancer 1992;50:223–9.
86. Iscovich JM, L'Abbe KA, Castelleto R, et al. Colon cancer in Argentina. I: Risk from intake of dietary items. Int J Cancer 1992;51:851–7.
87. Steinmetz KA, Potter JD. Food-group consumption and colon cancer in the Adelaide Case-Control Study. II. Meat, poultry, seafood, dairy foods and eggs. Int J Cancer 1993;53:720–7.
88. Centonze S, Boeing H, Leoci C, et al. Dietary habits and colorectal cancer in a low-risk area. Results from a population-based case-control study in southern Italy. Nutr Cancer 1994;21:233–46.
89. Kampman E, Verhoeven D, Sloots L, et al. Vegetable and animal products as determinants of colon cancer risk in Dutch men and women. Cancer Causes Control 1995;6:225–34.
90. La Vecchia C, Ferraroni M, Mezzetti M, et al. Attributable risks for colorectal cancer in northern Italy. Int J Cancer 1996;66:60–4.
91. Shannon J, White E, Shattuck AL, et al. Relationship of food groups and water intake to colon cancer risk . Cancer Epidemiol Biomarkers Prev 1996;5:495–502.
92. Franceschi S, Favero A, La Vecchia C, et al. Food groups and risk of colorectal cancer in Italy. Int J Cancer 1997;72:56–61.
93. Murata M, Tagawa M, Watanabe S, et al. Genotype difference of aldehyde dehydrogenase 2 gene in alcohol drinkers influences the incidence of Japanese colorectal cancer patients. Jpn J Cancer Res 1999;90:711–9.
94. Levi F, Pasche C, La Vecchia C, et al. Food groups and colorectal cancer risk. Br J Cancer 1999;79:1283–7.
95. Boutron-Ruault MC, Senesse P, Faivre J, et al. Foods as risk factors for colorectal cancer: a case-control study in Burgundy (France). Eur J Cancer Prev 1999;8:229–35.
96. Phillips RL, Snowdon DA. Dietary relationships with fatal colorectal cancer among Seventh-Day Adventists. J Natl Cancer Inst 1985;74: 307–17.
97. Hirayama T. Life-style and mortality : a large-scale census-based cohort study in Japan Basel, New York: Karger, 1990. 73–95.
98. Thun MJ, Calle EE, Namboodiri MM, et al. Risk factors for fatal colon cancer in a large prospective study. J Natl Cancer Inst 1992; 84:1491–500.
99. Willett WC, Stampfer MJ, Colditz GA, et al. Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women. N Engl J Med 1990;323:1664–72.
100. Bostick RM, Potter JD, Kushi LH, et al. Sugar, meat, and fat intake, and non-dietary risk factors for colon cancer incidence in Iowa women (United States). Cancer Causes Control 1994;5:38–52.
101. Kato I, Akhmedkhanov A, Koenig K, et al. Prospective study of diet and female colorectal cancer: the New York University Women's Health Study. Nutr Cancer 1997;28:276–81.
102. Knekt P, Jarvinen R, Dich J, et al. Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: a follow-up study. Int J Cancer 1999;80:852–6.
103. Willett W. Nutritional Epidemiology. Second Edition. New York: Oxford University Press, 1998. 514 p.
104. Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst 1981;66:1191–308.
105. Willett WC. Diet, nutrition, and avoidable cancer. Environ Health Perspect 1995;103 Suppl 8:165–70.
106. Platz EA, Willett WC, Colditz GA, et al. W.W.C.G.R.E.S. Proportion of colon cancer risk that might be preventable in a cohort of middle-aged US men. Cancer Causes Control 2000;11:579–88.
107. La Vecchia C. Population-attributable risk for colon cancer in Italy. Nutr Cancer 1999;33:196–200.

APPENDIX I – CORRECTION FACTORS BY GEOGRAPHICAL AREA AND PER CAPUT ENERGY INTAKE[1]

Geographical area	Survey		FAO energy (kcal/day)	Correction factor	
	Energy (kcal/day)	Year		Males	Females
North America				0.73	0.49
USA Females	1742		3562		
USA Males	2593		3562		
South America					
Brazil	2262	1974/75	2488	1.06	0.77
Caribbean				1.23	0.89
St Lucia	1881	1974	2067		
Trinidad Tobago	2948	1970	2481		
Europe (EPIC)				0.81	0.60
Eastern Europe				0.86	0.66
Poland males	2579	1982–85	3351		
Poland females	1886		3351		
Novosibirsk males	2907		3385		
Novosibirsk females	2028		3385		
Kaunas males	3232		3385		
Kaunas females	2792		3385		
High Income Asia				0.82	0.60
Japan	2034	1993	2893		
Middle Income Asia				0.96	0.70
Philippines	1769	1978	2149		
Low Income Asia				1.11	0.80
Bangladesh	1773	1973/74	1912		
Indonesia	1859	1987	2475		
Pakistan	2390	1984/85	2161		
Sri Lanka	2281	1981/82	2263		
Middle East Asia				0.75	0.54
Turkey	2105	1981/82	3285		
China	2467	1990	2668	1.08	0.79
India	2719	1971/72	2022	1.57	1.14
North Africa				1.04	0.76
Morocco	2466	1970/71	2442		
Tunisia	2275	1985	2935		
Sub-Saharian Africa				1.06	0.77
Cote d'Ivoire	2104	1979	2799		
Rwanda Rural	2444	1982/83	2279		
Togo	2026	1988/89	2235		
Average correction factor				1.17	0.85

[1]kcal/day, from surveys[60,61] and Food Balance Sheets (F.A.O.).

APPENDIX II – TOTAL MEAT, RED MEAT AND PROCESSED MEAT INTAKE AND COLORECTAL CANCER CASE-CONTROL STUDIES

Author, location	Design	Type of meat and partition	OR (95% CI)	Adjustment
Haenzsel *et al.,* 1973[68] Hawaii	Hawaiian-Japanese Colorectal 179 Control 357 Recruitment 1966–1970 FFQ[2]	Meat, total (times/month) ≤20 20–39 ≥40 Sausage and other processed pork (times/month) >6 6–11 12–23 ≥24	 1 2.2 2.4[5] 1 1.27 1.35 2.3[5]	
Dales *et al.,* 1978[69] USA	Colorectum 77 Controls 215 American Blacks Recruitment: 1973–1976 FFQ (89)[2]	All meat (times/month) >66 *vs.* ≤66 Nitrite-treated meats (times/month) >32 *vs.* ≤32	Unadjusted: 1.54 (0.90–2.66) Adjusted: 1.67 Unadjusted: 1.48 (0.87–2.51) Adjusted: 1.22	Age, gender, other foods, parity, smoking, others
Graham *et al.,* 1978[70] USA	White males Colon 256 Controls 783 Rectum 330 Controls 628 Recruitment: 1959–1965[3]	Meats, including fish (times/month) 0.–20 21–30 31–40 41–50 50+ Bacon: Not associated	Colon / Rectum 1 / 1 0.65 / 1.01 0.59 / 1.42 0.70 / 1.45 0.30 / 1.77	
Haenszel *et al.,* 1980[71] Japan	Colorectum 588 Controls 588[2]	Meat, total (times/month) ≥12 *vs.* <12	0.87 NS	Age, gender, prefecture
Manousos *et al.,* 1983[72] Greece	Colorectum 100 Controls 100 Recruitment: 1979–1980 FFQ (80)[2]	Meat, fish, eggs, novel protein Highest *vs.* lowest quartile: not reported. $p = 0.01$		

APPENDIX II – TOTAL MEAT, RED MEAT AND PROCESSED MEAT INTAKE AND COLORECTAL CANCER CASE-CONTROL STUDIES (CONTINUED)

Author, location	Design	Type of meat and partition	OR (95% CI)	Adjustment
Miller *et al.,* 1983[73] Canada	348 colon (171 male and 177 female) 194 rectum (114 male and 80 female) 542 hospital and 535 population controls 1976–1978	Sausages, cold cuts, luncheon meats and animal organs servings/week Males / Females <10.1 / <5.1 <29.1 / <17 ≥29.1 / ≥17	Males: Colon / Rectum 1 / 1 0.8 / 1.1 1.0 / 1.3	Age, gender, other foods, saturated fat Females: Colon / Rectum 1 / 1 0.9 / 0.9 1.0 / 1.2
Pickle *et al.,* 1984[74] USA	Colon 58 Rectum 28 Controls 176 Recruitment: 1970–1977 Mean age: 74 FFQ (57) Rural area[2]	Meat, total (serving/week) ≥12.6 *vs.* <12.6 Beef, pork, lamb, mutton, wild game (serving/week) ≥6 *vs.* <6 Bacon, sausage, lunch meat, corned beef (serving/week) ≥3 *vs.* <3	Colon: 1.71 Rectum: 1.06 Colon: 1.09 Rectum: 1.25 Colon: 1.16 Rectum: 1.37	Age, gender, ethnic group, others
Tajima and Tominaga, 1985[75] Japan	Colon 42 Rectum 51 Controls 186 Age 40–74 Recruitment: 1981–1983 FFQ[2]	Ham and sausage Low Medium High	Colon / Rectum 1 / 1 2.19 / 0.60 2.87[4] / 0.60	Gender, age
Macquart-Moulin *et al.,* 1986[76] France	Colorectal 399 Control 399 Recruitment: 1979–1984 Mean age = 65 FFQ (158)[2]	Fresh meat Quartiles Reference: lowest "Charcuterie" Quartiles Reference: lowest	1 1.32 1.40 0.89 1 1.31 0.88 0.89	Age, gender, total energy, weight
Kune *et al.,* 1987[77] Australia	Colon 392 Rectum 323 Controls 727 Recruitment: 1980–1981 Dietary history (+300)[3]	Meat (g/week) Males / Females <830 / <602 <1011 / <757 <1270 / <890 <1600 / <1080 <1600 / >1080	Males / Females 1 / 1 0.69 / 0.98 0.65 / 0.77 0.80 / 0.66 1.13 / 0.76	Age, gender
Vlajinac *et al.,* 1987[78] Belgrade	Colon 81 Controls 162 Hospital and neighbourhood controls age 24–85 Recruitment: 1984–1986 FFQ (49)	Meat (times/month) <24 24–42 43–63 64+ Nitrite-treated meats over and above the median	*vs.* Hospital / *vs.* Neighbours 1 / 1 1.25 / 0.63 1.34 / 1.26 2.34 / 9.20 Hospital:1.10 Neighbours: 0.81	
La Vecchia *et al.,* 1988[79] Italy	Colon 339 Rectum 236 Controls 778 Age < 75 Recruitment: 1985–1987 FFQ (29)[2]	Highest *vs.* lowest tertile Raw ham Ham Salami and sausages	Colon / Rectum 1.01 / 1.05 1.04 / 0.73 1.05 / 0.73	Age, gender, education, area, other foods
Young *et al.,* 1988[37] USA	Colon 353 Controls: 618 white Americans Age 35–89 Recruitment: 1981–1982 FFQ (25)[3]	Any meat-based meal Diet over 35 years 20 *vs.* 1/month Bacon, ham, lunchmeat Sausage, hot dogs, processed lunch meat	No differences between cases and controls 1.85 (1.33–2.58) OR not reported, $p < 0.15$	
Tuyns *et al.,* 1988[80] Belgium	Colon 453 Rectum: 365 Controls: 3669 Recruitment: 1978–1982 Age: 35–75 FFQ (extensive list)[3]	Fresh meat, smoked meat. <705 −906 −1175 +1175 g/w Meat, except poultry and rabbit <575 −767 −1015 +1015 g/w Charcuterie g/w 0 <50 50–125 >125	Colon / Rectum 1 / 1 1.00 / 1.00 0.98 / 0.67 0.82 / 0.75[4] Colon / Rectum 1 / 1 0.90 / 0.78 0.89 / 0.74 0.89 / 0.57[5] Colon / Rectum 1 / 1 1.16 / 1.38 0.83 / 0.94 0.90 / 0.98	Age, gender, province
Lee *et al.,* 1989[81] Singapore	Colorectum 203:426 Males 121:239 Females 82:187 Chinese origin Colon 77 males 55 females Rectum 44 males 27 females Recruitment: 1985–1987 FFQ (116)[2]	Red meat and poultry excluding fish and liver (g/day) Males / Females <59.8 / <30.3 <112.2 / <73.3 >112.2 / >73.37	Colorectum / Colon / Rectum 1 / 1 / 1 1.17 (0.75–1.80) / 1.13 (0.67–1.89) / 1.17 (0.61–2.23) 1.18 (0.76–1.83) / 1.30 (0.78–2.17) / 0.91 (0.46–1.81)	Age, gender, dialect, education

APPENDIX II – TOTAL MEAT, RED MEAT AND PROCESSED MEAT INTAKE AND COLORECTAL CANCER CASE-CONTROL STUDIES (CONTINUED)

Author, location	Design	Type of meat and partition	OR (95% CI)	Adjustment
		Pork, beef, mutton (g/day)		
		Males / Females	Colorectum / Colon / Rectum	
		<43.9 / <19.9	1 / 1 / 1	
		<79 / <47.5	1.18 (0.77–1.81) / 1.01 (0.60–1.70) / 1.43 (0.75–2.74)	
		>79 / >47.5	1.29 (0.84–1.97) / 1.41 (0.87–2.31) / 0.97 (0.48–1.92)	
		Cured or smoked luncheon meat		
		>1 time/wk *vs.* less	2.9 (1.2–7.1) $p = 0.03$	
Wohlebb *et al.,* 1990[82] USA	Colorectum 43 Controls 41 Males Age 45–75 FFQ (55)[2]	Cured or smoked bacon >1 time/wk *vs.* less	5.0 (0.99–25)	
		Fresh meat times/month	Colorectum	
		<16	1	
		<25	2.30	
		26–32	2.11	
		≥32	2.52[5]	
Benito *et al.,* 1990[83] Spain	Colon: 144 Males 72 Females 72 Rectum 130 Males 74 Females 56 Population controls 295 Age < 80 Recruitment: 1984–1988 FFQ (99)	Processed meat times/month / Colorectum	Colon / Rectum	Age, gender, weight 10 years prior, education, occupation, physical activity, other foods
		0 / 1	1 / 1	
		<11 / 1.35	1.97 / 1.98	
		11–22 / 1.42	1.99 / 2.05	
		≥22 / 1.36	2.87[5] / 2.42	
Hu *et al.,* 1991[84] China	Colon 111 Rectum 225 Controls 336 Recruitment: 1985–1988 FFQ (25)[2]	Meat intake before 1985 ≥5 kg/year *vs.* none	Meat intake before 1966 ≥2 kg/year *vs.* <2	
		Colon males not significant	Colon not significant	
		Colon females not significant	Male rectum not significant	
		Rectum Male 3.38 (1.65–6.95)	Females rectum 2.06 (1.13–3.75)	
		Rectum Females not significant		
Gerhardsson *et al.,* 1991[41] Sweden	Colon 452 Rectum 268 Controls: 624 Recruitment: 1986–1988 FFQ (55)[3]	Beef, pork, ham, bacon, sausages		Age, gender, protein, boiled and fried meat intake
		serving/year	Colon / Rect.	
		<85	1 / 1	
		<167	1.1 / 1.6	
		<215	1.3 / 1.3	
		≥215	1.3 / 1.7	
			1.4 / 2.4[4]	
		Bacon/smoked ham	Colon / Rectum	
		More seldom	1 / 1	
		1–3 ts/month	0.9 (0.7–1.3) / 1.5 (1.0–2.2)	
		>once/week	1.3 (0.8–1.9) / 1.7 (1.1–2.8)[4]	
		Sausage fried	Colon / Rectum	
		More seldom	1 / 1	
		1–3 times/month	0.9 (0.7–1.3) / 1.5 (1.0–2.2)	
		>once/week	1.3 (0.8–1.9) / 1.7 (1.1–2.8)[4]	
		Sausage oven-roasted	Colon / Rectum	
		More seldom	1 / 1	
		1–3 ts/month	1.0 (0.7–1.3) / 1.0 (0.7–1.6) 1.5	
		>once/week	1.0 (0.6–1.4) / (0.9–2.3)	
		Sausage boiled	Colon / Rectum	
		More seldom	1 / 1	
		1–3 ts/month >once/week	1.2 (0.8–1.7) / 1.3 (0.9–2.0)	
			1.2 (0.5–2.8) / 2.1 (0.9–4.9)[4]	
Bidoli *et al.,* 1992[85] Italy	Colon 123 Rectum 125 Controls 699 Mean age: Controls 56.4 Colon 57 Rectum 62 Recruitment: 1986–1990 FFQ[2]	Beef and pork	Colon / Rectum	Age, gender, social status
		Lowest tertile	1 / 1	
		Second tertile	1.5 / 1.5	
		Highest tertile	1.6 / 2.0[5]	
		Highest *vs.* lowest tertile	Colon / Rectum	
		Cured ham	1.4 NS / 1.6 NS	
		Boiled ham	1.3 NS / 1.2 NS	
		Salami and sausages	1.8[4] / 1.9[4]	
Peters *et al.,* 1992[39] USA	White men and women 746 colon cancer (327 females, 419 males) 746 hospital-based controls Incidence: 1983–86 FFQ (116)	Beef, pork or lamb as sandwuich, mixed or main dish)	Both genders: 1.04 (0.92–1.19)	Age, gender, social-class strata, macronutrients, alcohol, calcium, physical activity, weight, family history, pregnancy history
		RR per 10 servings/month	Males: 1.18[4]	
			Females: 1.14[5]	
		Bacon, hot dogs, salami, bologna, etc.	Both genders: 0.99 (0.93–1.06)	
			Males: 1.05	
		RR per 10 servings/month	Females: 1.12[5]	
Iscovich *et al.,* 1992[86] Argentina	Colon 110 Controls: 220 Recruitment: 1985–1987 Age: 35–80 FFQ (140)[3]	Fresh meat (times/year)		Age, gender, residence, other foods
		<269	1	
		269–381	0.93 (0.42–2.03)	
		382–392	0.30 (0.11–0.80)	
		>392	0.41 (0.19–0.91)[5]	

APPENDIX II – TOTAL MEAT, RED MEAT AND PROCESSED MEAT INTAKE AND COLORECTAL CANCER CASE-CONTROL STUDIES (CONTINUED)

Author, location	Design	Type of meat and partition		OR (95% CI)		Adjustment
		Red Meat (times/year)				
		<176		1		
		176–315		2.29 (1.03–5.08)		
		>315		0.82 (0.39–1.70)		
		Processed (times/year)				
		<16		1		
		16–76		0.83 (0.41–1.69)		
		76–198		0.86 (0.42–1.79)		
		>198		0.43 (0.21–0.89)[4]		
Steinmetz *et al.,* 1993[87] Australia	Colon Males 121 cases, 241 controls Females 99 cases, 197 controls Recruitment: 1979–1980 Age: 30–74 FFQ (165)[3]	Red meat, processed meat (servings/week)				Age, gender, occcupation, Quetelet index, alcohol intake
		Males	Females	Males	Females	
		≤7.4	≤6.1	1	1	
		7.5–10.9	6.2–8.1	0.53 (0.27–1.04)	0.57 (0.27–1.20)	
		11–14.4	8.2–11.2	0.71 (0.37–1.33)	1.17 (0.57–2.40)	
		≥14.5	≥11.3	1.18 (0.62–2.25)	0.95 (0.45–1.99)	
		Red meat (servings/week)				
		Males	Females	Males	Females	
		≤3.9	≤3.4	1	1	
		4.0–5.5	3.5–5.0	1.80 (0.92–3.52)	1.44 (0.70–2.93)	
		5.6–8.2	5.1–7.1	1.64 (0.82–3.27)	1.15 (0.57–2.32)	
		≥8.3	≥7.2	1.59 (0.81–3.13)	1.48 (0.73–3.01)	
		Processed meat (servings/week)				
		Males	Females	Males	Females	
		≤2.2	≤1.4	1	1	
		2.3–4.3	1.5–2.8	0.69 (0.35–1.37)	0.54(0.25–1.23)	
		4.4–7.6	2.9–4.3	0.68 (0.35–1.34)	0.81 (0.37–1.77)	
		≥7.7	≥4.4	1.03 (0.55–1.95)	0.77 (0.35–1.68)	
Centonze *et al.,* 1994[88] Italy	Colorectum 119 Controls 121 Rural Area Median age: 67 Recruitment: 1987–1989 FFQ (70)[3]	Meat, fish, eggs (g/day)				Age, gender, smoking, education, changes in diet
		<149		1		
		150–199		0.8 (0.41–1.54)		
		+199		0.74 (0.38–1.44)		
		Fresh Meat (g/day)				
		<87		1		
		88–131		1.16 (0.62–2.19)		
		>131		0.74 (0.37–1.45)		
		Processed (g/day)				
		2		1		
		≥3		1.01 (0.57–1.69)		
Kampman *et al.,* 1995[89] Netherlands	232: 259 Males 130:136 Females 102:123 Age < 75 Recruitment: 1989–1993 FFQ (289)[3]	Red meat (g/d)		Both genders		Age, gender, total energy, alcohol intake family history, others
		<52		1		
		52–72		0.80 (0.47–1.38)		
		73–94		0.91 (0.54–1.55)		
		>94		1.11 (0.65–1.90)		
		Males	Females	Males	Females	
		<60	<38	1	1	
		60–83	38–59	0.80 (0.39–1.61)	1.82 (0.75–4.46)	
		84–102	60–83	0.57 (0.27–1.30)	2.71 (1.15–6.38)	
		>102	>83	0.89 (0.43–1.81)	2.35 (0.97–5.56)	
				$p = 0.62$	$p = 0.04$	
La Vecchia *et al.,* 1996[90] Italy	Colon 828 Rectum 498 Controls: 2024 Hospital based Age: 20–74 Recruitment: 1985–1992 FFQ (29)	Red meat More than 4 times/week *vs.* less		Colorectum: 1.6 (1.4–1.9) Colon: 1.6 (1.3–1.9) Rectum: 1.6 (1.3–2.0)		Age, gender, education, area, other foods, energy, family history
	Colon Males 238:224 Females 186:190 Age: 30–62 Recruitment: 1985–1989 FFQ (71)[3]	Total meat (including fish) serving/day				Age, gender, total energy
		Males	Females	Males	Females	
		0.–1.5	0.–1.17	1	1	
		1.5–1.9	1.18–1.53	0.79 (0.44–1.41)	0.67 (0.36–1.24)	
		2–2.6	1.54–2.08	1.18 (0.68–2.05)	0.76 (0.40–1.45)	
		>2.6	>2.08	1.52 (0.84–2.77)	0.78 (0.39–1.55)	
Shannon *et al.,* 1996[91] USA	Colon Males 238:224 Females 186:190 Age 30–62 Recruitment 1985–1989 FFQ (71)[3]	Red meat serving/day				
		Males	Females	Males	Females	
		0.–0.78	0.–0.49	1	1	
		>0.78–1.2	>0.49–0.79	1 (0.58–1.74)	0.90 (0.50–1.64)	
		>1.2–1.7	>0.79–1.2	1.05 (0.61–1.83)	1.03 (0.55–1.90)	
		>1.7	>1.2	1.48 (0.82–2.66)	0.72 (0.37–1.38)	
Franceschi *et al.,* 1997[92] Italy	Colon 1225 Rectum 728 Controls 4154 Age: 19–74 Recruitment: 1992–1996 FFQ (79)[3]	Red meat serving/wk		Colorectum		Age, gender, education, total energy, physical activity, others
		<2.3		1		
		<3.5		0.98 (0.83–1.17)		
		<4.8		1.12 (0.94–1.34)		
		<6.3		1.0 (0.83–1.21)		
		>6.3		1.14 (0.93–1.39)		
		OR per 1 serving/day		Colorectum 1.09 (0.90–1.31) Colon 1.06 (0.85–1.32) Rectum 1.16 (0.88–1.52)		
		Processed meat serving/wk		Colorectum		
		<1		1		
		<2		1.21 (1.03–1.42)		

APPENDIX II – TOTAL MEAT, RED MEAT AND PROCESSED MEAT INTAKE AND COLORECTAL CANCER CASE-CONTROL STUDIES (CONTINUED)

Author, location	Design	Type of meat and partition	OR (95% CI)	Adjustment
		<3	1.06 (0.89–1.26)	
		<4	1.24 (1.02–1.49)	
		>4	1.02 (0.84–1.24)	
		OR per 1 serving/day	Colorectum 0.97 (0.79–1.18) Colon: 1.08 (0.87–1.36) Rectum: 0.78 (0.57–1.06)	
Le Marchand *et al.,* 1997[54] Hawaii	Prevalent and incidents Colorectum (Males 698 Females 494)	Red meat Colorectum (quartiles, reference: lowest) Tertiles (reference: lowest)	Males: 1-1.2-1.5-1.6 (1.0–2.5) Females: 1-0.8-0.7-0.7 (0.4–1.2)	Age, gender, ethnicity, family history, alcohol, tobacco, BMI, total energy, others
	Right colon (Males 197 Females 164) Left colon (Males 270 Females 194) Rectum (Males 221 Females 129) Controls 1192 Multiethnic Recruitment: 1987–1989 FFQ (>280)[3]	Right colon Left colon Rectum Processed meat (quartiles) Right colon Left colon Rectum	1-1.5-2.3[4] 1-0.9-1.1 1-1.2-1.3 1-1.7-2.2 2.3 (1.5–3.4)[5] 1-1.1-1.6 1-1.6-1.4 1-1.1-2.2	1-1.2-0.8 1-0.8-0.8 1-0.90.7 1-0.8-1.1 1.2 (0.8–2.0) 1-1.2-1.0 1-0.8-1.1 1-0.8-0.8
Augustsson *et al.,* 1999[43] Sweden	Colon 521 Rectum 249 Controls 553 Age: 51–77 Recruitment: 1992–1994 FFQ (188)[3]	Total meat and fish intake Quintiles. Reference category: lowest	Colon 1.4 (0.9–2.2) 1.4 (0.9–2.1) 1.7 (1.1–2.6) 0.9 (0.5–1.4)	Rectum 1.4 (0.9–2.3) 1.4 (0.8–2.3) 1.4 (0.9–2.4) 1.0 (0.6–1.6)	Age, gender, energy
Murata *et al.,* 1999[93] Japan	Colon 265 Rectum 164 Controls 794 Recruitment: 1989–1997 FFQ[2]	Total meat excluding fish. Every day *vs.* rare	Colon: 1.41 (1.13–1.77)[5] Rectum: 1.33 (1.01–1.77)[4]	Age, alcohol, tobacco, gender, eating attitude other foods
Kampman *et al.,* 1999[47] USA	Colon 1542 cases/1860 controls Age: 30–79 Males 868/989 Females 674/871 Recruitment: 1992–1995 FFQ (800)[3]	Red meat: beef and ham (servings/week) Males / Females ≤2.2 / ≤1.5 2.3–3.7 / 1.6–2.5 3.8–5.6 / 2.6–4.0 5.7–8.8 / 4.1–6.2 >8.8 / >6.2	Males / Females 1 / 1 0.8 (0.6–1.0) / 1.1 (0.8–1.5) 1.1 (0.8–1.0) / 1.3 (0.9–1.8) 1.0 (0.7–1.4) / 1.3 (0.9–1.8) 0.9 (0.7–1.3) / 1.0 (0.7–1.5)	Age, gender, total energy, BMI, dietary fiber, tobacco, other
		Processed meat: bacon, sausages, cold cuts Males / Females ≤0.5 / 1 0.6–1.0 / 1.1 (0.8–1.6) 1.1–1.8 / 1.2 (0.9–1.8) 1.9–3.1 / 1.3 (1.0–1.8) >3.1 / 1.4 (1.0–1.9)	Males / Females ≤0.2 / 1 0.3–0.5 / 1.3 (1.0–1.9) 0.6–0.9 / 1.2 (0.9–1.7) 1.0–1.7 / 1.3 (0.9–1.8) >1.7 / 1.1 (0.8–1.6)	
Levi *et al.,* 1999[94] Switzerland	Colon 119 Rectum 104 Control 491 Mean age: 63 Recruitment: 1992–1997 FFQ (70)[2]	Red meat (serving/week) <2.25 2.25–3.75 >3.75	Colorectum 1 1.27 (0.81–2.02) 2.06 (1.29–3.30)[5]	Education, tobacco, alcohol, BMI, vegetables, total energy, physical activity
		OR for 1 serving/day	Colorectum 1.54 (1.28–1.85) Colon 1.63 (1.30–2.04) Rectum 1.50 (1.2–1.88)	
		Pork and processed meat (serving/week) >2.25 2.25–3.75 >3.75	Colorectum 1 1.12 (0.68–1.85) 2.33 (1.42–3.830)[5]	
		OR for 1 serving/day	Colorectum 1.27 (1.13–1.43) Colon 1.34 (1.17–1.53) Rectum 1.18 (1.02–1.37)	
Boutron-Ruault *et al.,* 1999[95] France	Right colon: 43 Left colon: 63 Rectum: 65 Controls: 309 Age 30–79 Recruitment: 1985–1990 Dietary history[3]	Fresh meat g/d Males / Females 82.1 / 56.5 <105.0 / <81.4 <127.1 / <102.6 >127.1 / >102.6	Both genders 1 1.2 (0.7–2.0) 1.0 (0.6–1.8) 1.2 (0.6–2.1)	Age, gender, total energy
		Delicatessen (g/day) Males / Females <19.2 / <11.2 <34.7 / <21.2 <55.3 / <33.3 >55.3 / >33.3	Both genders 1 1.6 (0.9–2.9) 1.2 (0.6–2.2) 2.4 (1.3–4.5)[5]	

[1]FFQ, food frequency questionnaire. Number of items between parentheses.–[2]Hospital-based.–[3]Population-based.–[4]$p < 0.05$.–[5]$p < 0.01$.

APPENDIX III – COHORT STUDIES

Author, location	Design	Type of meat and partition	OR (95% CI)			Adjustment
Phillips and Snowdon, 1985[96] Seventh-day Adventist, USA	Colorectum Cancer Mortality Colon 147 Rectum 35 Cohort: 25493 subjects Age > 35 Recruitment: 1960–1980 Follow-up 20 years FFQ (21)	Meat (times/week) <1 1–3 ≥4	 1 1.4 (1.0–1.9) 0.9 (0.6–1.5)			Age, gender
Hirayama, 1990[97] Japan	Colorectal Cancer Mortality Age 40 or older Intestine: 256 men, 318 women Rectum: 316 men, 247 women Cohort: 265118 subjects Follow-up: 1966–1982	Meat Daily Occasional Rare None	Males Intestine 1 1.86 (1.17–2.97) 1.52 (0.90–2.57) 1.89 (0.84–2.47)	Rectum 1 1.50 (1.01–2.22) 1.47 (0.95–2.28) 1.54 (0.74–3.20)		
			Females Intestine	Rectum		
		Daily Occasional Rare None	1 0.83 (0.59–1.18) 0.74 (0.50–1.10) 0.95 (0.57–1.56)	1 1.20 (0.76–1.91) 1.08 (0.65–1.79) 1.41 (0.77–2.60)		
Thun *et al.,* 1992[98] Cancer Prevention Study, USA	Colon Cancer Mortality Deaths: 2757 Subjects 1185124 Mean age: 57 Recruitment: 1982 Follow-up: 2 years FFQ (42)	Meat excluding fish and poultry Quintiles References: lowest Red meat (g/day)	Males 1 1.12 1.08 1.01 1.21	Females 1 0.92 1.06 0.91 1.05		
Willett *et al.,* 1990[99] The Nurses Health Cohort Study, USA	Colon: 150 cases Cohort: 8875 women Age: 34–59 (512 488 person/years) Recruitment: 1980–1986 FFQ (61)	 <59 59–83 84–105 106–133 ≥134	 1 1.16 (0.67–1.99) 0.25 (0.73–2.13) 1.13 (0.65–1.97) 1.77 (1.09–2.88)[2]			Age, energy
		Processed meat <1/month 1–3/month 1/w 2–4/w ≥4/w	 1 1.09 (0.70–1.69) 1.45 (0.91–2.31) 1.86 (1.16–2.98) 1.21 (0.53–2.72)[2]			
Giovannucci *et al.,* 1994[19] Health Professionals Follow-up Study, USA	Colon: 205 cases Cohort: 47949 men (737910 person/years) Age: 40–75 Recruitment: 1986 Follow-up 6 years	Red meat (g/day) 18.5 42.9 64.1 88.5 129.5	 1 0.97 (0.62–1.54) 0.98 (0.62–1.56) 1.21 (0.77–1.88) 1.71 (1.15–2.55)[2]			Age, obesity, total energy, family history, alcohol, tobacco, physical activity, others
		Processed meat None 1–3/month 1/w 2–4/w ≥5/w	 1 1.25 (0.87–1.80) 1.40 (0.92–2.13) 1.67 (1.06–2.61) 1.16 (0.44–3.04)			
Goldbohm *et al.,* 1994[40] Netherlands	Case-cohort Males Colon 157 Cohort: 58279 Females Colon 155 Cohort: 62573 Age 55–69 Recruitment: 1986–1990 Follow-up 3.3 years FFQ (150)	Fresh red meat and poultry Men, Women g/day 53,43 84/72 101,91 123,107 158,145	Men 1 1.09 (0.58–2.04) 1.62 (0.89–2.93) 0.98 (0.51–1.91) 0.87 (0.43–1.77)	Women 1 0.83 (0.44–1.56) 1.03 (0.58–1.84) 1.05 (0.57–1.93) 0.88 (0.45–1.69)	Both genders 1 0.92 (0.59–1.44) 1.24 (0.81–1.90) 0.98 (0.62–1.55) 0.84 (1.51–1.37)	Age, gender, total energy, other types of meat
		Both genders: OR per 5 g/day: 0.98 (0.93–1.03)				
		Processed meat Processed Meat g/day 0.–10 10–20 >20	 Men 1.25 (0.59–2.70) 1.45 (0.67–3.12) 1.84 (0.85–3.95)	 Women 1.22 (0.66–2.26) 1.48 (0.77–2.87) 1.66 (0.82–3.35)	 Both genders 1.23 (0.76–1.98) 1.43 (0.87–2.35) 1.72 (1.03–2.87)[2]	
Bostick *et al.,* 1994[100] Iowa Women Health's Study, USA	Colon: 212 Cohort: 35215 Women (167447 person/years) Age: 55–69 Recruitment: 1986–1990 FFQ (127)	Total eggs and meat (serving/week) <9 9–11 11.5–14 14.5–18 >18	 1 0.83 (0.54–1.26) 1.02 (0.69–1.52) 0.71 (0.44–1.13) 0.88 (0.52–1.49)			Age, gender, total energy, other foods, others
		Red meat (serving/week) <4 4–6 6.5–8 8.5–11 >11	 1 1.13 (0.76–1.69) 1.20 (0.77–1.87) 0.88 (0.54–1.42) 1.04 (0.62–1.76)			
		Processed meat (serving/week) 0 0.5 1 2–3 >3	 1 1.0 (0.73–1.38) 1.07 (0.71–1.61) 0.81 (0.46–1.44) 1.51 (0.72–3.17)			

APPENDIX III – COHORT STUDIES (CONTINUED)

Author, location	Design	Type of meat and partition	OR (95% CI)		Adjustment
Gaard *et al.,* 1996[48] Norway	Colon: 143 cases 19% (48) Cohort 570842 person/years Age 20–53 Recruitment: 1977–1983 Mean follow-up 11.4 FFQ (80)	Excluding fish (meals/week) ≤2 3 4 ≥5	Males 1 1.33 1.44 0.80	Females 1 1.33 1.40 1.87	Age
Kato *et al.,* 1997[101] New York University Women's Health Study, USA	Colorectal Cohort: 15785 women (105044 person-years) Recruitment: 1985–1991 Age: 34–65 FFQ (70)	Red meat Quartiles Reference: lowest category Ham, sausages Quartiles Reference: lowest category	1 1.28 (0.72–2.28) 1.27 (0.71–2.28) 1.23 (0.68–2.22) 1 1.39 (0.81–2.38) 1.38 (0.79–2.42) 1.09 (0.59–2.02)		Age, total energy, education, others
Chen *et al.,* 1998[57] Physicians Health Study, USA	Nested case-control Males Colorectum 212:221 Age: 40–84 Recruitment: 1982 13 years follow-up	Red meat (srving/day) ≤5 >0.5–1 >1	1 0.98 (0.64–1.52) 1.17 (0.68–2.02)		
Hsing *et al.,* 1998[44] Lutheran Brotherhood Cohort, USA	Colorectum Cancer Mortality in white males Colon: 120 Rectum: 25 286731 person-years Recruitment: 1966 20 y follow-up FFQ (35)	Red meat (time/month) <15 15–19 20–29 30–59 ≥60	1 1.2 (0.6–2.2) 1.5 (0.9–2.5) 1.4 (0.8–2.5) 1.9 (0.9–4.3) p trend = 0.1		Age, smoking status, alcohol intake, total energy
Singh and Fraser, 1998[42] Adventist Health Study, USA	Colorectum: 157 (135 colon 22 recto-sigmoidal junction) Cohort: 32051 Age > 25 Recruitment: 1976–1982 FFQ (51)	Meat Never ≥1 time/wk ≥1 time/wk Red meat Never <1 time/wk ≥1 time/wk	1 1.50 (0.92–2.45) 1.85 (1.16–2.87)[2] 1 1.58 (1.01–2.45) 1.41 (0.9–2.21)		BMI, physical activity, parental history of colon cancer, tobacco alcohol,
Knekt *et al.,* 1999[102] Finland	Colorectum 73 Cohort: 9985 subjects Recruitment: 1966–1972 Follow-up until 1990 (21 years) Dietary history	Meat and meat-products (cured) Quartiles. Reference: lowest	1 1.48 (0.77–2.84) 1.28 (0.63–2.57) 1.84 (0.98–3.47)		
Pietinen *et al.,* 1999[50] ATBC Prevention Study, Finland	Cases: 185 Cohort: 27111 Male smokers Age: 50–69 Recruitment: 1988 (Follow up 8 years) FFQ (276)	Red meat (g/day) 79 114 143 203 Processed meat (g/day) 26 50 73 122	1 1.1 (0.8–1.7) 1.0 (0.7–1.6) 1.1 (0.7–1.7) 1 1.5 (1.0–2.2) 1.2 (0.7–1.8) 1.2 (0.7–1.8)		Age, tobacco years, BMI, alcohol, education, physical activity, others

[1]FFQ, Food frequency questionnaire. Number of items between parentheses.–[2]$p < 0.05$.–3$p < 0.01$.–[4]$p < 0.05$.–[5]$p < 0.01$.

2. Méthodes de cuisson de la viande et risque de cancer.

Basé sur:

Meat cooking and cancer risk. In: Nutrition and Lifestyle: Opportunities for Cancer Prevention pp 181-6. Edited by E. Riboli and R. Lambert. IARC Scientific Publications No 156 IARC Press Lyon 2002.

Les méthodes de cuisson à haute température, tels que la friture, le grillage et le barbecue, ainsi que l'habitude de manger les viandes bien cuites se sont avérées en relation avec le risque de certains cancers (Tableau 1). La recherche en laboratoire a suggéré que l'un de mécanismes qui pourrait expliquer la relation entre la consommation de viande rouge et le cancer colorectal serait son contenu en amines hétérocycliques (HCAs) et en hydrocarbures polycycliques aromatisés (PAHs).

Les HCAs sont des substances potentiellement cancérogènes formées lorsque la viande est cuite à haute température en quantités directement proportionnelles au temps de cuisson (127). Les PAHs se trouvent dans la viande et le poisson qui ont été exposés pendant sa cuisson directement au charbon ou autres matières combustibles (128;129).

Nous décrivons ici l'approche multidisciplinaire développée par Sinha et collaborateurs (130-132) pour estimer les niveaux de HCAs et PAHs contenus dans la viande selon méthode de cuisson. Nous décrivons aussi les résultats obtenus par l'application de cette méthodologie dans des études épidémiologiques.

Afin de développer des instruments utilisables dans des études épidémiologiques de grande taille, Sinha et collaborateurs ont déterminé en laboratoire les niveaux de HCAs et PAHs contenus dans près de 2500 échantillons de viandes cuites selon 120 méthodes et degrés de cuisson différents. Cette étude a montré aussi que les niveaux des HCAs dans la viande dépendent fondamentalement de la température et du temps de cuisson (130;132).

La deuxième étape a consisté en une recherche de biomarqueurs de l'exposition aux HCAs alimentaires chez l'homme. Des échantillons biologiques ont été collectés au cours d'une étude métabolique chez des volontaires sains avec des régimes alimentaires contenant des HCAs en concentrations strictement contrôlées. Les HCAs libres ou totaux dans les urines, l'activité mutagénique de l'urine et des adduits à l'ADN ont été examinés. Aucun biomarqueur n'a été identifié en raison de la faible corrélation entre les HCAs alimentaires et les valeurs en urines, l'absence d'activité mutagénique détectable dans les urines 12 heures après l'ingestion de la viande et le manque de sensitivité des adduits à l'ADN même pour des niveaux des très élevés des deux principaux HCAs dans les viandes (MeIQx et PhIP)(131).

La troisième étape a vu la conception d'un questionnaire alimentaire avec l'inclusion d'une liste détaillée de méthodes de cuisson : indicateurs de la température de cuisson, du degré de cuisson de la viande, indicateur du temps de la cuisson par exemple. Des photographies ont été utilisées pour améliorer la description du degré de cuisson. Les questionnaires, combinés avec les valeurs des HCAs et PAHs déterminés dans le laboratoire ont été utilisés dans plusieurs études épidémiologiques.

Les HCAs contenus dans la viande se sont révélés en relation avec le risque d'adénomes colorectaux (133), de cancer du poumon –bien que les auteurs n'excluent pas la possibilité d'un effet de confusion résiduel avec le tabac (134) - et du cancer de l'estomac (135). Les deux études de cancer du sein réalisées n'ont pas abouti à des résultats concordants (136;137) [Tableau 2].

Le risque potentiel que les HCAs par voie alimentaire peuvent poser à l'homme dépend non seulement du type de HCAs et des concentrations des HCAs dans la viande, mais aussi de leur activation métabolique (138). L'activation initiale est la N-oxydation par le cytochrome P450 1A2 (CYP1A2) du foie. Le métabolite N-hydroxylaminé est ultérieurement acétylé par les acétyltransférase NAT1 ou NAT2. Dans l'étude métabolique chez les volontaires sains, les

phénotypes CYP1A2 et NAT2 ont été déterminés par excrétion de métabolites de la caféine. L'activité de l'enzyme NAT2 n'a pas varié avec l'exposition aux HCAs alimentaires, mais l'activité de l'enzyme CYP1A2 a augmenté chez les sujets qui venaient de suivre un régime riche en viande très cuite. L'augmentation de l'activité a été plus importante chez les sujets qui avaient des niveaux d'activité de cette enzyme élevés avant l'exposition aux HCAs, et cette augmentation d'activité a été moins marquée chez les sujets présentant une activité enzymatique faible avant l'exposition. Ceci laisse supposer l'existence d'un effet d'induction des HCAs sur cette enzyme, ainsi que d'autres effets.

Pris dans son ensemble, les résultats obtenus suggèrent l'existence d'une relation entre les mutagènes ou carcinogènes formés dans la viande pendant sa cuisson et le risque de cancer et justifient la réalisation d'ultérieures études.

Meat cooking and cancer risk

Sinha R.[1], Norat T.[2]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Executive Plaza North, Rm. 443, 6130 Executive Blvd., Rockville, MD 20892. [2]Unit of Nutrition and Cancer, International Agency for Research on Cancer, 150, Cours Albert Thomas, 69372, Lyon Cedex 08, France.

A report by an international panel of experts entitled *Food, Nutrition and the Prevention of Cancer: A Global Perspective* concluded that "diets containing substantial amounts of red meat probably increase the risk of colorectal cancer" and "that such diets possibly increase the risk of pancreatic, breast, prostate, and renal cancers" (World Cancer Research Fund, 1997). The association with red meat intake may be due to a combination of factors such as content of fat, protein, and iron, and/or meat preparation (e.g. cooking or preserving methods).

Laboratory results have shown that meats cooked at high temperatures contain heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs), which are mutagenic and carcinogenic in animals (Ohgaki *et al.*, 1985; Adamson,1990; Felton & Knize, 1990; Skog, 1993; Ito *et al.*, 1991; Ghoshal *et al.*, 1994; Culp *et al.*, 1998). Epidemiological studies (Schiffman *et al.*, 1990; Gerhardsson De Verdier *et al.*, 1991; Steineck *et al.*, 1993; Knekt *et al.*, 1994; Muscat & Wynder, 1994; Ronco *et al.*, 1996) have found a suggestive but inconsistent relationship between the way meat is cooked and colon cancer risk (Table 1). Cooking methods or meat doneness have been examined in epidemiological studies as a surrogate measure of mutagens formed during high-temperature cooking of meat, e.g. HCAs and PAHs. The techniques of meat cooking associated with increased cancer risk were high-temperature cooking methods (frying, broiling, or grilling/barbecuing) and doneness level or surface browning of meat and intake of gravy made from meat drippings. In these studies, the information obtained on meat cooking practices could not differentiate between factors that had an important influence on the production of HCAs and PAHs.

We will describe our approach to estimating meat-cooking mutagens and the main results we have obtained on the association of cancer risk with meat intake, meat cooking methods and certain mutagens formed during cooking.

Assessing exposure to HCAs and PAHs

We set up a multidisciplinary approach to develop tools to estimate dietary intake of HCAs and PAHs. The first step was to develop databases for HCA and PAH content in meat. Approximately 2500 individual pieces of meat were cooked to provide data for 120 categories by cooking method and doneness that were ultimately used to create the HCA database (Knize *et al.*, 1995, 1996; Sinha *et al.*, 1995a; Sinha *et al.*, 1998a,b).

We found that the measured values of the specific HCAs varied with meat type, cut of meat, cooking method, and doneness level and that the different HCAs were formed in varying amounts. The results suggest that questions on meat intake not including details of type or cut of meat, cooking technique, and doneness level are likely to misclassify HCA exposure. For example, for the same level of doneness, steaks, hamburger patties, and roast beef had substantially different levels of HCAs. The three high-temperature cooking methods (pan-frying, oven broiling, and grilling/barbecuing) produced varying levels of HCAs (Fig. 1). We also measured mutagenic activity in the meat samples using the Ames salmonella test (strain 98). Mutagenic activity has the advantage of being a biological measure that integrates all classes of mutagens according to their mutagenic potential.

HCA production resulted from two important elements: temperature and time. By developing the HCA database, we found that cooking technique could serve as a reasonable proxy of temperature and doneness level as a surrogate for time. Using existing Food Frequency Questionnaires (FFQs), we embedded the cooking methods and doneness levels within each meat line item. As doneness can be subjective, we included photographs of different levels of doneness showing both the inside and outside for various meat items (Sinha & Rothman, 1997).

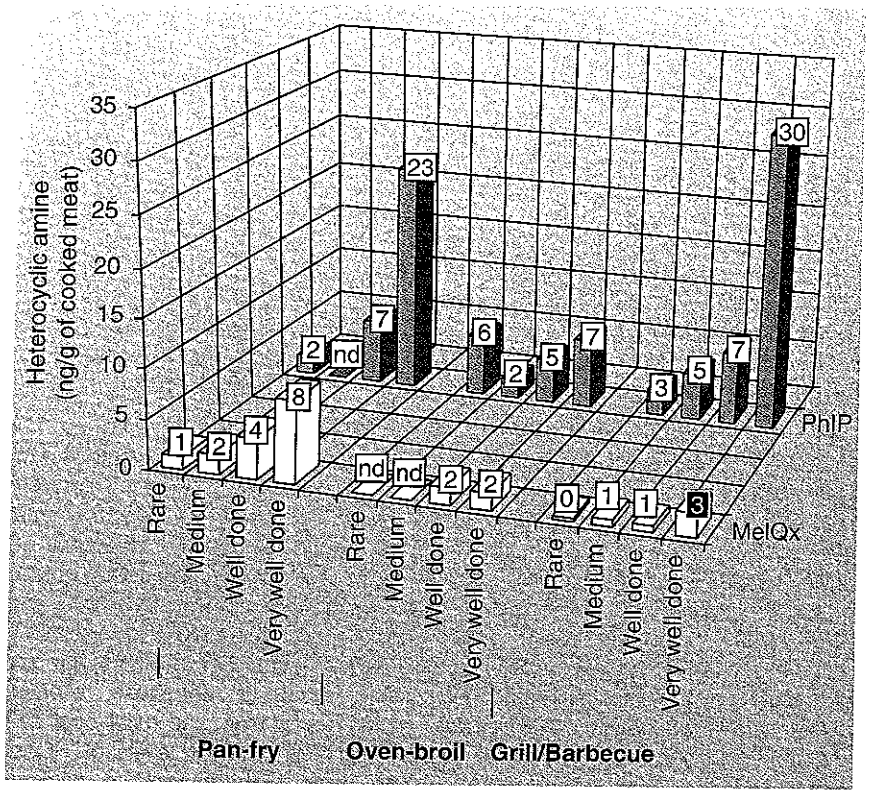


Figure 1
Meat cooking and content in heterocyclic amines

PhIP is 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
MeIQx is 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline

Development of biomarkers of HCA exposure

In a metabolic study we collected multiple biological samples to test for new biological markers and examine HCA metabolism pathways in individuals who consumed meats containing known amounts of HCAs. We evaluated several markers of HCA intake in the urine by measuring mutagenic activity (free and acid hydrolysed), HCAs (free and acid hydrolysed) and metabolites of HCA (Sinha *et al.*, 1995b ; Stillwell *et al.*, 1997, 1999a, 1999b;). Urinary free HCAs or total (acid-hydrolysed urine samples includes free, N2-glucuronide and sulfamate metabolites) correlated modestly with the amount of HCA consumed in the metabolic study, with the Spearman correlation coefficients between 0.4 and 0.6. HCAs and mutagenic activity in the urine were not detectable 12 h after consuming the high-HCA meal. This indicates that HCAs in urine may not be ideal measures of usual intake in etiological studies. We measured DNA adducts to examine if they could be used as a marker of biologically effective dose of HCAs, but DNA adducts were not sensitive enough, even in subjects who consumed high levels of MeIQx (2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline) and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine). Thus, at present, it is unlikely with current methods that we will be able to measure HCA adducts in a free-living population, whose intake is likely to be much lower than that of subjects in the metabolic study.

The cancer risk posed to humans from HCAs in the diet may depend upon the extent to which the compounds are activated *in vivo*. The initial activation step is thought to be N-oxidation by liver CYP1A2. The N-hydroxylamine metabolite is O-acetylated in the liver itself or transported to the appropriate target organ, where it is acetylated by the polymorphic acetyltransferases, *NAT1* or *NAT2*. The acetylated compound can form adducts and cause DNA damage. CYP1A2 and *NAT2* activities can be measured by evaluating excretion of caffeine metabolites in urine after caffeine consumption In the metabolic study, CYP1A2 and *NAT2* activities were measured at baseline and at the end of the low- and high-temperature cooked-meat periods *NAT2* activity remained unchanged throughout the study, while CYP1A2 activity increased in most of the subjects after consuming high-temperature cooked meat, suggesting induction by some compound(s) formed during high-temperature cooking. There was a high within-person correlation for CYP1A2: subjects with low enzymatic activity after eating low-temperature cooked meat tended to have relatively low activity even after consuming high-temperature cooked meat and the subjects who had high enzymatic activity tended to stay relatively high. This suggests a fixed component in the regulation of CYP1A2 and the need to consider both a fixed and an inducible component of this enzyme in epidemiological studies.

Due to the ubiquitous nature of PAHs in foods, estimating dietary consumption is considerably more challenging than estimating intake of HCAs, which are formed in large

Table 1. Epidemiological evidence of the possible association between cooked meat and cancer

Relative risk for very well done meat/high browning vs rare meat/not browning

Author	Cooking method	RR (CI)	
Schiffman *et al*, 1990		3 5 (1 3–9.6)	a
Gerhardsson *et al*, 1991		2 0 (1 2–3.6)	a
Lang *et al*, 1994		2 1 (1 1–4 1)	
Muscat *et al*, 1994		1 2 (0 6–2 4)	
Kampmann *et al*, 1999		1 2 (0 9–1.5)	

Relative risk for high vs low consumption of meat according to cooking methods

Lyon *et al*, 1988	Frying	1 2 (0 8–1 9)	
	Broiling	0 7 (0 8–2 1)	b
Young *et al*, 1988	Frying	1 3 (1 0–1 8)	b
	Broiling	0 7 (0 5–1 0)	
Peters *et al*, 1989	Deep frying	2 1 (1 0–4 6)	b
Wohlebb *et al*, 1990	BBQ	3 3 (1 2–9 2)	a

[a]Statistically significant.
[b]Borderline statistically significant

Table 2. HCA carcinogenicity in animal and epidemiologic studies

Site	Animal studies	Epidemiological studies
Colon	IQ, MeIQ, PhIP	MeIQx, PhIP[a]
Lung	IQ, MeIQx	MeIQx
Mammary/breast	PhIP	PhIP

PhIP is 2-amino-1-methyl-6-phenylimidazo[4 5-b]pyridine.
MeIQx is 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline

[a]Borderline statistically significant

quantities only in high-temperature cooked meat Humans can be exposed to PAHs via grilled and smoked foods, foods grown in polluted environments, ambient air contact, and tobacco smoking. Thus, in estimating exposure to PAHs, it is crucial to develop biomarkers that integrate exposures from different sources. We are currently investigating a number of possible biomarkers: urinary PAH metabolites as a measure of dietary exposure and Benzo(a)pyrene-DNA adduct as a marker for long-term PAH exposure from all sources.

Epidemiologic studies

Colorectal adenomas

We investigated the role of meat intake, meat-cooking techniques, and meat-cooking mutagens in a hospital-based case–control study of colorectal adenomas in Bethesda (Sinha *et al*, 2001). This was the first study to use the detailed FFQ meat-cooking module. We found a nonsignificant increased risk of colorectal adenomas of 4% per 10 g/day (or 2.5 oz/week) of total meat intake. This increased risk for total meat partitioned into a significant 11% per 10 g/day risk increase for consumption of red meat and a nonsignificant decrease in risk of 5% per 10 g/day for white meat intake. The partitioned risk associated with well done/very well done red meat was 29% risk increase per 10 g/day. High-temperature cooking methods were also associated with increased risk; 26% per 10 g/day of grilled red meat and 15% per 10 g/day of pan-fried red meat consumption. The risk was further elevated to 85% per 10 g/day among subjects who ate well done/very well done grilled meat. Risk of colorectal adenomas was doubled in the highest compared to the lowest quintile of intake of DiMeIQx, MeIQx, and PhIP. The excess risk was confined to the fifth quintile for DiMeIQx and MeIQx, and to both the fourth and fifth quintiles for PhIP. When adjusting each HCA for the other two, the risk estimates became attenuated for all three because they were modestly to highly correlated. However, the trend for MeIQx intake remained statistically significant. There was an increase in colorectal adenoma risk in relation to estimated total meat-derived mutagenic activity. The risk associated with mutagenic activity was minimally affected when adjusted for PhIP (which became nonsignificant) and was weakened when adjusted for MeIQx (which was attenuated to an even greater extent; data not shown). Mutagenic activity remained significantly associated with increased risk even when adjusted for intake of red meat or well-done red meat. In contrast, the red meat and well-done red meat associations were no longer statistically significant when adjusted for total mutagenic activity, suggesting that mutagenic activity explained the meat relationships.

Lung cancer

Some epidemiological studies have found that diets high in fat, saturated fat, or cholesterol are associated with an increased risk of lung cancer. Meat consumption is highly correlated with the intake of saturated fat and cholesterol. In the Missouri Women's Study, a population-based lung cancer case–control study, lung cancer was associated with higher intake of total meat, red meat, well-done red meat, and fried red meat (Sinha *et al.*, 1998c). Because MeIQx has been found to induce lung tumours in rodents, we compared risks for the 90th and 10th percentiles of intake (95% confidence interval, CI). We observed significant excess risks for MeIQx (OR, 1.5; CI, 1.1–2.0) but not for DiMeIQx (OR, 1.2; CI, 0.9–1.6) or PhIP (OR, 0.9; CI, 0.8–1.1). Interestingly, MeIQx consumption was associated with an increased risk of lung cancer for the non-smokers and the light/moderate smokers but not for the heavy smokers (Sinha *et al.*, 2000b). The increased risk of lung cancer associated with higher intake of meat, well-done/fried meat, and MeIQx consumption needs to be viewed in the context of other risk factors such as smoking. Smoking is by far the strongest risk factor for lung cancer and its impact cannot be minimized even when other modest risk factors are found. Therefore, we remain concerned about residual confounding from smoking.

Breast cancer

In carcinogenicity experiments with rodents, PhIP consistently induced mammary tumours. In a nested case–control study within the Iowa Women's Health Study, subjects completed the meat-cooking module and preference for level of doneness by using a series of colour photographs. Breast cancer risk was associated with well-done/very well-done red meat (Zheng *et al.*, 1998). We estimated HCA intake and found that risk of breast cancer was elevated across increasing quintiles of PhIP consumption, whereas MeIQx and DiMeIQx were not associated in these analyses (Sinha *et al.*, 2000a). When PhIP and very well-done red meat were adjusted for each other, PhIP remained significantly associated with breast cancer risk but very well-done meat did not, indicating that PhIP may be the relevant component of very well-done red meat.

In a case–control study among women (Susceptibility to Breast Cancer: Dietary and other Factors (Delphina *et al.*, 2000) with suspicious breast masses, no significant associations between red meat intake and breast cancer were found for any doneness preference. White meat intake was significantly protective (for >67 g/day vs <26 g/day (OR, 0.46; CI, 0.23–0.94), as were well-done, pan-fried or barbecued chicken, and other chicken. The subjects with estimated intakes of PhIP in the highest quartile (>240 ng/day) versus the lowest quartile (<31 ng/day) were at a significantly decreased risk of breast cancer (OR, 0.42; CI, 0.02–0.88). The protective effect of PhIP was no longer significant after adjusting for chicken intake. Furthermore, there was no association between *NAT2* genotype and breast cancer.

These two studies appear to contradict each other: PhIP was associated with increased risk of breast cancer in the Iowa Women's Health study, whereas intake of chicken and PhIP were protective in the Californian study. The types of meats consumed and the control subjects used in the two studies, however, were quite different. In Iowa, the risk of breast cancer was associated with very well-done red meat consumption and the HCAs were estimated from three red meats only (steak, hamburger, and bacon). In the California study, the women ate very small amounts of red meat, so PhIP intake was mainly from chicken. Our estimate of PhIP intake in the California study may be a surrogate for chicken consumption rather than an accurate estimate of this compound. Results from the FFQ validation study and database development work indicate that there may be substantial misclassification in estimating PhIP intake from chicken. Estimating chicken intake using the existing FFQs may not be optimal and variability in PhIP content of cooked chicken can be substantial. Better methods to estimate PhIP in chicken are needed.

Stomach cancer

In a population-based case–control study (Ward *et al.*, 1997), high intake of red meat was associated with an increased risk of stomach cancer. Overall, broiling or frying of beef, chicken, or pork was not associated with risk of this tumour. Barbecuing/grilling, reported as the usual cooking method for a small number of study participants, was associated with an elevated risk. After excluding those who reported usually barbecuing/grilling, a source of both PAHs and HCAs, the doneness level was evaluated as a surrogate for HCA exposure. Compared to a preference for rare/medium rare beef, ORs were 2.4 for medium, 2.4 for medium-well and 3.2 for well-done beef preferences, with a significant positive trend. The finding that well-done and grilled meats are associated with an increased risk of stomach cancer suggests that dietary HCAs and PAHs play an etiological role. To assess the role of HCA and PAH intake, future studies of stomach cancer will need more detailed questions on meat-cooking techniques.

Final considerations

We have found that red meat intake is associated with increased risk of colorectal adenomas as well as lung and stomach cancers. Grilled meat intake, a possible surrogate for HCAs and PAHs,

increased the risk of colorectal adenomas and stomach cancer, whereas intake of fried meat, which contains mainly HCAs, was associated with lung cancer. With greater degrees of red meat doneness, a proxy for a higher level of meat-cooking carcinogens, an increase in risks for colorectal adenomas, lung, breast, and stomach cancers was observed. The data suggest some degree of organ specificity for HCAs and are consistent with those obtained in animal carcinogenicity studies (Table 2).

To elucidate the relationship between meat-cooking mutagens/carcinogens and cancer risk further, future research should also be conducted in populations with a wide range of consumption of different types of meat and cooking methods, such as in different European countries (European Prospective Investigation on Cancer), groups characterized by rapidly changing diets with increasing meat consumption, such as in Japan and China, and groups with high meat intake using high-temperature cooking methods, such as in some South American countries and Australia/New Zealand.

Using 24-h recalls from the EPIC calibration study, we began to collaborate in the development of meat-cooking modules tailored to country-specific cohorts in the overall study.

References

Adamson, R.H. (1990) Mutagens and carcinogens formed during cooking of food and methods to minimize their formation. In: DeVita, V.T., Hellman, S. & Rosenberg, S.A., eds, *Cancer Prevention*. Philadelphia, J.B. Lippincott Company, pp 1–7

Culp, S.J., Gaylor, D.W., Sheldon, W.G., Goldstein, L.S. & Beland, F.A. (1998) A comparison of the tumor induced by coal tar and benzo[a]pyrene in a 2-year bioassay *Carcinogenesis*, **19**, 117–124

Delfino, R.J., Sinha, R., Smith, C., West, J., White, E., Lin, H.J., Liao, S.Y., Gim, J.S., Ma, H.L., Butler, J. & Anton-Culver, H. (2000) Breast cancer, heterocyclic aromatic amines from meat and N-acetyltransferase 2 genotype *Carcinogenesis*, **21**, 607–615

Felton, J.S. & Knize, M.G. (1990) New mutagens from cooked food *Prog. Clin. Biol. Res.*, **347**, 19–38

Ghoshal, A., Preisegger, K.H., Takayama, S., Thorgeirsson, S.S. & Snyderwine, E.G. (1994) Induction of mammary tumours in female Sprague-Dawley rats by the food-derived carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and effect of dietary fat. *Carcinogenesis*, **15**, 2429–2433

Ito, N., Hasegawa, R., Sano, M., Tamano, S., Esumi, H., Takayama, S., Tamano, S., Esumi, H., Takayama, S. & Sugimura, T. (1991) A new colon and mammary carcinogen in cooked food, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). *Carcinogenesis*, **12**, 1503–1506

Gerhardsson De Verdier, M., Hagman, U., Peters, R.K., Steineck, G. & Overvik, E. (1991) Meat, cooking methods and colorectal cancer: a case-referent study in Stockholm. *Int. J. Cancer*, **49**, 520–525

Knekt, P., Steineck, G., Jarvinen, R., Hakulinen, T. & Aromaa, A. (1994) Intake of fried meat and risk of cancer: a follow-up study in Finland. *Int. J. Cancer*, **59**, 756–760

Knize, M.G., Sinha, R., Rothman, N., Brown, E.D., Salmon, C.P., Levander, O.A., Cunningham, P.L. & Felton, J.S. (1995) Fast-food meat products have relatively low heterocyclic amine content. *Food Chem. Toxicol.*, **33**, 545–551

Knize, M.G., Sinha, R., Salmon, C.P., Mehta, S.S., Dewhirst, K.P. & Felton, J.S. (1996) Formation of heterocyclic amine mutagen/carcinogens during cooking of muscle meat. *J. Muscle Food*, **35**, 433–441

Muscat, J.E. & Wynder, E.L. (1994) The consumption of well-done red meat and the risk of colorectal cancer. *Am. J. Public Health*, **84**, 856–858

Ohgaki, H., Hasegawa, H., Kato, T., Suenaga, M., Sato, S., Takayama, S. & Sugimura, T. (1985) Carcinogenicities in mice and rats of IQ, MeIQ, and MeIQx. *Princess Takamatsu Symp.*, **16**, 97–105

Ronco, A., De Stefani, E., Mendilaharsu, M. & Deneo-Pellegrini, H. (1996) Meat, fat and risk of breast cancer: a case–control study from Uruguay *Int. J. Cancer*, **65**, 328–331

Schiffman, M.H., Van Tassell, R. & Andrews, A.W. (1990) Epidemiologic studies of fecal mutagenicity, cooked meat ingestion, and risk of colorectal cancer. *Prog. Clin. Biol. Res.*, **340**, 205–214

Sinha, R., Rothman, N., Brown, E.D., Salmon, C.P., Knize, M.G., Swanson, C.A., Rossi, S.C., Mark, S.D., Levander, O.A. & Felton J.S. (1995a) High concentrations of the carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) occur in chicken but are dependent on the cooking method. *Cancer Res.*, **55**, 4516–4519

Sinha, R., Rothman, N., Mark, S.D., Murray, S., Brown, E.D., Levander, O.A., Davies, D.S., Lang, N.P., Kadlubar, F.F. & Hoover, R.N. (1995b) Lower levels of urinary 2-amino-3,8-dimethylimidazo[4,5-f]-quinoxaline (MeIQx) in humans with higher CYP1A2 activity. *Carcinogenesis*, **16**, 2859–2861

Sinha, R. & Rothman, N. (1997) Exposure assessment of heterocyclic amines (HCAs) in epidemiologic studies. *Mutat. Res.*, **376**, 195–202

Sinha, R., Knize, M.G., Salmon, C.P., Brown, E.D., Rhodes, D., Felton, J.S., Levander, O.A. & Rothman, N. (1998a) Heterocyclic amine content of pork products cooked by different methods and to varying degrees of doneness *Food Chem. Toxicol.*, **36**, 289–297

Sinha, R., Rothman, N., Salmon, C.P., Knize, M.G., Brown, E.D., Swanson, C.A., Rhodes, D., Rossi, S., Felton, J.S. & Levander, O.A. (1998b) Heterocyclic aromatic amine content of beef cooked by different methods and degrees of doneness and beef gravy made from roast. *Food Chem. Toxicol.*, **36**, 279–287

Sinha, R., Kulldorff, M., Curtin, J., Brown, C.C., Alavanja, M.C.R. & Swanson, C.A. (1998c) Fried, well-done red meat and risk of lung cancer in women (United States). *Cancer Causes Control*, **9**, 621–630

Sinha, R., Gustafson, D.R., Kulldorff, M., Wen, W.Q., Cerhan, J.R. & Zheng, W.

(2000a) 2-Amino-1-methyl-6-phenylimidazo [4,5-b]pyridine, a carcinogen in high-temperature cooked meat and breast cancer *J Natl Cancer Inst*, **92**, 1352–1354

Sinha, R., Kulldorff, M., Swanson, C.A., Curtin, J., Brownson, R.C. & Alavanja, M.C.R. (2000b) Dietary heterocyclic amines and the risk of lung cancer among Missouri women. *Cancer Res.*, **60**, 3753–3756

Sinha, R., Kulldorff, M., Chow, W.H., Denobile, J. & Rothman, N., (2001) Dietary intake of heterocyclic amines, meat derived mutagenic activity, and risk of colorectal adenomas *Cancer Epidemiol. Biomarkers Prev.* **10**, 559–562

Skog, K. (1993) Cooking procedures and food mutagens: a literature review *Food Chem Toxicol.*, **31**, 655–675

Steineck, G., Gerhardsson De Verdier, M. & Overvik, E. (1993) The epidemiological evidence concerning intake of mutagenic activity from the fried surface and the risk of cancer cannot justify preventive measures *Eur. J. Cancer Prev.*, **2**, 293–300

Stillwell, W.G., Kidd, L.C., Wishnok, J.S., Tannenbaum, S.R. & Sinha, R. (1997) Urinary excretion of unmetabolized and phase II conjugates of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in humans: relationship to cytochrome P4501A2 and N-acetyltransferase activity. *Cancer Res.*, **57**, 3457–3464

Stillwell, W.G., Turesky, R.J., Sinha, R., Skipper, P.L., & Tannenbaum, S.R. (1999a) Biomonitoring of heterocyclic aromatic amine metabolites in human urine. *Cancer Lett.*, **143**, 145–148

Stillwell, W.G., Turesky, R.J., Sinha, R. & Tannenbaum, S.R. (1999b) N-oxidative metabolism of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) in humans: excretion of the N2-glucuronide conjugate of 2-hydroxyamino-MeIQx in urine. *Cancer Res.*, **59**, 5154–5159

Ward, M.H., Sinha, R., Heineman, E.F., Rothman, N., Markin, R., Weisenburger, D.D., Rothman, N., Markin, R., Weisenburger, D.D., Correa, P. & Zahm, S.H. (1997) Risk of adenocarcinoma of the stomach and esophagus with meat cooking method and doneness preference. *Int J. Cancer*, **71**, 14–19

World Cancer Research Fund/American Institute for Cancer Research. (1997) *Food, Nutrition and the Prevention of Cancer: A Global Perspective* Washington, D.C., World Cancer Research Fund/American Institute for Cancer Research

Zheng, W., Gustafson, D.R., Sinha, R., Cerhan, J.R., Moore, D., Hong, C.P., Anderson, K.E., Kushi, L.H., Sellers, T.A. & Folsom, A.R. (1998) Well-done meat intake and the risk of breast cancer *J. Natl Cancer Inst.*, **90**, 1724–1729

CHAPITRE III. LA CONSOMMATION DE VIANDE ET DU POISSON DANS LES POPULATION DE L'ETUDE PROSPECTIVE EUROPEENNE SUR LE CANCER ET LA NUTRITION (EPIC) .

1. **L'Etude Prospective Européenne sur le Cancer et la Nutrition (EPIC) : population de l'étude et méthodes de collection de données.** *European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr. 2002; 5:1113-24*

2. **Consommation de viande parmi les populations de l'Etude Prospective Européenne sur le Cancer et la Nutrition (EPIC) : résultats des rappels alimentaires de 24 heures.** *Meat consumption in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts: results from 24-hour dietary recalls. Public Health Nutr. 2002; 5 :1243-58*

3. **Méthodes de cuisson de la viande et du poisson dans des populations de l'Europe-résultats de l'Etude Prospective Européenne sur la Nutrition et le Cancer (EPIC).** *Cooking of meat and fish in Europe-results from the European Prospective Investigation into Cancer and Nutrition (EPIC). Eur J Clin Nutr. 2002 ; 56 :1216-30*

1. L'Etude Prospective Européenne sur le Cancer et la Nutrition (EPIC) : population de l'étude et méthodes de collection de données.

Basé sur:

European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr. 2002; 5:1113-2.

L'Etude Prospective Européenne sur le Cancer et la Nutrition est une étude prospective de cohorte multicentrique, qui a pour objectif d'étudier la relation entre la nutrition et le cancer, aussi bien que d'autres maladies. Lancée en 1992, cette étude est le fruit de la collaboration de chercheurs de vingt-quatre centres de recherche dans dix pays européens : le Danemark, la France, l'Allemagne, la Grèce, l'Italie, les Pays-Bas, la Norvège, l'Espagne, la Suède et le Royaume Uni. EPIC regroupe 519 978 participants (366 521 femmes et 153 457 hommes), la plupart dans une tranche d'âge de 35 à 70 ans. Le recrutement des participants a eu lieu entre 1992 et 2000 (Tableaux 1 et 2).

L'éligibilité des participants à la cohorte a été basée sur des critères géographiques ou administratifs et les populations de chaque cohorte sont des échantillons de convenance des sujets ayant accepté de participer. Dans la majorité des centres, les participants appartiennent à la population générale d'adultes résidants dans une ville donnée ou un secteur géographique, mais pour certains centres, des spécificités existent : la cohorte française est basée sur les membres de l'assurance maladie pour les enseignants (Mutuelle Générale de l'Education Nationale –MGEN-), les cohortes italiennes et espagnoles incluent des membres des associations locales des donneurs du sang, les cohortes à Utrecht (Pays-Bas) et à Florence (Italie) sont composées de femmes recrutées par des programmes de dépistage du cancer du sein. A Oxford, la moitié de la population a été recrutée parmi des associations des

végétariens et végétaliens. En France, en Norvège, à Utrecht (Pays-Bas) et à Naples, seulement des femmes ont été recrutées.

Pour l'identification de cas incidents de cancer, les sujets sont suivis tout au long de l'étude à travers des registres du cancer dans sept des pays participants : la Danemark, la Pays-Bas, l'Espagne, la Norvège, la Suède et le Royaume Uni. Dans les trois pays restants, la France, l'Allemagne et la Grèce plusieurs méthodes ont été combinées, telles que l'utilisation de registres d'assurance maladie, registres de pathologie et courriers aux participants ou à leurs proches.

Au moment de leur recrutement, des données sur le régime alimentaire habituel, le tabagisme, la consommation d'alcool, l'activité physique, la vie reproductive, les antécédents de maladie et d'autres caractéristiques des participants ont été obtenus par questionnaire (Tableaux 3 et 4). Les questionnaires étaient auto-administrés dans la majorité des centres mais dans certains, l'information a été recueillie lors d'un entretien par un enquêteur. Les participants ont été invités aux centres pour la prise des mesures anthropométriques, de tension artérielle et de sang. Le sang a été séparé en fractions de plasma, sérum, globules blancs et globules rouges et stockés dans l'azote liquide (-198 ºC) pour de futures analyses (Tableau 5).

Les questionnaires alimentaires ont été développés et validés pour tenir en compte des particularités régionales des habitudes alimentaires. Des questionnaires détaillés contenants jusqu'à 260 items avec estimation individuelle de la portion ont été auto-administrés dans les centres au nord de l'Italie, aux Pays-Bas et en Allemagne et appliqués par des enquêteurs en Grèce. Des questionnaires semblables, mais structurés par repas ont été utilisés en Espagne et à Ragusa (Italie) par des enquêteurs, et par auto-administration en France. Au Danemark, en Norvège, à Naples (Italie) et à Umeå en Suède, les questionnaires utilisés étaient de type sémi-quantitatifs (avec une taille de la portion standard pour tous les individus). Les deux centres britanniques ont appliqué un questionnaire sémi-quantitatif et un journal de

consommation sur sept jours. A Malmö (Suède), un journal de consommation de repas chaud sur quatorze jours et un questionnaire non-quantitatif ont été utilisés.

Les questionnaires de fréquence alimentaire spécifiques à chaque centre permettent de classer les sujets par rapport à leur apport alimentaire à l'intérieur de chaque centre, mais ces valeurs ne sont pas strictement comparables entre les centres. Pour cette raison, une deuxième mesure de l'alimentation consistant en un rappel de 24 heures a été appliquée sur un échantillon de 5 à 12% des sujets de chaque centre. Cette deuxième mesure, à l'aide d'un logiciel développé spécifiquement à cet effet (EPIC-Soft) a été utilisée comme méthode de référence pour calibrer les données des questionnaires alimentaires entre les centres. De plus, un tableau standardisé de composition des aliments a été utilisé pour la standardisation du calcul de nutriments.

Tous les individus qui ont accepté de participer à EPIC ont donné leur consentement écrit de participation. L'étude a été approuvée par les Comités d'Ethiques de tous les centres participants. L'étude EPIC a été financée par la Communauté Européenne et des associations régionales et nationales des pays participants.

EPIC est la plus grande étude en cours dans le monde pour la recherche prospective du cancer et d'autres maladies qui a collecté des échantillons de sang, ainsi que des données sur les habitudes alimentaire et le mode de vie de participants. Grâce à sa taille et l'hétérogénéité de sa population d'étude, EPIC a la puissance statistique nécessaire pour permettre des analyses selon localisation cancéreuse, le type histologique des tumeurs, le stade tumoral et les caractéristiques de la population, ainsi que pour étudier les interactions entre facteurs alimentaires, de mode de vie, métaboliques et génétiques. Aussi, EPIC a requis de gros efforts pour garantir la qualité et la comparabilité des données, des autres mesures et des échantillons biologiques.

Public Health Nutrition: 5(6B), 1113–1124 DOI: 10.1079/PHN2002394

European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection

E Riboli[1,*], KJ Hunt[2], N Slimani[1], P Ferrari[1], T Norat[1], M Fahey[1], UR Charrondière[1], B Hémon[1], C Casagrande[1], J Vignat[1], K Overvad[3], A Tjønneland[4], F Clavel-Chapelon[5], A Thiébaut[5], J Wahrendorf[6], H Boeing[7], D Trichopoulos[8,9], A Trichopoulou[8], P Vineis[10], D Palli[11], HB Bueno-de-Mesquita[12], PHM Peeters[13], E Lund[14], D Engeset[14], CA González[15], A Barricarte[16], G Berglund[17], G Hallmans[18], NE Day[19], TJ Key[20], R Kaaks[21] and R Saracci[1,22]

[1]Unit of Nutrition and Cancer, International Agency for Research on Cancer (IARC–WHO), 150 cours Albert-Thomas, 69372 Lyon Cedex 08, France: [2]Division of Clinical Epidemiology, University of Texas Health Science Center, San Antonio, TX, USA: [3]Department of Epidemiology and Social Medicine, University of Aarhus, Denmark: [4]Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark: [5]INSERM, U521, Institute Gustave Roussy, Villejuif, France: [6]German Cancer Research Centre, Heidelberg, Germany: [7]Department of Epidemiology, German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany: [8]Department of Hygiene and Epidemiology, School of Medicine, University of Athens, Greece: [9]Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA: [10]Department of Biomedical Sciences and Human Oncology, University of Turin, Italy: [11]Molecular & Nutrition Epidemiology Unit, CSPO, Scientific Institute of Tuscany, Florence, Italy: [12]Department of Epidemiology, National Institute of Public Health and the Environment, Bilthoven, The Netherlands: [13]Julius Center for General Practice and Patient Oriented Research, University of Utrecht, The Netherlands: [14]Institute of Community Medicine, University of Tromsø, Norway: [15]Department of Epidemiology, Catalan Institute of Oncology, Barcelona, Spain: [16]Service of Surveillance and Epidemiological Control, Institute of Public Health of Navarra, Pamplona, Spain: [17]Department of Medicine, Lund University, Malmö University Hospital, Sweden: [18]Public Health and Clinical Medicine, University Hospital of Northern Sweden, Umeå, Sweden: [19]Department of Public Health and Primary Care, School of Clinical Medicine, University of Cambridge, UK: [20]Cancer Research UK, Epidemiology Unit, University of Oxford, UK: [21]Hormones and Cancer Group, IARC–WHO, Lyon, France: [22]Division of Epidemiology, IFC National Research Council, Pisa, Italy

Abstract

The European Prospective Investigation into Cancer and Nutrition (EPIC) is an ongoing multi-centre prospective cohort study designed to investigate the relationship between nutrition and cancer, with the potential for studying other diseases as well. The study currently includes 519 978 participants (366 521 women and 153 457 men, mostly aged 35–70 years) in 23 centres located in 10 European countries, to be followed for cancer incidence and cause-specific mortality for several decades. At enrolment, which took place between 1992 and 2000 at each of the different centres, information was collected through a non-dietary questionnaire on lifestyle variables and through a dietary questionnaire addressing usual diet. Anthropometric measurements were performed and blood samples taken, from which plasma, serum, red cells and buffy coat fractions were separated and aliquoted for long-term storage, mostly in liquid nitrogen. To calibrate dietary measurements, a standardised, computer-assisted 24-hour dietary recall was implemented at each centre on stratified random samples of the participants, for a total of 36 900 subjects. EPIC represents the largest single resource available today world-wide for prospective investigations on the aetiology of cancers (and other diseases) that can integrate questionnaire data on lifestyle and diet, biomarkers of diet and of endogenous metabolism (e.g. hormones and growth factors) and genetic polymorphisms. First results of case–control studies nested within the cohort are expected early in 2003. The present paper provides a description of the EPIC study, with the aim of simplifying reference to it in future papers reporting substantive or methodological studies carried out in the EPIC cohort.

Keywords
Nutrition
Cancer
Chronic diseases
Cohort study
Anthropometry
Biological samples
EPIC study
Europe

*Corresponding author: Email ntr@iarc.fr

The existence of a relationship between nutrition and cancer was first shown clearly in the 1940s in a series of experimental studies in which severe energy restriction markedly reduced the occurrence of cancers in mice[1]. In the 1960s, following the development of cancer registries, ecological studies drew attention to the large world-wide variations in cancer incidence, and provided first suggestions that these variations might be related to differences in lifestyle, particularly diet[2–4]. In the 1970s and 1980s, a large number of traditional case–control studies were conducted to identify dietary risk factors with greater specificity, and since the late 1980s these studies have been followed by a series of prospective cohort studies[5].

In spite of several decades of research, comparatively few nutrition-related factors have been established unequivocally as playing a causal role in human cancer occurrence. These established factors include obesity and alcohol consumption[5]. In fact, epidemiological studies on nutrition and cancer have faced several methodological problems. Dietary habits are difficult to assess accurately and dietary exposures relevant to the aetiology of today's cancer incidence or mortality may have occurred over many years. Food patterns and specific food components, macro- and micronutrients, may all play aetiological roles and their effects may also be modified by other lifestyle factors such as physical activity or childbearing patterns. Case–control studies may be flawed by differential bias between cases and controls in the recall of dietary habits, and case–control studies that use biomarkers of diet or metabolism may also be flawed because the markers may be altered by the presence or diagnosis of a tumour. In principle, prospective cohort studies are not subject to these two major forms of bias. But, unless they are very large in size, they are inadequate to generate informative data on the aetiology of less common forms of cancer, such as those of the oesophagus, gall bladder, thyroid gland, ovary or endometrium. Even for the common forms of cancer, such as those of the lung, colon–rectum, breast, prostate and stomach, prospective cohort studies may prove less than adequate as soon as the aetiological investigation focuses on sub-types characterised by localisation, histology or other biological traits.

Finally, a drawback of prospective studies has been the fact that so far they have often been conducted within populations with relatively homogeneous lifestyles and dietary patterns. This homogeneity, combined with relatively large errors in dietary intake assessments, may make it very difficult to demonstrate moderate associations of specific aspects of diet with cancer risk.

In an attempt to overcome these various limitations, the International Agency for Research on Cancer (IARC) initiated the European Prospective Investigation into Cancer and Nutrition (EPIC) – a multi-centre prospective cohort study in Western Europe[6–10]. The study has been supported from its beginning by the Europe Against Cancer programme of the European Union. Initiated in 1992, this study has grown progressively into a collaborative endeavour between 23 centres in 10 European countries. The principal aim of EPIC is to investigate, in a prospective manner, the aetiology of cancers at various sites (as well as other forms of chronic disease) in relation to diet and lifestyle, taking advantage both of the contrast in cancer rates and dietary habits between centres and countries and of the large overall size of the study, which makes it possible to explore interactions between nutritional, genetic, hormonal and lifestyle factors.

Enrolment of the EPIC cohort participants and collection of baseline questionnaire data, anthropometric measurements and blood samples have now been completed for all countries. By May 2002, the follow-up for cancer incidence had already led to the identification of large numbers (1000–4500 cases) of subjects who developed cancer after cohort enrolment at one of the major sites (lung, colon–rectum, prostate and breast). This represents a total of about 16 000 incident cases. In previous reports, the rationale for the EPIC study and its future perspectives have been discussed[6,9,10]. In the present paper, we describe in some detail the individual EPIC study cohorts and their source populations, as well as the baseline information and biological samples collected from the participants of each cohort.

Methods

Outline

EPIC is an ongoing multi-centre prospective cohort study. The prospective cohort approach includes the collection of baseline questionnaire and interview data on diet and non-dietary variables, as well as anthropometric measurements and blood samples for long-term storage from apparently healthy populations. The cohort participants are followed over time for the occurrence of cancer and other diseases, as well as for overall mortality, to allow incidence and mortality comparisons by exposure variables. At regular intervals, follow-up questionnaires are used to update information on selected aspects of lifestyle that are known or strongly suspected to be related to cancer risk and that may have changed over time. The EPIC study has recruited 519 978 participants, in 23 centres located in 10 European countries. The study started with 17 research centres in seven core EPIC countries (France, Germany, Greece, Italy, The Netherlands, Spain and the UK). Subsequently, these were joined by centres in three Scandinavian countries (Sweden, Denmark and Norway) and one centre in Italy (Naples) that were conducting broadly similar prospective studies. The enrolment of subjects included in all EPIC centres took place between 1992 and 2000.

Source populations, invitation and study logistics

Participant eligibility within each cohort was based essentially on geographic or administrative boundaries. The source populations were identified according to age, gender and, optionally, other criteria (Table 1)[11–17]. The age range was generally from 35 to 70 years (Table 1). The actual study populations are samples of convenience of volunteers agreeing to participate, but not required to be random samples of defined populations; moreover, only some of the centres have maintained records of all the individuals invited to participate. As shown in Table 1, in the majority of study centres, subjects were invited from the general adult population residing in a given town or geographical area. There were, however, exceptions to this recruitment scheme. The French cohort was based on members of the health insurance for teachers (with the aim of facilitating follow-up for incidence of cancer and other diseases); components of the Italian and Spanish cohorts included members of local blood donor associations; the cohorts in Utrecht (The Netherlands) and Florence (Italy) included women invited for a local population-based breast cancer screening programme. In Oxford (UK) half of the cohort was recruited among subjects who did not eat meat, including vegans (who consume no animal products), lacto-ovo vegetarians and fish eaters (i.e. consumers of fish but not meat). In France, Norway, Utrecht (The Netherlands) and Naples (Italy) only women were recruited.

Centre-specific information on geographical/political area, source population, eligibility criteria and enumeration of invited participants are shown in Table 1, while Table 2 provides centre- and gender-specific information on study population size, enrolment dates and participant age at enrolment.

As a rule, participants were invited to participate either by mail or in person (Table 1). Individuals who agreed to participate signed an informed consent agreement and were mailed a questionnaire on diet and a questionnaire on lifestyle. Most participants completed these questionnaires at home and were then invited to a study centre for an examination. This included collection of the two completed questionnaires, venepuncture, anthropometry and measurement of blood pressure. For the blood pressure measurements, uniform procedures were recommended but no standard method or common type of instrument was introduced[18]. Among the seven initial EPIC countries, the centres in Italy (except Ragusa), the UK (except Oxford), The Netherlands and Germany followed these procedures. In France, a study that started in 1990 included lifestyle questions with self-reported anthropometry measurements; the participants enrolled in EPIC are those who answered the dietary questionnaire. A subset of the French cohort (20 725 women close to a metropolitan area) later came to a field centre, donated a blood sample, and underwent blood pressure and anthropometry measurements. In Spain and Ragusa (Italy), the recruited participants received the non-dietary questionnaire by mail, and were invited to a study centre for an examination that included collection of the lifestyle questionnaire, venepuncture, anthropometry and blood pressure measurements (in Spain only in sub-sample of the cohort), as well as an interviewer-administered computer-driven dietary questionnaire. Finally, in Greece, participants were initially invited by mail, sent a questionnaire, and asked to come for an examination at a study centre; however, recruitment numbers were so low that active recruitment was initiated. In contrast to most other EPIC centres, actively recruited Greek participants had their EPIC study centre examination at enrolment and all completed an interviewer-administered questionnaire on diet and a questionnaire on lifestyle. In Denmark and Malmö (Sweden), the participants filled in dietary questionnaires at home and lifestyle questionnaires at the study centres. In Umeå (Sweden), both questionnaires were completed at the study centre. In Norway, participants completed an initial mailed questionnaire unrelated to EPIC, completed a subsequent mailed questionnaire for EPIC, and then had blood samples mailed to the study centre in Tromsø for processing.

Dietary intake assessments

Dietary intake was assessed by a number of different instruments that had been developed and validated previously in a series of studies within the various source populations participating in EPIC (Table 3)[19–23]. Following the results of the methodological studies and taking into account the local context, three dietary assessment methods were adopted:

1. Extensive self-administrated quantitative dietary questionnaires, containing up to 260 food items and estimating individual average portions systematically, were used in northern Italy, The Netherlands, Germany and Greece (where dietary questionnaires were interviewer-administered). Questionnaires, similar in content to the self-administered quantitative dietary questionnaires but structured by meals, were used in Spain, France and Ragusa (south Italy). To increase compliance, the centres in Spain and Ragusa performed a face-to-face dietary interview using a computerised dietary program, whereas the dietary questionnaire was self-reported in France.
2. Semi-quantitative food-frequency questionnaires (with the same standard portion(s) assigned to all subjects) were used in Denmark, Norway, Naples in Italy and Umeå in Sweden.
3. Combined dietary methods were used in the UK and Malmö (Sweden). The two British centres used both a semi-quantitative food-frequency questionnaire and a 7-day record, whereas a method combining a short non-quantitative food-frequency questionnaire with a 14-day record on hot meals (lunches and dinners) was developed in Malmö.

Table 1 Source populations, eligibility criteria and recruitment procedures of the cohorts: the European Prospective Investigation into Cancer and Nutrition (EPIC) study

Country	Geographic/political area (centre)	Source population* (description)	Target eligibility criteria	Initial contact	Enumeration of invited
Core initial EPIC cohorts					
Greece	Greece: nation-wide	Active recruitment of the general population	Apparently healthy men and women aged 25–82	In person and by mail	No
Spain	Granada: province	Blood donors, general population (recruited through census, health centres)	Residents: men aged 40–64, women aged 35–64	In person and by mail	No
	Murcia: region	Blood donors and their partners (67% of cohort), general population of two towns (23%), civil servants (5%), employees of two companies (3%), participants in a cardiovascular risk study (2%)	Residents: men aged 40–65, women aged 35–65	In person and by mail	No
	Navarra: Pamplona city and Navarra region	Blood donors, general population	Residents: men aged 40–65, women aged 35–65	Mail	Yes
	San Sebastian: city and Gipuzkoa province	Blood donors, employees of selected enterprises (recruited through census of selected municipalities)	Residents: men aged 40–65, women aged 35–65	In person and by mail	Yes
	Asturias: region	Blood donors, regional civil servants and general population	Men aged 40–64, women aged 35–64	Mail	Yes
Italy	Ragusa: province	Local blood donors association, population-based recruitment in four towns (Monterosso, Giarratana, Ispica and Chiaramonte), local teachers union, and other sources	Residents: men aged 40–65, women aged 35–65	Mail	Yes
	Florence: province	Breast cancer screening participants (CSPO), men and women from the general population	Residents: men aged 35–64, women aged 35–64, without prevalent cancer	In person and by mail	No
	Turin: city	Blood donors, employees, volunteers, medical test users at national health service	Residents: men aged 40–74, women aged 35–74, without prevalent cancer	In person	No
	Varese: province	Volunteers from resident general population, mostly an extension of an ongoing study (ORDET)	Men aged 40–65, women aged 35–65	In person and by mail	No
France	France	Nation-wide health insurance programme (MGEN): teachers and school workers enrolled in an ongoing study prior to EPIC	Women aged 40–65 in 1990 with informed consent to obtain MGEN info on non-respondents	Mail	Yes

Germany	Heidelberg and surrounding areas	General population	Residents: men aged 40–65, women aged 35–65, completed questionnaires and examination	Mail	Yes
	Potsdam and surrounding areas	General population	Residents: men aged 40–65, women aged 35–65, completed questionnaires and examination	Mail	Yes
Netherlands	Bilthoven: Amsterdam, Doetinchem and Maastricht (three cities)	Population-based age- and sex-stratified samples of the general population	Residents: men and women aged 20–60 in Amsterdam and Maastricht, and aged 20–65 in Doetinchem	Mail	Yes
	Utrecht: district	Population-based breast cancer screening participants	Residents: women aged 49–70	Mail	Yes
United Kingdom	Cambridge: Norfolk	Population-based patients of general practitioners	Listed by general practitioners: men and women aged 45–74	Mail	Yes
	Oxford: (1) local counties; (2) 'health-conscious' from England, Wales, Scotland and Northern Ireland	(1) Population based in collaboration with general practitioners; (2) vegetarians, vegans and other health-conscious individuals in collaboration with vegetarian societies and magazines	(1) Listed by general practitioners: men and women aged 40–65; (2) men and women aged 20+, but targeted at those aged 35+	Mail	No
Associated EPIC cohorts					
Italy	Naples	Female volunteers from resident general population	Women aged 30–69	In person and by mail	No
Denmark	Aarhus	Population-based	Born in Denmark: men and women aged 50–64, without prevalent cancer	Mail	Yes
	Copenhagen	Population-based	Born in Denmark: men and women aged 50–64, without prevalent cancer	Mail	Yes
Sweden	Malmö: city	Population-based	Residents: men aged 50–72, women aged 46–72	Mail	Yes
	Umeå: the Västerbotten county	Population-based	Residents: men and women aged 30, 40, 50 or 60	Mail	Yes
Norway	Tromsø: national sample	Population-based	Women born in Norway between 1943 and 1957	Mail	Yes

*Under source population, the term 'population-based' implies that participants were invited as a random sample of their population, while the term 'general population' implies that volunteers were invited from the general population.

Table 2 Characteristics of the cohorts: the European Prospective Investigation into Cancer and Nutrition (EPIC) study

Country	Centre		*n**	Enrolment period (date)	Enrolment age (years) (1st–99th percentile)
Core initial EPIC cohorts					
Greece		Male	11 954	1994–1999	30–76
		Female	16 618	1994–1999	30–76
Spain	Granada	Male	1796	1992–1996	35–65
		Female	6083	1992–1996	35–65
	Murcia	Male	2685	1992–1996	38–65
		Female	5831	1992–1996	35–65
	Navarra	Male	3908	1992–1995	40–64
		Female	4176	1992–1995	35–64
	San Sebastian	Male	4158	1992–1995	40–65
		Female	4259	1992–1995	35–65
	Asturias	Male	3085	1992–1995	40–65
		Female	5459	1992–1995	35–65
Italy	Ragusa	Male	3053	1993–1997	37–65
		Female	3350	1993–1997	35–65
	Florence	Male	3514	1993–1998	35–65
		Female	10 083	1992–1998	35–65
	Turin	Male	6047	1993–1998	35–65
		Female	4557	1993–1998	35–65
	Varese	Male	2557	1995–1997	40–65
		Female	9526	1993–1997	35–72
France		Female	72 996	1993–1997	43–68
Germany	Heidelberg	Male	11 929	1994–1998	40–65
		Female	13 617	1994–1998	35–65
	Potsdam	Male	10 904	1994–1998	38–65
		Female	16 644	1994–1998	35–65
Netherlands	Bilthoven	Male	10 280	1993–1997	21–63
		Female	12 435	1993–1997	21–64
	Utrecht	Female	17 357	1993–1997	49–70
United Kingdom	Cambridge	Male	13 698	1993–1998	41–76
		Female	16 744	1993–1998	41–76
	Oxford	Male	13 214	1994–2000	22–83
		Female	44 284	1993–2000	21–79
Associated EPIC cohorts					
Italy	Naples	Female	5062	1993–1997	35–68
Denmark	Aarhus	Male	8433	1995–1997	50–65
		Female	8721	1995–1997	50–65
	Copenhagen	Male	18 746	1993–1997	50–65
		Female	21 154	1993–1997	50–65
Sweden	Malmö	Male	11 063	1991–1996	47–72
		Female	17 035	1991–1996	45–73
	Umeå	Male	12 433	1992–1996	30–60
		Female	13 299	1992–1996	30–60
Norway	Tromsø	Female	37 231	1998–1998	41–56

* By April 2002.

The EPIC study aims to increase the overall statistical power of identifying diet–disease relationships by combining study populations that have different types of diets and lifestyles and different cancer incidence rates, resulting in increased overall ranges of dietary exposures and cancer risks. Any global statistical analysis that takes account of the total range of dietary exposures of all sub-cohorts combined requires that the dietary assessments obtained in each of the sub-cohorts be comparable on an absolute scale. Such comparability, however, can be compromised by the use of different dietary assessment methods across the 23 EPIC centres. To overcome this problem, it was decided to collect additional dietary intake data by a computer-assisted 24-hour dietary recall (EPIC-SOFT) in representative sub-samples of 5–12% of study participants in each of the sub-cohorts (about 1.5% in the British cohorts). In total, 24-hour recalls were collected from 36 900 EPIC participants[24–26]. The baseline dietary assessments conducted on all EPIC participants, used locally to estimate long-term usual dietary intake, will be used to rank subjects within centres, while the 24-hour dietary recall can be used as a reference method to correct for systematic between-centre over- or underestimations in the baseline dietary assessments[27]. The calibration study, its rationale and its standardisation are described in detail elsewhere[25–31]. A common food composition database for a number of nutrients, standardised across the European countries involved in EPIC, is currently being developed[32,33].

Table 3 Baseline dietary assessment: the European Prospective Investigation into Cancer and Nutrition (EPIC) study

Location	Assessment tool(s) and its(their) structure	Administered	Number of items*
Core initial EPIC cohorts			
France	Quantitative dietary questionnaire structured by meals†	Self	210
Northern Italy‡	Quantitative dietary questionnaire structured by meal courses§	Self	236
Italy, Ragusa	Quantitative dietary questionnaire structured by meals, computer-driven†	Face-to-face	266
Spain	Quantitative dietary questionnaire structured by meals, computer-driven†	Face-to-face	736¶
United Kingdom	(1) Semi-quantitative FFQ‖; (2) 7-day records (diaries)	Self	170
Netherlands	Quantitative dietary questionnaire§	Self	213
Greece	Quantitative dietary questionnaire§	Face-to-face	260¶
Germany	Quantitative dietary questionnaire§	Self	254
Associated EPIC cohorts			
Sweden, Malmö	Combination of semi-quantitative FFQ‖ and 14-day record of hot meals	Self**	2443††
Sweden, Umeå	Semi-quantitative FFQ‖	Self	98
Denmark	Semi-quantitative FFQ‖	Self	173
Norway	Semi-quantitative FFQ‖	Self	88
Italy, Naples	Semi-quantitative FFQ‖	Face-to-face	158

FFQ – food-frequency questionnaire.
* Number of items is defined as the number of foods plus the number of standard mixed recipes.
† Questionnaire structured by main meals (breakfast, lunch, dinner, between-meal food consumption occasions) with meal-specific food frequency and portion.
‡ Florence, Turin and Varese.
§ Individual average portion sizes were estimated using series of photographs, standard units and/or household measurements.
¶ Open-ended sections in the questionnaire.
‖ The same standard portion(s) were assigned to all subjects. In Denmark, sex-specific mean portions were used to quantify standard mixed recipes.
** Self-reported during the main examination at the centre, and checked immediately by the interviewer.
†† Essentially open-ended dietary assessment method.

Questionnaire data on non-dietary variables

Apart from diet, questionnaire data were collected on a large number of lifestyle and health factors that are of interest in studies on nutrition and cancer, as they may be related to nutritional status or may be known or suspected cancer risk factors. For the seven initial EPIC countries, a common set of questions and possible answers was agreed upon and translated into national questionnaires. This included questions on education and socio-economic status; current job, current and past occupation which might have led to exposure to carcinogens; history of previous illness, disorders or surgical operations; lifetime history of tobacco smoking; lifetime history of consumption of alcoholic beverages; physical activity (occupational, walking, cycling, gardening, housework, physical exercise, climbing stairs); menstrual and reproductive history; and use of exogenous hormones for contraception and postmenopausal replacement therapy (Table 4). In Denmark, Sweden and Norway and in the Naples centre in Italy, which joined EPIC at a later stage, questionnaires on non-dietary variables had been developed quite independently of those in the initial EPIC countries. Nevertheless, their questionnaires do cover to a large extent the same variables, even if these were not defined in exactly the same manner as for the rest of EPIC. A comprehensive re-coding scheme was developed for standardisation of the questionnaire variables from these study centres, to make the codes as close as possible to those of the core EPIC lifestyle questions.

Anthropometric measurements

In all EPIC centres, except France, the Oxford cohort and Norway, height, weight, and waist and hip circumference were measured on all subjects using similar protocols (in Umeå, only weight and height were measured). In addition, in Italy, Spain, Utrecht, Greece, Germany and Denmark, sitting height was measured. In France and Oxford, weight, height, waist and hip (and sitting height in France) were measured only for a restricted number of participants, but self-reported weight and height were obtained from all individuals. In Oxford, self-reported measurements also included waist and hip circumferences. In Norway only self-reported height and weight are available[34].

Biological samples

Biological samples including blood plasma, blood serum, white blood cells and erythrocytes were collected from 385 747 of the 519 978 EPIC study participants (Table 5). The procedure for storage of blood samples differed between the seven initial EPIC countries and the three Scandinavian countries that joined EPIC at a later stage.

In the former countries and in Naples (Italy), blood samples were aliquoted into 28 plastic straws containing 0.5 ml each (12 plasma with sodium citrate, eight serum, four erythrocytes, four buffy coat for DNA). To ensure a high degree of standardisation, the same materials (syringes, straws, etc.) were purchased centrally and distributed to the centres. The samples were then split into two mirror halves of 14 aliquots each. One set was stored

Table 4 Non-dietary information: the European Prospective Investigation into Cancer and Nutrition (EPIC) study

Anthropometry	All centres except Umeå (Sweden) and Tromsø (Norway) have either self-reported (France and part of the UK) or measured information on weight, height, hip circumference and waist circumference. In Umeå (Sweden) and Tromsø (Norway) information is available on weight and height only. Sitting height measurements were obtained in France, Italy, Spain, Utrecht, Greece, Germany and Denmark
Reproductive history	All core* centres (except Bilthoven, which has limited information) have detailed information including, but not limited to, information on menopausal status, pregnancies, miscarriages, induced abortion, infertility, and hormone use for both birth control and menopause. Of the associated participants, the Danish and Norwegian centres have complete information, the centre in Malmö (Sweden) has the majority of information, and the centre in Umeå (Sweden) has this information on about half of the cohort, which is now increasing via follow-up
Physical activity	All core* centres have information on type of physical activity at work, physical exercise to keep fit and vigorous physical activity, as well as time spent on specific activities including walking, cycling, gardening, housework, and number of stairs climbed per day. Of the associated participants, the Danish centres have complete information, the centre in Malmö (Sweden) has the majority of the information, and the centre in Umeå (Sweden) is limited to information on type of physical activity at work. The centres in Umeå and Tromsø have additional questions on physical activity, which are not completely adapted to the core questionnaire
Tobacco smoking	All centres have information on smoking status (current, past, never), as well as information on amount of cigarettes smoked. In addition, all centres (except those in The Netherlands and Norway) have information on current and past cigar and pipe smoking
Alcohol consumption	The core* centres have information on past amount of wine, beer/cider, fortified wine and spirit/liquor consumed. In addition, for Cambridge, Bilthoven and Greece, information on current levels of consumption for each of these types of alcohol is available as non-dietary variable. Of the associated participants, the Danish and Naples centres have complete information whereas the centres in Malmö (Sweden) and Norway have information on current alcohol consumption only. No information on past alcohol consumption is available in Umeå (Sweden). However, for all EPIC centres, additional information on current alcohol consumption is available from the dietary questionnaires
Occupational history	The centres in Italy, Spain, Cambridge, Greece, Germany and Denmark have information on occupational history. The Norwegian centre has information on current occupation
Socio-economic status	All centres have information on highest school level achieved
Previous illnesses	All centres have information on heart disease and diabetes, while the majority (both core* and associated participants) of centres have information on stroke, hypertension, hyperlipidaemia, gall stones, polyps of the large bowl, hysterectomy, oopherectomy and breast surgery, as well as information on age of onset of each of these events

*Core centres include centres in France, Italy (except Naples), Spain, UK, The Netherlands, Greece and Germany. The associated participants include centres in Sweden, Denmark, Norway and Naples (Italy).

locally, and one transported to IARC to be stored in liquid nitrogen (at −196°C) in a central biorepository.

In Norway the biological samples were collected in twenty 0.5 ml plastic straws; for 9197 subjects, 12 of the 16 plasma and two of the four buffy coat samples were shipped to IARC for storage in the central repository. In Sweden and Denmark, blood samples were stored in tubes (not in plastic straws) and for practical reasons are stored only in local repositories (the central EPIC repository at IARC is not suitable for storing tubes). In Sweden, the samples are kept in freezers at −70°C, and in Denmark in nitrogen vapour (−150°C).

The central biological bank located at IARC currently contains around 3.8 million straws with blood aliquots from 275 861 EPIC participants. The straws of each participant are stored together successively inside a tube, goblet, canister and container. The canisters are arranged in colour-coded concentric circles located in each of 33 liquid nitrogen containers. Each straw is labelled with the participant's ID and colour-coded to indicate its contents; in addition, the tube, goblet and canister are colour-coded to aid in identifying the samples. Finally, a computer software program indicates the container, canister, goblet, and the location of the goblet and the canister within each container to track the stored biological samples of each participant.

Follow-up for changes in lifestyle and health conditions

After their initial enrolment, cohort members are contacted at regular intervals every 3–4 years to obtain information on various aspects of lifestyle that are known or strongly suspected of being related to cancer risk, and that may change over time. This includes tobacco smoking, alcohol drinking, physical activity, weight, menstruation, pregnancies, menopause, and other variables. In addition, a series of questions was added on whether the subjects had suffered from any major diseases. In most EPIC centres, the first follow-up is currently ongoing and in several it has been completed.

Follow-up for cancer incidence and overall mortality

Follow-up aimed at identifying cancer cases occurring among the EPIC cohort is based on population cancer registries in seven of the participating countries (Denmark, Italy, The Netherlands, Norway, Spain, Sweden and the UK) and on a combination of methods including health insurance records, cancer and pathology registries, and on active follow-up through study subjects and their next-of-kin in three countries (France, Germany and Greece). A working group created in 1996 (End-Point Committee) prepared a detailed protocol for the collection and

Table 5 Biological samples: the European Prospective Investigation into Cancer and Nutrition (EPIC) study

Centre		*n*	Age range (years) (1st–99th percentile)	Female (%)	Achievement rate* (%)	Samples collected† (number of 0.5 ml straws desired)				Storage location	
						Plasma	Serum	White blood cells	Erythrocytes	IARC	Local
Core EPIC cohorts											
Greece	Nation-wide	28 500	29–76	58.2	99.8	12	8	4	4	Yes	Yes
Spain	Granada	6892	35–66	77.0	87.5	12	8	4	4	Yes	Yes
	Murcia	8146	35–65	68.7	95.7	12	8	4	4	Yes	Yes
	Navarra	7799	36–64	51.5	96.5	12	8	4	4	Yes	Yes
	San Sebastian	8325	36–65	50.6	98.9	12	8	4	4	Yes	Yes
	Asturias	8417	35–65	64.0	98.5	12	8	4	4	Yes	Yes
Italy	Ragusa	6396	35–65	52.3	99.9	12	8	4	4	Yes	Yes
	Florence	13 597	35–65	74.2	100.0	12	8	4	4	Yes	Yes
	Turin	10 604	35–64	43.0	100.0	12	8	4	4	Yes	Yes
	Varese	12 073	36–72	78.9	99.9	12	8	4	4	Yes	Yes
France		20 725	43–68	100.0	31.0‡	12	8	4	4	Yes	Yes
Germany	Heidelberg	24 235	36–64	52.6	94.9	12	8	4	4	Yes	Yes
	Potsdam	26 444	35–66	59.8	95.9	12	8	4	4	Yes	Yes
Netherlands	Bilthoven	19 388	21–64	54.0	93.1§	12	8	4	4	Yes	Yes
	Utrecht	16 930	49–69	100.0	96.9	12	8	4	4	Yes	Yes
United Kingdom	Cambridge	24 035	41–76	54.3	93.8¶	12	8	4	4	Yes	Yes
	Oxford	19 103	23–73	76.7	96.1‖	12	8	4	4	Yes	Yes
Associated EPIC cohorts											
Italy	Naples	5055	34–68	100.0	99.9	12	8	4	4	No	Yes
Denmark	Aarhus	17 094	50–65	50.8	99.7	T[b]	T[b]	T[b]	T[b]	No	Yes
	Copenhagen	39 037	50–65	52.7	97.8	T[b]	T[b]	T[b]	T[b]	No	Yes
Sweden	Malmö	28 023	46–73	60.6	99.7	T[a]	T[a]	T[a]	T[a]	No	Yes
	Umeå	25 732	30–61	51.7	100.0	T[a]	T[a]	T[a]	T[a]	No	Yes
Norway	Tromsø	9197	40–55	100.0	~60.0**	16	NC	4	NC	Yes	Yes

T[a] – stored in 2 ml tubes at −80°C; T[b] – stored in 1 ml tubes in nitrogen vapour at a temperature between −150°C and −160°C; NC – not collected.
* In all centres, except those in France, the UK, Bilthoven (Netherlands) and Norway, all EPIC participants were invited to donate blood (Table 2 contains the denominator used to calculate the achievement rate, which represents the percentage of participants with partial or complete stored biological samples out of those asked to donate blood).
† In the core centres, biological samples are distributed equally between IARC and local storage, and are stored in straws at −196°C.
‡ In France, 66 858 EPIC participants living near a metropolitan area were asked to give blood.
§ In Bilthoven, 13 451 EPIC participants recruited from Amsterdam or Doetinchem after 11 May 1993, and 7364 EPIC participants recruited from Maastricht after 2 June 1993, were asked to donate blood.
¶ In Cambridge (UK), 25 633 EPIC participants who attended a study exam were asked to give blood.
‖ In Oxford (UK), enrolment of the participants recruited by general practitioners from the local counties was based on a willingness to donate blood and the achievement rate is 96.1%; among the 'health-conscious' sub-cohort, 24.4% donated blood.
** In Norway, collection of biological samples is currently underway and will continue until samples have been collected from 12 000 participants.

standardisation of clinical and pathological data on each cancer site: *Guidelines for Collection of End-point Data in the EPIC Study* (IARC, 1998). In parallel, data on total and cause-specific mortality are collected at the EPIC study centres through mortality registries or active follow-up and death-record collection.

Storage, management and quality control of the EPIC database

The EPIC data are housed centrally at IARC in the EPIC ORACLE database. For practical reasons, 14 centres in the 10 participating countries act as co-ordinating centres that interact with IARC for centralisation of the EPIC data (in particular, all Spanish and Italian data are centralised in Barcelona and Milan, respectively). The database comprises individual EPIC data, as well as the computer software (ORACLE) and the programs that store, track and manage the database.

The EPIC core information concerning non-dietary lifestyle variables and anthropometry is stored in the EPIC ORACLE database, using the centre-specific variable names and formats as well as variable names and formats standardised across EPIC. Centre-specific data were loaded into the ORACLE system, and transformed into the standard EPIC variables on which logical and substantive quality control checks were then run. Figure 1 summarises the process.

For dietary data, a common format and classification system was proposed to enable centralised data management and a series of pooled analyses. The food items reported in each EPIC dietary questionnaire were classified in their respective food groups using the same system as that used to classify the food items reported in the EPIC-SOFT 24-hour dietary recalls (the so-called EPIC-SOFT food classification system)[35]. However, other classification criteria may be considered on an individual basis depending on the purposes of specific analyses. In addition, the frequency of consumption of each item, the number of portions consumed on each occasion and the (standard) portion sizes were also stored in the central

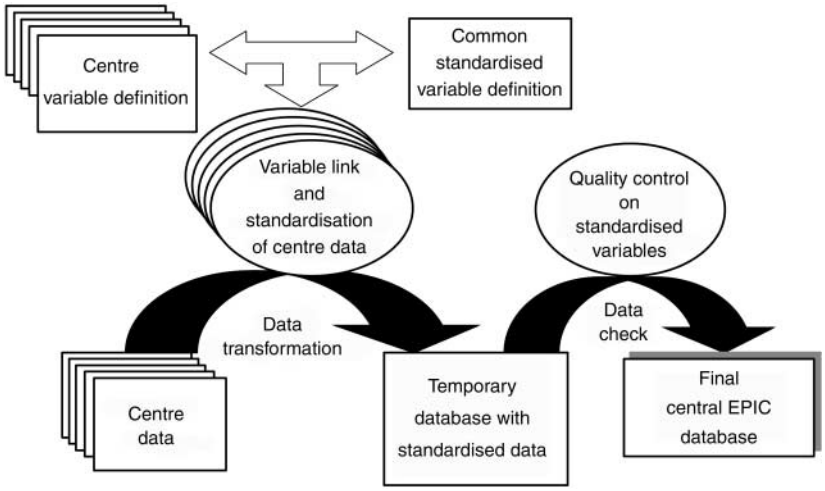


Fig. 1 Flow chart of lifestyle and personal data: the European Prospective Investigation into Cancer and Nutrition study

ORACLE database; hence, the total quantity of each food was calculated from this information as grams consumed per day. For the 24-hour dietary recalls, used as common reference calibration measurements, the same standardised software (i.e. EPIC-SOFT) methodology was used in all centres to collect and subsequently store, retrieve and export these data. The same format file was therefore used to load and store the 24-hour dietary recall data in the central EPIC database.

The storage, management and interrelationships between the various components of the EPIC dietary data are shown schematically in Fig. 2.

Personal identifying information, as available at local centres, is not transferred to the IARC co-ordinating centre. Informed consent was provided by each participant, and projects using the EPIC resource need to be cleared by both the IARC and local ethical review committees.

Concluding remarks

Approximately 10 years after its inception, the EPIC study baseline information and biological samples have been collected, centralised and, when applicable, standardised.

As a large prospective cohort with stored biological samples, EPIC is now starting to generate specific studies investigating cancer aetiology in relation to diet and lifestyle factors, and this will continue over the next 10 years and beyond. When biological samples are involved, these studies mostly use the nested case–control approach. In addition, information on vital status and cause of death can be used to address endpoints other than cancer, in particular cardiovascular diseases, as well as survival after cancer diagnosis.

The very magnitude of the individual and total cohorts, the related lengthy period of subject recruitment and the variety of local facilities have made it impossible to standardise all of the procedures strictly, as would be possible for smaller studies. However, considerable effort has been put into ensuring maximum comparability within and between cohorts, in particular where dietary information is concerned, by means of the large calibration sub-sample. The storage of biological samples

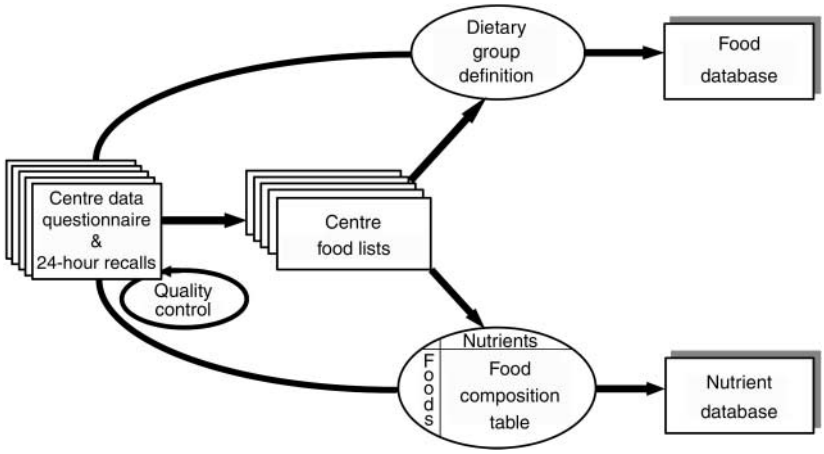


Fig. 2 Flow chart of dietary data: the European Prospective Investigation into Cancer and Nutrition study

in multiple aliquots in liquid nitrogen represents the best available technology for maintaining long-term stability.

A multi-centre cohort the size of EPIC offers the substantial advantage of enabling informative studies on common cancers (as well as other common causes of deaths) not only overall but also in specific subsets of the total population, within which aetiological factors may differ. Also, sufficient numbers will accrue to enable meaningful investigation of rarer cancers. Finally, the variations in disease rates, diet and lifestyles across the populations included in EPIC raise interesting methodological issues on the one hand and, on the other, the opportunity to capitalise simultaneously on the within- and between-centre variability to increase the capacity of the study to clarify the complex role of nutrition in the causation and prevention of cancer.

Acknowledgements

The work described in this paper was carried out with financial support of the 'Europe Against Cancer' Programme of the European Commision (SANCO); Ligue contre le Cancer (France); Société 3M (France); Mutuelle Générale de l'Education Nationale; Institut National de la Santé et de la Recherche Médicale (INSERM); Institute Gustave Roussy; German Cancer Aid; German Cancer Research Centre; German Federal Ministry of Education and Research; Danish Cancer Society; Health Research Fund (FIS) of the Spanish Ministry of Health; the Spanish Regional Governments of Andalucia, Asturias, Basque Country, Murcia and Navarra; Cancer Research UK; Medical Research Council, UK; Stroke Association, UK; British Heart Foundation; Department of Health, UK; Food Standards Agency, UK; Wellcome Trust, UK; Greek Ministry of Health; Greek Ministry of Education; Italian Association for Research on Cancer; Italian National Research Council; Dutch Ministry of Public Health, Welfare and Sports; Dutch Prevention Funds; LK Research Funds; Dutch ZON (Zorg Onderzoek Nederland); World Cancer Research Fund; Swedish Cancer Society; Swedish Scientific Council; Regional Government of Skane, Sweden; Norwegian Cancer Society; Norwegian Research Council. Partial support for the publication of this supplement was provided by the Centre de Recherche et d'Information Nutritionnelles (CERIN).

In addition, we wish to thank all study participants for their co-operation and all interviewers who participated in the fieldwork studies in each EPIC centre.

References

1 Tannenbaum A. Initiation and growth of tumors; introduction: effects of underfeeding. *Am. J. Cancer* 1940; **39**: 335–50.
2 Doll R, Payne P, Waterhouse J. *Cancer Incidence in Five Continents: A Technical Report*. Berlin: Springer, 1966.
3 Doll R, Payne P, Waterhouse J. *Cancer Incidence in Five Continents*. Berlin: Springer, 1970.
4 Armstrong B, Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. *Int. J. Cancer* 1975; **15**: 617–31.
5 World Cancer Research Fund/American Institute of Cancer Research (WCRF/AICR). *Food, Nutrition and the Prevention of Cancer: A Global Perspective*. Washington, DC: WCRF/AICR, 1997.
6 Riboli E. Nutrition and cancer: background and rationale of the European Prospective Investigation into Cancer and Nutrition (EPIC). *Ann. Oncol.* 1992; **3**: 783–91.
7 Riboli E. The European Prospective Investigation into Cancer and Nutrition: perspectives for cancer prevention. *Nestle Nutr. Workshop Ser. Clin. Perform. Programme* 2000; **4**: 117–30.
8 Riboli E. The European Prospective Investigation into Cancer and Nutrition (EPIC): plans and progress. *J. Nutr.* 2001; **131**: 170S–5S.
9 Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. *Int. J. Epidemiol.* 1997; **26**(Suppl. 1): S6–14.
10 Riboli E, Kaaks R. Invited commentary: the challenge of multi-center cohort studies in the search for diet and cancer links. *Am. J. Epidemiol.* 2000; **151**: 371–4.
11 Berglund G, Elmstahl S, Janzon L, Larsson SA. The Malmö Diet and Cancer Study. Design and feasibility. *J. Intern. Med.* 1993; **233**: 45–51.
12 Clavel-Chapelon F, van Liere MJ, Giubout C, Niravong MY, Goulard H, Le Corre C, *et al*. E3N, a French cohort study on cancer risk factors. E3N Group. Etude Epidémiologique auprès de femmes de l'Education Nationale. *Eur. J. Cancer Prev.* 1997; **6**: 473–8.
13 Hjartåker A, Lund E. Relationship between dietary habits, age, lifestyle, and socio-economic status among adult Norwegian women. The Norwegian Women and Cancer Study. *Eur. J. Clin. Nutr.* 1998; **52**: 565–72.
14 Boeing H, Wahrendorf J, Becker N. EPIC–Germany – a source for studies into diet and risk of chronic diseases. *Ann. Nutr. Metab.* 1999; **43**: 195–204.
15 Boeing H, Korfmann A, Bergmann MM. Recruitment procedures of EPIC–Germany. European Investigation into Cancer and Nutrition. *Ann. Nutr. Metab.* 1999; **43**: 205–15.
16 Day N, Oakes S, Luben R, Khaw KT, Bingham S, Welch A, *et al*. EPIC–Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. *Br. J. Cancer.* 1999; **80**(Suppl. 1): 95–103.
17 Keinan-Boker L, van Noord PAH, van der Schouw YT, Koot NVCM, Bueno-de-Mesquita HB, Riboli E, *et al*. Prospect–EPIC Utrecht: study design and characteristics of the cohort population. *Eur. J. Epidemiol.* 2002; in press.
18 Schulze MD, Kroke A, Saracci R, Boeing H. The effect of measurement procedure differences on the comparability of blood pressure estimates in multi-centre studies. *Blood Press. Monit.* 2000; **7**: 95–104.
19 Overvad K, Tjønneland A, Haraldsdóttir J, Bang S, Ewertz M, Møller-Jensen O. Development of a semi-quantitative food frequency questionnaire to assess food, energy and nutrient intake in Denmark. *Int. J. Epidemiol.* 1991; **20**: 906–12.
20 Bingham SA, Gill C, Welch A, Day K, Cassidy A, Khaw KT, *et al*. Comparison of dietary assessment methods in nutritional epidemiology: weighted records v. 24 h recalls, food-frequency questionnaires and estimated-diet records. *Br. J. Nutr.* 1994; **72**: 619–43.
21 Margetts BM, Pietinen P, Riboli E, eds. EPIC: European Prospective Investigation into Cancer and Nutrition: validity studies on dietary assessment methods [special issue]. *Int. J. Epidemiol.* 1997; **26**(Suppl. 1): S1–189.
22 Riboli E, Elmståhl S, Saracci R, Gullberg B, Lindgärde F. The

Malmö Food Study: validity of two dietary assessment methods for measuring nutrient intakes. *Int. J. Epidemiol.* 1997; **26**: S161–71.

23 Kroke A, Klipstein-Grobusch K, Voss S, Moseneder J, Thielecke F, Noack R, *et al*. Validation of a self-administered food-frequency questionnaire administered in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study: comparison of energy, protein, and macronutrient intakes estimated with the doubly labeled water, urinary nitrogen, and repeated 24-h dietary recall methods. *Am. J. Clin. Nutr.* 1999; **70**: 439–47.

24 Slimani N, Deharveng G, Charrondière RU, van Kappel AL, Ocké MC, Welch A, *et al*. Structure of the standardized computerized 24-h diet recall interview used as reference method in the 22 centers participating in the EPIC project. European Prospective Investigation into Cancer and Nutrition. *Comput. Meth. Programs Biomed.* 1999; **58**: 251–66.

25 Slimani N, Ferrari P, Ocké M, Welch A, Boeing H, Liere M, *et al*. Standardization of the 24-hour diet recall calibration method used in the European Prospective Investigation into Cancer and Nutrition (EPIC): general concepts and preliminary results. *Eur. J. Clin. Nutr.* 2000; **54**: 900–17.

26 Slimani N, Kaaks R, Ferrari P, Casagrande C, Clavel-Chapelon F, Lotze G, *et al*. European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study: rationale, design and population characteristics. *Public Health Nutr.* 2002; **5**(6B): 1125–45.

27 Kaaks R, Riboli E. Validation and calibration of dietary intake measurements in the EPIC project: methodological considerations. European Prospective Investigation into Cancer and Nutrition. *Int. J. Epidemiol.* 1997; **26**(Suppl. 1): S15–25.

28 Kaaks R, Plummer M, Riboli E, Esteve J, van Staveren W. Adjustment for bias due to errors in exposure assessments in multicenter cohort studies on diet and cancer: a calibration approach. *Am. J. Clin. Nutr.* 1994; **59**: 245S–50S.

29 Kaaks R, Riboli E, Esteve J, van Kappel AL, van Staveren WA. Estimating the accuracy of dietary questionnaire assessments: validation in terms of structural equation models. *Stat. Med.* 1994; **13**: 127–42.

30 Kaaks R, Riboli E, van Staveren W. Sample size requirements for calibration studies of dietary intake measurements in prospective cohort investigations. *Am. J. Epidemiol.* 1995; **142**: 557–65.

31 Kaaks R, Riboli E, van Staveren W. Calibration of dietary intake measurements in prospective cohort studies. *Am. J. Epidemiol.* 1995; **142**: 548–56.

32 Slimani N, Charrondière UR, van Staveren W, Riboli E. Standardization of food composition databases for the European Prospective Investigation into Cancer and Nutrition (EPIC): general theoretical concept. *J. Food Comp. Anal.* 2000; **13**: 567–84.

33 Charrondière UR, Vignat J, Møller A, Ireland J, Becker W, Church S, *et al*. The European Nutrient Database (ENDB) for nutritional epidemiology. *J. Food Comp. Anal.* 2002; **15**(4): 435–51.

34 Haftenberger M, Lahmann PH, Panico S, González CA, Seidell JC, Boeing H, *et al*. Overweight, obesity and body fat distribution in 50- to 64-year-old participants in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public Health Nutr.* 2002; **5**(6B): 1147–62.

35 Ireland J, van Erp-Baart AMJ, Charrondière UR, Møller A, Smithers G, Trichopoulou A, for the EFCOSUM Group. Selection of a food classification system and a food composition database for future food consumption surveys. *Eur. J. Clin. Nutr.* 2002; **56**(Suppl. 2): S33–45.

2. Consommation de viande dans les populations de l'Etude Prospective Européenne sur le Cancer et la Nutrition (EPIC) : résultats des rappels alimentaires de 24 heures.

Basé sur:

Meat consumption in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts: results from 24-hour dietary recalls. Public Health Nutr. 2002; 5 :1243-5.

Plusieurs théories sur l'origine de l'espèce humaine font appel à la modification du régime alimentaire composé par des aliments d'origine végétale vers un régime riche en viande, pour expliquer l'augmentation rapide de la capacité du cerveau du genre Homo (139). Aujourd'hui, les viandes font partie des régimes alimentaires de la plupart de populations et constituent les sources fondamentales de protéines de haute qualité, fer héminique et zinc. La viande est aussi source d'autres nutriments, tels que les vitamines du groupe B, et en particulier, de la vitamine B12. En même temps, certaines substances contenues dans les viandes, qu'elles en soient des éléments constituants, tels que les acides gras saturés ou le cholestérol, ou qu'elles en soient des substances ajoutées ou formées au cours de la cuisson ou de la préservation de la viande – le sel, le nitrate/nitrite, les amines hétérocycliques- peuvent jouer un rôle préjudiciable pour la santé (140).

Le rôle de la viande dans l'étiologie de maladies cardiovasculaires et du cancer est sujet à débat. Pour les maladies cardiovasculaires, un effet nocif est attribué aux calories apportées par les lipides de la viande et à l'influence des acides gras saturés sur le cholestérol plasmatique (141). Les hypothèses concernant le rôle de la viande dans l'étiologie des cancers font référence aux carcinogènes potentiels formés pendant la cuisson et la préservation de la viande –amines hétérocycliques, hydrocarbures polycycliques aromatisés- ou de manière endogène, à partir de substrats apportés par la viande -composés N-nitrosés (142). Plusieurs

études ont suggéré l'implication de la viande rouge dans l'étiologie des cancers du sein, du colon-rectum, de la prostate, du pancréas et des reins (50;69).

Les profils de consommation de viande en Europe ont été décrits à l'aide des bilans alimentaires collectés par l'Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO-UN), ainsi que des enquêtes de ménages (143). Dans notre étude, nous décrivons les profils de consommation habituelle de viandes dans les populations de l'étude EPIC, selon les données recueillies à l'aide de rappels de 24 heures dans un échantillon de la population de l'étude. Les variations journalières du régime alimentaire impliquent que les valeurs recueillies avec un rappel de 24 heures ne sont pas représentatives de la consommation individuelle habituelle, mais elles permettent d'obtenir une estimation plus précise des apports alimentaires moyens de la population que l'estimation fournie par les questionnaires semi-quantitatifs qui ont été appliqués dans l'ensemble de la population EPIC.

Dans notre étude, la viande rouge se rapporte au bœuf, à l'agneau et au porc ; la viande préservée se rapporte en général, à la viande traitée par fumaison, salaison ou d'autres procédures pour sa préservation -saucisses, jambon, hamburgers, lardon -. Pour le veau, la classification en viande rouge ou blanche dépend de l'âge de l'animal et des pratiques en matière d'abattage et d'alimentation. La situation étant différente dans les populations de l'étude, nous avons inclue le veau dans la catégorie viande rouge (Figure 1).

Au travers des pays, nous avons observé une variabilité aussi bien des apports en viande que du type de viande (Figures 2 et 3). Sans compter la cohorte dite « *Health Conscious* » qui compte une proportion élevée de végétariens et de végétaliens en Oxford avec une très faible consommation moyenne de viande rouge, les apports de viande étaient en moyenne les plus bas dans la cohorte de la Grèce (47 et 79 grammes par jour chez les femmes et les hommes, respectivement) et les plus haut dans le nord de l'Espagne, particulièrement à San Sébastian

(124 et 234 grammes par jour, respectivement), suivi des autres centres du nord de l'Espagne (Asturies, Navarre). Ceci diffère de la situation trouvée dans les centres au sud de l'Espagne (Grenade, Murcia), où la consommation de viande rouge est plus faible. En moyenne, la consommation de viande à Naples (Italie) était inférieure à celle des centres EPIC situés plus au nord d'Italie, aussi bien pour le bœuf et les volailles que pour la viande traitée. Cependant, Raguse (Sicile, Italie) ne suit pas ce gradient nord-sud, avec des valeurs comparables à celles des populations du nord de l'Italie (Tableau 1a et 1b). Les cohortes allemandes ont les valeurs moyennes les plus élevés de consommation de saucisses, suivies des cohortes de la Suède, du Danemark et des Pays-Bas ; la consommation de lardons était la plus élevée au Royaume Uni (Tableau 2a et 2b).

Appart le centre et le sexe, d'autres facteurs associés avec la consommation de viande dans les populations étudiées sont l'âge, l'index de masse corporelle, le jour de la semaine, le tabagisme et les apports caloriques totaux (Tableaux 3). Nous avons constaté dans toutes les cohortes que la consommation de viande est plus faible chez les hommes et les femmes plus âgées. D'autre part, la consommation de viande rouge est aussi plus élevée chez les sujets avec un index de masse corporelle plus élevée, ainsi que chez les fumeurs. Ces associations persistent après avoir contrôlé les apports caloriques totaux (Tableau 4).

Public Health Nutrition: 9(4), 449–464 DOI: 10.1079/PHN2005861

Dietary intake of different types and characteristics of processed meat which might be associated with cancer risk – results from the 24-hour diet recalls in the European Prospective Investigation into Cancer and Nutrition (EPIC)

Jakob Linseisen[1,*], Sabine Rohrmann[1], Teresa Norat[2], Carlos A Gonzalez[3], Miren Dorronsoro Iraeta[4], Patrocinio Morote Gómez[5], Maria-Dolores Chirlaque[6], Basilio G Pozo[7], Eva Ardanaz[8], Irene Mattisson[9], Ulrika Pettersson[9], Richard Palmqvist[10], Bethany Van Guelpen[11], Sheila A Bingham[12], Alison McTaggart[13], Elizabeth A Spencer[14], Kim Overvad[15], Anne Tjønneland[16], Connie Stripp[16], Françoise Clavel-Chapelon[17], Emmanuelle Kesse[17], Heiner Boeing[18], Kerstin Klipstein-Grobusch[18], Antonia Trichopoulou[19], Effie Vasilopoulou[19], George Bellos[20], Valeria Pala[21], Giovanna Masala[22], Rosario Tumino[23], Carlotta Sacerdote[24], Mariarosaria Del Pezzo[25], H Bas Bueno-de-Mesquita[26], Marga C Ocke[26], Petra HM Peeters[27], Dagrun Engeset[28], Guri Skeie[28], Nadia Slimani[2] and Elio Riboli[2]

[1]German Cancer Research Centre, Division of Clinical Epidemiology, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany: [2]International Agency for Research on Cancer, Unit of Nutrition, Lyon, France: [3]Department of Epidemiology, Catalan Institute of Oncology, Barcelona, Spain: [4]Department of Health of the Basque Government, Public Health Division of Gipuzkoa, San Sebastian, Spain: [5]Health Council and Health Services Asturias, Public Health Directorate, Oviedo, Spain: [6]Epidemiology Department, Health Council of Murcia, Spain: [7]Andalusian School of Public Health, Granada, Spain: [8]Institute of Public Health, Navarra Cancer Registry, Pamplona, Spain: [9]Department of Medicine, Surgery and Orthopaedics, Lund University, Malmö, Sweden: [10]Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden: [11]Department of Public Health and Clinical Medicine, Nutrition Research, Umeå University, Umeå, Sweden: [12]Medical Research Council Dunn Human Nutrition Unit, Cambridge, UK: [13]University of Cambridge, Institute of Public Health, Strangeways Research Laboratory, Cambridge, UK: [14]Cancer Research UK Epidemiology Unit, University of Oxford, Oxford, UK: [15]Aarhus University, Department of Epidemiology and Social Medicine, Aarhus, Denmark: [16]Danish Cancer Society, Institute of Cancer Epidemiology, Copenhagen, Denmark: [17]INSERM, E3N–EPIC Group, Institute Gustave Roussy, Villejuif, France: [18]German Institute of Human Nutrition, Department of Epidemiology, Potsdam-Rehbrücke, Germany: [19]University of Athens Medical School, Department of Hygiene and Epidemiology, Athens, Greece: [20]Coropi Health Center, Greek Ministry of Health, Athens, Greece: [21]Epidemiology Unit, Italian National Cancer Institute, Milan, Italy: [22]Molecular and Nutritional Epidemiology Unit, CSPO, Florence, Italy: [23]Cancer Registry, Azienda Ospedaliera 'Civile MP Arezzo', Ragusa, Italy: [24]University of Turin, Turin, Italy: [25]Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy: [26]National Institute of Public Health and the Environment, Centre for Nutrition and Health, Bilthoven, The Netherlands: [27]Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, The Netherlands: [28]Institute of Community Medicine, University of Tromsø, Tromsø, Norway

Submitted 21 December 2004: Accepted 10 August 2005

Abstract

Objective: There is increasing evidence for a significant effect of processed meat (PM) intake on cancer risk. However, refined knowledge on how components of this heterogeneous food group are associated with cancer risk is still missing. Here, actual data on the intake of PM subcategories is given; within a food-based approach we considered preservation methods, cooking methods and nutrient content for stratification, in order to address most of the aetiologically relevant hypotheses.

Design and setting: Standardised computerised 24-hour diet recall interviews were collected within the framework of the European Prospective Investigation into Cancer and Nutrition (EPIC), a prospective cohort study in 27 centres across 10 European countries.

*Corresponding author: Email j.linseisen@dkfz-heidelberg.de

Subjects: Subjects were 22 924 women and 13 031 men aged 35–74 years.
Results: Except for the so-called 'health-conscious' cohort in the UK, energy-adjusted total PM intake ranged between 11.1 and 47.9 g day^{-1} in women and 18.8 and 88.5 g day^{-1} in men. Ham, salami-type sausages and heated sausages contributed most to the overall PM intake. The intake of cured (addition of nitrate/nitrite) PM was highest in the German, Dutch and northern European EPIC centres, with up to 68.8 g day^{-1} in men. The same was true for smoked PM (up to 51.8 g day^{-1}). However, due to the different manufacturing practice, the highest average intake of $NaNO_2$ through PM consumption was found for the Spanish centres (5.4 mg day^{-1} in men) as compared with German and British centres. Spanish centres also showed the highest intake of NaCl-rich types of PM; most cholesterol- and iron-rich PM was consumed in central and northern European centres. Possibly hazardous cooking methods were more often used for PM preparation in central and northern European centres.
Conclusions: We applied a food-based categorisation of PM that addresses aetiologically relevant mechanisms for cancer development and found distinct differences in dietary intake of these categories of PM across European cohorts. This predisposes EPIC to further investigate the role of PM in cancer aetiology.

Keywords
EPIC
Diet
Processed meat
24-Hour dietary recall
Europe
Nutrients
Preservation
Cooking methods

Several studies have already shown a positive association between meat consumption and different types of cancer, especially colorectal cancer[1]. In a recent review a higher risk of colorectal cancer was calculated for a high intake of processed meat (PM) and red meat; additionally, a strong dose–response relationship between PM intake and colorectal cancer risk (1.36, 95% confidence interval 1.15–1.61, for consumption of 30 g day^{-1} vs. no consumption) existed[2]. An even stronger association was provided by a recent meta-analysis, where Sandhu *et al.*[3] showed a significant 49% increased risk for a daily increase of 25 g of PM. In addition, associations between PM consumption and gastric cancer[4–6], lung cancer[7], cancer of the upper aerodigestive tract[8,9], prostate cancer[10,11] and cancer of the lower urinary tract[12] have been observed. There are also reports linking maternal intake of cured meat to the risk of brain tumours in childhood[13,14].

Several mechanisms have been hypothesised to explain associations between the consumption of PM and cancer risk; not all of them, however, are specific for PM. In general, it may be mediated through (1) an increased intake of (pre-)carcinogens or their precursors as well as (2) a high intake of specific nutrients enhancing the development of carcinogenic processes. Suggested mechanisms include the production of heterocyclic aromatic amines (HCA) during cooking[15], an increased content of polycyclic aromatic hydrocarbons (PAH) by certain cooking and smoking methods applied to PM[16], the presence of preformed *N*-nitroso compounds (NOC) from nitrates and nitrites added for preservation purposes, endogenous production of NOC by means of the high haem content in PM produced from red meat[17], and the content of lipid (fatty acids, cholesterol) oxidation products arising during processing and storage[18].

Concerning nutrient effects, curing increases drastically the originally low NaCl content of fresh meat and a high intake of salted food is associated with the risk of gastric cancer[1]. Further, high haem iron intake may be linked to carcinogenesis via enhancement of endogenous NOC production and oxidative damage to macromolecules, including lipids and DNA[19]. Therefore, also categorisation of PM by lipid content seems a promising approach. In particular, formation of cholesterol oxidation products during preparation and storage of PM has been described[18,20,21] and a link between 7β-hydroxycholesterol and lung cancer risk has been suggested[22].

To the best of our knowledge, a systematic analysis of factors that might contribute to a higher cancer risk due to high PM consumption has not been conducted so far. The European Prospective Investigation into Cancer and Nutrition (EPIC) offers the opportunity to study the diversity of food and nutrition habits in Europe because detailed nutrition information on type and preparation of the consumed PM is available for a subset of EPIC participants. Therefore, the aim of the present work is to describe PM intake in categories which reflect suggested aetiological factors and mechanisms that might help to explain the association of PM intake and cancer risk. We applied a food-based approach considering preservation methods, cooking methods and nutrient content for stratification; the definition of the subgroups and categories that we were able to build is given in the following section. The results may be the basis for more refined aetiological analyses in EPIC to disentangle the different mechanistic hypotheses on the effect of PM.

Material and methods

Subjects

The EPIC cohort study includes about half a million subjects from 10 European countries (France, Italy, Spain, Greece, The Netherlands, the United Kingdom, Germany, Denmark, Sweden, Norway)[23]. Information on the usual

individual dietary intakes of all participants was assessed using different dietary questionnaires developed and validated in each participating country. In order to adjust (at the group level) for systematic measurement error between countries, highly standardised 24-hour recalls were performed in a sub-sample (5–12% of the whole cohort) of each cohort as an additional dietary measurement[23,24]. Results presented in this paper are based on 24-hour recalls from 35 955 subjects (22 924 women and 13 031 men) participating in the EPIC calibration study between 1995 and 1998 (except Norway: 1999–2000). The age of the participants ranged from 35 to 74 years at recruitment. The distribution of study participants over the 27 study centres in 10 European countries is given in Table 1. The initial 23 EPIC coordinating centres were redefined in France, the UK and Norway. In the UK, the 'health-conscious group' and the subjects recruited from the general population both in Cambridge and Oxford ('general population group') were considered as two separate population groups. In France and Norway, where the study subjects were scattered all over the country, four and two geographical regions were defined, respectively. In France, Norway, Utrecht/The Netherlands and Naples/Italy, women only were recruited. A detailed description of further characteristics of study participants is given elsewhere[24].

Dietary assessment

A computerised 24-hour diet recall interview software, EPIC-SOFT, was developed as a calibration instrument by the International Agency for Research on Cancer in collaboration with all EPIC study centres[25]. The program was adapted for each participating country in terms of foods and recipes included. EPIC-SOFT provides a common structure and interview interface for an optimised standardisation of the dietary interview procedure within and between EPIC centres. According to common food groups and food subgroups, the countries generated a list of the single food items expected to be consumed by their participants. The open design allowed iterative update of the food item list. Furthermore, national data on the energy, fat, carbohydrate and alcohol contents of the food items were inserted to allow a rapid quality check of individual total energy and macronutrient intakes at the end of the interview while the subject was still present. In the current investigation these data are used to calculate energy intake (for adjustment).

The current report describes the intake of the EPIC-SOFT food subgroup 'processed meat', which is a subgroup of the group 'meat' (the remainder of the 'meat' group being classified as 'fresh meat'). The subgroup 'processed meat' encompasses all meat items that were further processed for preservation by salting (addition of NaCl), curing (addition of NaCl containing nitrite or nitrate), smoking, marinating or cooking, or that have been bought as a ready-to-eat product (including those with an unknown recipe). The food items of the subgroup 'processed meat' were reclassified in different ways according to current hypotheses related to their preparation and preservation methods with the support from experts of each participating country.

The first classification scheme referred to the following groups, mainly with respect to the preparation and preservation method: raw (i.e. preserved but not cooked) ham, cooked ham, bacon, raw and spreadable sausage, salami-type sausage and heated sausage. Additionally, data for minced PM (such as hamburger, fricadel, meat balls) and PM cuts (e.g. 'schnitzel', slices of cold roasted meat, roasted meat in aspic) are given. In EPIC, minced meat and meat cuts were usually attached to fresh meat (mainly pork and beef; being part of a recipe); however, when bought as a ready-to-eat product it was attributed to the subgroup 'processed meat'.

Second, PM food items were aggregated using the preservation method: curing (i.e. addition of NaCl which contains nitrite/nitrate) and smoking. In countries for which analytical data on the nitrite/nitrate concentration in ready-to-eat products were available (Germany, Spain, UK), mean nitrite/nitrate intake by PM consumption was calculated. For Germany, the analytical data gained between 1994 and 1998 by the Federal Institute of Meat Science, Kulmbach, were used; for the UK, data were taken from the MAFF UK Food Surveillance Info Sheet 142 (February 1998) providing analytical data for bacon and cured meat products collected in 1996/1997; for Spain, country-specific data were evaluated and average $NaNO_2$ contents calculated with assistance from a national expert in meat science.

Third, PM was classified according to the nutrient concentration as rich in salt (NaCl), haem and myoglobin iron (Fe), or cholesterol. For each country, single PM food items were listed by decreasing NaCl, Fe or cholesterol content as provided by the national EPIC centres and based on national food composition data. As valid surrogates at the food level, raw ham plus salami-type sausages (NaCl-rich; >3 g NaCl/100 g PM), liver-containing PM (cholesterol-rich; >90 mg cholesterol/100 g) as well as blood- and liver-containing PM (haem and myoglobin Fe-rich; >3 mg Fe/100 g) were identified. The contribution of PM intake to dietary fat intake was given in an earlier report[26] and was not further explored here.

Fourth, PM and PM subgroups were classified according to the application of cooking methods that can cause increased formation of the carcinogenic or potentially carcinogenic agents PAH and HCA. These so-called 'possibly hazardous cooking methods' (HCM) include barbecuing, frying and grilling. A detailed description of the use of cooking methods for the preparation of meat and fish was given elsewhere[27].

Due to differences in the extent of mis-(under-)reporting between centres[28], all results were adjusted for total energy intake (continuous variable). Furthermore, adjustment within centres or countries was performed to correct for deviations from an ideal sampling of the 24-hour recalls

(weekday, season) as well as for age. For weekdays, two discrete levels (Monday–Friday; Saturday and Sunday) and for season four discrete levels were applied (weighting). Age was included as a continuous variable. The presentation of the results is stratified for sex and centre. Calculations were done by means of SPSS® for Windows™ Release 10.0.7 (SPSS Inc., Chicago, IL, USA) and SAS System® for Windows™ Release 8.00 (SAS Institute Inc., Cary, NC, USA).

Results

Overall PM intake

Adjusted mean total PM intake showed a high range of variation across EPIC (Tables 1a and 1b). Except for the 'health-conscious' cohort (a large proportion of whom were vegetarian) in the UK (4.9 g day^{-1} in women, 13.4 g day^{-1} in men), the lowest intake was found in Greece (11.1 and 18.8 g day^{-1} in women and men, respectively) and the highest in north-western Norway (women, 47.9 g day^{-1}) and Potsdam/Germany (men, 88.5 g day^{-1}). Overall, heated sausages, ham (raw and cooked) and salami-type sausages contributed most to total PM intake.

PM intake by preservation method

With respect to PM products that have not been cooked ('raw'), i.e. raw ham, raw and spreadable sausages and salami-type sausages, the consumption of raw ham and salami-type sausages was highest in the Spanish centres (Tables 1a and 1b). Considerable amounts of raw and spreadable sausages were consumed in the UK cohort (general population). Heated sausages were the preferably consumed types of PM in the cohorts of The Netherlands, Germany and the Nordic countries (Denmark, Sweden and Norway).

Most raw PM has been cured, i.e. preserved by means of addition of salt (NaCl) containing nitrate and/or nitrite. However, cooked sausages were often cured as well. In general, the intake of cured PM is higher in the EPIC cohorts of central and northern Europe than in the southern cohorts (Tables 2a and 2b). Smoking of PM was more often applied in central and northern countries than in the French, Italian or Spanish centres. Almost all smoked PM has been cured as well.

$NaNO_2$ intake by PM consumption was estimated for three EPIC countries for which analytical data were available. The mean $NaNO_2$ intake varied between 0.1 and 3.3 mg day^{-1} in women and 0.8 and 5.4 mg day^{-1} in men in the different centres (Table 3). The highest intake was calculated for the Spanish EPIC cohorts, whereas the intake was considerably lower in the German as well as the British cohorts.

PM intake by nutrient content

Salting, i.e. addition of NaCl, is a common preservation method and PM can be categorised according to NaCl content. The consumption of NaCl-rich PM (raw ham and salami-type sausages) was found to be highest in the Spanish centres (>20 g day^{-1} among men, >10 g day^{-1} among women), followed by the North Italian centres of Florence, Varese and Turin, while it was lowest in the British cohorts (Tables 4a and 4b).

The intake of haem and myoglobin iron-rich PM is reflected by the extent of consumption of liver- and blood-containing PM. The intake was highest in Danish and Swedish centres (Tables 4a and 4b). Considering only blood-containing PM, the Spanish centres of Granada (men, 7.3 g day^{-1}) and San Sebastian (women, 3.7 g day^{-1}) showed the highest consumption level.

Liver-containing PM has the highest cholesterol content among the PM food items. Men in the Danish cohorts consumed on average >10 g of liver-containing PM daily, followed by Malmö/Sweden and Potsdam/Germany. At the low end, liver-containing PM was rarely consumed in the Italian and Spanish centres (Tables 4a and 4b).

PM intake by cooking method

PM was cooked differently in the EPIC centres and, with few exceptions, HCM (frying, grilling, barbecuing) were more often used in northern and central European than in the southern EPIC centres (Tables 5a and 5b). While barbecued and grilled PM were consumed rarely, fried PM contributed most to the consumption. However, in the UK, the French centres and some Italian centres, grilling is the most frequently used HCM. PM most often prepared by these three cooking methods was bacon and ham, minced PM and sausages. Especially in the centres of central and northern Europe sausages contributed most to the total consumption of PM prepared by HCM. PM that was cured and prepared by HCM was consumed in higher amounts in Navarra/Spain (women: 12.0 g day^{-1}; men: 16.1 g day^{-1}) as well as in the Swedish and Norwegian centres. In these centres, the intake of cured and smoked PM cooked by the three high-temperature methods was high as well.

Discussion

To the best of our knowledge this is the first report that describes systematically the consumption of different types of PM and its characteristics in Europe by several factors that might be potential risk factors for different types of cancer when larger amounts of PM – mostly from red meat – are consumed. The results of this study clearly show the diversity and heterogeneity that exist within the EPIC cohorts.

Due to the lack of similar studies, comparisons of our results, as assessed by means of a 24-hour recall in a

Table 1a Mean daily intake (g day^{-1}; adjusted)* of processed meat (PM) and its subgroups in women across 10 European countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study (24-hour recall)

		Total PM		Ham, raw†		Ham, cooked		Bacon		Sausages, raw† and spreadable		Sausages, salami-type		Sausages, heated		PM, minced		PM, cuts	
EPIC country and centre	*n*	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Greece																			
Greece	1374	11.1	1.3	1.3	0.3	0.6	0.5	0.4	0.2	0.2	0.2	1.4	0.3	5.1	0.9	0.7	0.5	1.3	0.4
Spain																			
Granada	304	28.7	2.7	5.3	0.7	4.4	1.0	0.7	0.5	1.1	0.4	6.3	0.6	5.3	1.9	4.6	1.0	1.0	0.9
Murcia	324	24.7	2.7	4.0	0.7	5.3	1.0	0.1	0.5	1.0	0.4	6.4	0.6	3.6	1.9	1.2	1.0	3.1	0.9
Navarra	271	35.4	2.8	7.1	0.8	7.0	1.0	1.3	0.5	0.1	0.4	9.7	0.7	5.8	2.0	3.2	1.1	1.3	0.9
San Sebastian	244	28.2	3.0	3.9	0.8	7.5	1.1	0.5	0.5	2.7	0.4	7.8	0.7	0.7	2.1	4.1	1.2	1.1	1.0
Asturias	300	30.2	2.6	5.0	0.7	6.7	0.9	1.1	0.4	0.0	0.0	9.9	0.6	4.9	1.8	1.3	1.0	1.4	0.9
Italy																			
Ragusa	138	18.0	4.0	0.1	1.1	2.4	1.5	0.0	0.7	0.0	0.0	5.9	0.9	8.6	2.8	0.0	0.0	1.2	1.3
Naples	403	16.9	2.3	4.4	0.6	2.9	0.8	0.6	0.4	0.1	0.3	3.3	0.5	5.4	1.7)	0.1	0.9	0.2	0.8
Florence	785	19.7	1.7	5.8	0.4	3.3	0.6	0.4	0.3	1.2	0.2	3.6	0.4	4.4	1.2	0.1	0.6	1.0	0.6
Turin	392	21.1	2.4	4.4	0.6	7.2	0.9	0.6	0.4	0.0	0.3	4.2	0.6	3.3	1.7	0.1	0.9	1.3	0.8
Varese	794	28.0	1.7	4.4	0.4	8.3	0.6	1.8	0.3	0.2	0.2	5.4	0.4	5.7	1.2	0.1	0.6	2.1	0.5
France																			
South coast	612	27.3	1.9	1.8	0.5	9.6	0.7	1.0	0.3	0.0	0.0	2.6	0.4	11.3	1.3	0.1	0.7	0.9	0.6
South	1396	27.2	1.3	1.2	0.3	8.8	0.5	1.1	0.2	0.0	0.0	1.9	0.3	13.0	0.9	0.2	0.5	1.0	0.4
North-west	622	32.0	1.9	1.8	0.5	10.9	0.7	2.2	0.3	0.0	0.0	0.9	0.4	15.0	1.3	0.2	0.7	1.0	0.6
North-east	2009	27.6	1.0	1.7	0.3	8.8	0.4	1.7	0.2	0.0	0.0	1.8	0.2	11.7	0.7	0.9	0.4	1.2	0.3
Germany																			
Heidelberg	1087	41.6	1.4	2.6	0.4	4.8	0.5	0.6	0.2	0.5	0.2	5.5	0.3	22.4	1.0	4.4	0.6	0.8	0.5
Potsdam	1063	42.1	1.4	1.6	0.4	2.6	0.5	0.7	0.2	1.8	0.2	4.3	0.3	25.1	1.0	5.0	0.6	1.0	0.5
The Netherlands																			
Bilthoven	1086	38.2	1.4	1.5	0.4	7.3	0.5	2.7	0.2	0.5	0.2	1.2	0.3	13.4	1.0	5.0	0.6	6.7	0.5
Utrecht	1874	36.0	1.1	2.9	0.3	6.3	0.4	3.2	0.2	1.5	0.2	0.8	0.3	13.6	0.8	2.6	0.4	5.0	0.4
United Kingdom																			
General population	571	24.3	2.0	0.2	0.5	5.2	0.7	6.0	0.3	6.2	0.3	0.9	0.5	2.3	1.4	3.0	0.8	0.4	0.6
'Health-conscious'	197	4.9	3.3	0.0	0.0	1.6	1.2	2.1	0.6	0.8	0.5	0.0	0.0	0.4	2.4	0.0	1.3	0.0	0.0
Denmark																			
Copenhagen	1485	24.7	1.2	2.4	0.3	2.7	0.4	1.0	0.2	0.0	0.0	1.6	0.3	13.0	0.9	1.3	0.5	2.7	0.4
Aarhus	510	21.8	2.1	2.2	0.6	1.5	0.8	2.0	0.4	0.0	0.0	2.1	0.5	11.7	1.5	0.2	0.8	2.1	0.7
Sweden																			
Malmö	1711	43.5	1.2	5.9	0.4	4.5	0.4	1.6	0.2	0.4	0.2	3.1	0.3	19.1	0.8	2.9	0.4	6.0	0.4
Umeå	1574	43.4	1.2	8.6	0.3	3.2	0.4	0.7	0.2	0.2	0.2	0.8	0.3	19.2	0.8	4.6	0.5	6.0	0.4
Norway																			
South & East	1136	44.2	1.4	2.1	0.4	5.2	0.5	0.8	0.2	0.0	0.0	1.9	0.3	20.1	1.0	12.4	0.5	1.8	0.5
North & West	662	47.9	1.8	2.7	0.5	5.4	0.7	0.9	0.3	0.0	0.0	1.5	0.4	21.9	1.3	12.1	0.7	3.6	0.6

* Results are given as mean and standard error of mean (SEM), adjusted for energy intake and age as well as for weekday and season of the 24-hour recall assessment.
† Raw: preserved, not cooked.

Table 1b Mean daily intake (g day^{-1}; adjusted)* of processed meat (PM) and its subgroups in men across eight European countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study (24-hour recall)

		Total PM		Ham, raw†		Ham, cooked		Bacon		Sausages, raw† and spreadable		Sausages, salami-type		Sausages, heated		PM, minced		PM, cuts	
EPIC country and centre	*n*	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Greece																			
Greece	1312	18.8	1.5	2.0	0.5	1.8	0.5	0.6	0.3	0.3	0.3	2.6	0.6	8.7	1.4	1.2	0.7	1.7	0.5
Spain																			
Granada	243	48.3	4.7	11.4	1.1	2.7	1.3	0.1	0.8	7.3	0.8	14.1	1.5	6.4	3.4	4.3	1.8	2.0	1.3
Murcia	386	43.0	4.4	7.7	1.0	4.4	1.3	0.0	0.8	1.5	0.8	12.9	1.4	9.0	3.2	2.6	1.7	5.0	1.2
Navarra	444	51.5	3.2	14.9	0.8	6.4	0.9	2.4	0.6	0.1	0.6	16.6	1.0	4.4	2.4	5.9	1.2	0.9	0.9
San Sebastian	490	41.5	3.1	8.1	0.7	4.3	0.9	5.0	0.5	2.0	0.6	17.7	1.0	0.0	0.0	5.0	1.2	1.8	0.9
Asturias	214	54.6	3.5	7.4	0.8	3.5	1.0	2.4	0.6	0.0	0.6	20.6	1.1	5.5	2.6	6.5	1.3	8.7	1.0
Italy																			
Ragusa	168	22.1	5.3	1.0	1.2	4.4	1.5	0.0	0.9	0.0	0.0	7.7	1.7	9.3	3.9	0.0	0.0	0.0	0.0
Florence	271	25.9	4.2	8.4	1.0	3.8	1.2	0.6	0.7	2.2	0.7	7.3	1.3	2.9	3.0	0.0	0.0	1.0	1.2
Turin	677	32.1	2.6	4.3	0.6	8.4	0.8	2.0	0.5	0.4	0.5	10.5	0.8	4.8	1.9	0.0	0.0	1.7	0.7
Varese	328	43.4	3.8	5.4	0.9	11.5	1.1	3.1	0.7	0.0	0.0	10.1	1.2	12.1	2.8	0.0	0.0	1.9	1.1
Germany																			
Heidelberg	1033	81.2	2.1	4.1	0.5	7.9	0.6	1.0	0.4	1.7	0.4	11.1	0.7	47.7	1.6	6.4	0.8	1.5	0.6
Potsdam	1235	88.5	1.9	4.1	0.5	2.7	0.6	1.8	0.3	3.9	0.3	12.7	0.6	49.8	1.4	10.9	0.7	2.7	0.5
The Netherlands																			
Bilthoven	1024	70.3	2.2	3.0	0.5	13.7	0.6	4.8	0.4	1.8	0.4	3.3	0.7	26.7	1.6	10.4	0.8	6.6	0.6
United Kingdom																			
General population	404	41.1	3.4	0.3	0.8	9.4	1.0	8.9	0.6	12.8	0.6	1.0	1.1	4.1	2.5	4.3	1.3	0.1	1.0
'Health-conscious'	114	13.4	6.4	0.4	1.5	1.8	1.8	3.8	1.1	0.8	1.1	1.1	2.0	3.9	4.7	1.5	2.4	0.2	1.8
Denmark																			
Copenhagen	1356	50.0	1.0	2.5	0.4	4.5	0.5	2.0	0.3	0.0	0.0	4.6	0.6	31.4	1.4	1.0	0.7	4.1	0.5
Aarhus	567	47.1	2.9	2.5	0.7	3.5	0.8	4.0	0.5	0.0	0.0	4.2	0.9	28.8	2.1	0.1	1.1	4.2	0.8
Sweden																			
Malmö	1421	66.6	1.9	7.3	0.5	7.2	0.5	1.4	0.3	0.4	0.3	7.6	0.6	30.7	1.4	4.6	0.7	7.3	0.5
Umeå	1344	67.9	1.9	7.3	0.4	4.4	0.5	1.2	0.3	0.5	0.3	1.9	0.6	40.4	1.4	5.8	0.7	6.5	0.5

*Results are given as mean and standard error of mean (SEM), adjusted for energy intake and age as well as for weekday and season of the 24-hour recall assessment.
†Raw: preserved, not cooked.

Table 2a Mean daily intake (g day^{-1}; adjusted)* of cured, smoked and cured & smoked (c&s) processed meat (PM) and PM subgroups in women across eight European countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study (24-hour recall)

	Total PM						Ham and bacon				Salami-type sausages				Heated sausages				Blood- and liver-containing sausages			
	Cured		Smoked		C&s		Cured		C&s		Cured		C&s		Cured		C&s		Cured		C&s	
EPIC country and centre	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Spain																						
Granada	20.8	2.3	1.5	1.8	1.5	1.7	10.3	1.2	0.6	1.0	6.3	0.6	0.2	0.4	4.0	1.7	0.6	1.3	0.8	0.7	0.1	0.3
Murcia	16.9	2.3	0.0	0.0	0.0	0.0	9.4	1.2	0.0	0.0	6.4	0.6	0.0	0.0	1.0	1.7	0.0	0.0	0.2	0.7	0.0	0.0
Navarra	28.6	2.4	5.3	1.9	5.3	1.8	15.2	1.3	0.3	1.0	9.7	0.6	3.0	0.4	3.7	1.7	1.9	1.4	0.8	0.8	0.0	0.0
San Sebastian	20.3	2.5	0.0	0.0	0.0	0.0	11.9	1.4	0.0	0.0	7.8	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.8	0.0	0.0
Asturias	26.9	2.2	2.4	1.7	2.2	1.7	12.6	1.2	0.0	0.0	9.9	0.6	0.0	0.0	3.3	1.6	1.1	1.3	0.8	0.7	0.0	0.0
Italy																						
Ragusa	17.4	3.4	0.0	0.0	0.0	0.0	2.5	1.8	0.0	0.0	5.9	0.9	0.0	0.0	8.6	2.5	0.0	0.0	0.1	1.1	0.0	0.0
Naples	16.5	2.0	0.8	1.5	0.8	1.5	7.7	1.1	0.4	0.9	3.3	0.5	0.1	0.3	5.2	1.4	0.3	1.1	0.3	0.6	0.0	0.0
Florence	18.4	1.4	0.5	1.1	0.5	1.8	9.4	0.8	0.3	0.6	3.6	0.4	0.0	0.2	4.0	1.0	0.1	0.8	0.7	0.5	0.0	0.0
Turin	20.6	2.0	1.1	1.5	1.1	1.5	12.2	1.1	0.4	0.9	4.2	0.6	0.1	0.3	2.9	1.5	0.5	1.2	0.2	0.6	0.0	0.0
Varese	27.0	1.4	1.5	1.1	1.4	1.1	14.4	0.8	1.1	0.6	5.4	0.4	0.1	0.2	4.9	1.0	0.2	0.8	0.5	0.4	0.0	0.0
France																						
South coast	25.6	1.6	12.6	1.2	12.6	1.2	12.2	0.9	10.5	0.7	2.6	0.4	0.3	0.2	10.3	1.2	1.9	0.9	2.8	0.5	0.5	0.2
South	24.6	1.1	11.5	0.8	11.5	0.8	11.0	0.6	9.1	0.5	1.9	0.3	0.2	0.2	10.8	0.8	2.2	0.6	2.8	0.3	0.3	0.1
North-west	30.2	1.6	16.0	1.2	16.0	1.2	14.9	0.9	11.9	0.7	0.9	0.4	0.2	0.2	13.5	1.2	3.9	0.9	3.1	0.5	0.2	0.2
North-east	24.5	0.9	13.9	0.7	13.8	0.7	11.9	0.5	10.5	0.4	1.8	0.2	0.3	0.1	10.2	0.6	3.1	0.5	2.9	0.3	0.4	0.1
Germany																						
Heidelberg	31.8	1.2	21.5	0.9	21.5	0.9	7.7	0.7	3.0	0.5	5.5	0.3	5.1	0.2	17.5	0.9	12.8	0.7	2.9	0.4	2.7	0.2
Potsdam	33.0	1.2	24.8	0.9	24.7	0.9	4.7	0.7	2.1	0.5	4.3	0.3	4.3	0.2	21.4	0.9	16.4	0.7	5.5	0.4	4.3	0.2
The Netherlands																						
Bilthoven	28.3	1.2	19.5	0.9	19.1	0.9	11.1	0.7	11.2	0.5	1.2	0.3	0.6	0.2	9.9	0.9	5.2	0.7	4.1	0.4	0.3	0.2
Utrecht	28.6	0.9	20.1	0.7	19.8	0.7	12.1	0.5	11.8	0.4	0.8	0.3	0.1	0.1	11.6	0.7	6.3	0.5	5.3	0.3	0.2	0.1
United Kingdom																						
General population	16.6	1.6	12.4	1.3	12.3	1.3	11.4	0.9	11.2	0.7	0.9	0.4	0.7	0.3	2.0	1.2	0.4	1.0	1.4	0.5	0.0	0.0
'Health-conscious'	4.1	2.8	3.8	2.2	3.7	2.2	3.7	1.5	3.7	1.2	0.0	0.0	0.0	0.0	0.3	2.0	0.0	0.0	0.3	0.9	0.0	0.0
Sweden																						
Malmö	39.1	1.0	25.6	0.8	25.3	0.7	12.0	0.5	6.8	0.4	3.1	0.3	2.8	0.2	18.2	0.7	11.5	0.6	6.0	0.3	0.2	0.1
Umeå	37.2	1.0	27.1	0.8	27.1	0.8	12.5	0.5	8.9	0.4	0.8	0.3	0.8	0.2	17.6	0.7	12.9	0.6	4.8	0.3	0.2	0.1
Norway																						
South & East	34.3	1.2	25.1	0.9	24.7	0.9	8.2	0.6	8.4	0.5	1.9	0.3	2.2	0.2	18.2	0.9	12.9	0.7	4.7	0.4	0.1	0.2
North & West	38.2	1.5	30.3	1.2	28.5	1.2	8.9	0.8	9.2	0.7	1.5	0.4	1.8	0.2	21.0	1.1	16.2	0.9	4.3	0.5	0.1	0.2

* Results are given as mean and standard error of mean (SEM), adjusted for energy intake and age as well as for weekday and season of the 24-hour recall assessment.
As shown for total PM, nearly all smoked meat is also cured; therefore, data for 'smoked PM' is not given for the subgroups. Summing up of cured (or c&s) PM over subgroups gives the figure for total cured (or c&s) PM, with minor deviations due to adjustment procedures.

Table 2b Mean daily intake (g day^{-1}; adjusted)* of cured, smoked and cured & smoked (c&s) processed meat (PM) and PM subgroups in men across six European countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study (24-hour recall)

	Total PM						Ham and bacon				Salami-type sausages				Heated sausages				Blood- and liver-containing sausages			
	Cured		Smoked		C&s		Cured		C&s		Cured		C&s		Cured		C&s		Cured		C&s	
EPIC country and centre	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Spain																						
Granada	34.2	3.7	1.1	2.9	1.1	2.9	14.2	1.7	0.0	0.0	14.1	1.5	0.0	0.0	6.1	2.7	1.4	2.4	2.5	1.2	0.0	0.0
Murcia	30.3	3.4	0.9	2.7	0.9	2.7	12.2	1.6	0.0	0.0	12.9	1.4	0.0	0.0	5.1	2.5	1.1	2.2	1.1	1.1	0.0	0.0
Navarra	41.1	2.6	6.0	2.0	6.0	2.0	23.3	1.2	0.3	0.8	16.6	1.0	6.1	0.6	1.1	1.9	0.0	0.0	0.7	0.8	0.0	0.0
San Sebastian	33.7	2.5	0.0	0.0	0.0	0.0	17.9	1.2	0.0	0.0	17.9	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Asturias	41.0	2.8	6.6	2.2	5.5	2.2	12.7	1.3	0.0	0.0	20.7	1.1	0.0	0.0	1.4	2.0	0.0	0.0	0.0	0.0	0.0	0.0
Italy																						
Ragusa	22.8	4.2	0.0	0.0	0.0	0.0	5.6	2.0	0.0	0.0	7.8	1.7	0.0	0.0	9.5	3.1	0.0	0.0	0.0	0.0	0.0	0.0
Florence	25.6	3.3	0.0	0.0	0.0	0.0	13.0	1.5	0.0	0.0	7.4	1.3	0.0	0.0	3.0	2.4	0.0	0.0	1.0	1.0	0.0	0.0
Turin	31.2	2.1	1.1	1.6	1.1	1.6	14.9	1.0	1.3	0.7	10.5	0.8	0.0	0.0	4.0	1.5	0.0	0.0	0.4	0.7	0.0	0.0
Varese	41.4	3.0	0.0	0.0	0.0	0.0	19.9	1.4	0.0	0.0	10.1	1.2	0.0	0.0	9.9	2.2	0.0	0.0	0.0	0.0	0.0	0.0
Germany																						
Heidelberg	63.2	1.7	42.6	1.3	42.6	1.3	12.7	0.8	4.9	0.5	11.1	0.7	9.7	0.4	36.2	1.2	25.8	1.1	8.2	0.5	7.1	0.4
Potsdam	68.8	1.5	51.9	1.2	51.8	1.2	8.5	0.7	5.7	0.5	12.6	0.6	12.3	0.4	41.7	1.1	29.5	1.0	11.9	0.5	10.7	0.0
The Netherlands																						
Bilthoven	56.7	1.8	38.5	1.4	37.9	1.4	21.2	0.8	21.7	0.6	3.4	0.7	1.3	0.4	20.5	1.3	11.9	1.1	7.6	0.6	0.4	0.4
United Kingdom																						
General population	25.1	2.7	19.5	2.1	19.5	2.1	18.5	1.3	18.1	0.9	1.0	1.1	0.7	0.7	2.9	2.0	0.7	1.7	2.2	0.9	0.1	0.6
'Health-conscious'	10.0	5.1	7.3	4.0	7.2	4.0	5.9	2.4	5.4	1.6	1.0	2.0	0.4	1.3	2.7	3.7	1.2	3.2	0.6	1.6	0.3	1.1
Sweden																						
Malmö	59.9	1.5	38.3	1.2	38.1	1.2	15.4	0.7	7.9	0.5	7.5	0.6	7.0	0.4	29.5	1.1	18.8	1.0	10.0	0.5	0.5	0.3
Umeå	58.1	1.5	44.3	1.2	44.2	1.2	12.8	0.7	8.1	0.5	1.9	0.6	1.8	0.4	36.4	1.1	29.9	0.9	6.7	0.5	0.5	0.3

*Results are given as mean and standard error of mean (SEM), adjusted for energy intake and age as well as for weekday and season of the 24-hour recall assessment.
As shown for total PM, nearly all smoked meat is also cured; therefore, data for 'smoked PM' is not given for the subgroups. Summing up of cured (or c&s) PM over subgroups gives the figure for total cured (or c&s) PM, with minor deviations due to adjustment procedures.

Table 3 Mean daily intake of nitrite/nitrate (calculated as mg $NaNO_2$ per day, adjusted*) by consumption of processed meat in women and men from European Prospective Investigation into Cancer and Nutrition (EPIC) centres in Spain, Germany and the United Kingdom (24-hour recalls)

	$NaNO_2$ intake (mg day^{-1})		
EPIC country and centre	Mean	SEM	95% CI
Women			
Spain			
Granada	2.35	0.17	2.03–2.67
Murcia	1.97	0.16	1.65–2.29
Navarra	3.35	0.17	3.01–3.69
San Sebastian	2.59	0.18	2.23–2.95
Asturias	3.26	0.16	2.94–3.57
Germany			
Heidelberg	1.35	0.09	1.18–1.52
Potsdam	1.39	0.09	1.22–1.56
United Kingdom			
General population	0.63	0.12	0.39–0.86
'Health-conscious'	0.10	0.20	0.00–0.50
Men			
Spain			
Granada	3.85	0.31	3.24–4.47
Murcia	3.20	0.29	2.63–3.77
Navarra	5.17	0.22	4.75–5.60
San Sebastian	4.62	0.21	4.20–5.03
Asturias	5.44	0.23	4.98–5.89
Germany			
Heidelberg	2.82	0.14	2.54–3.10
Potsdam	3.13	0.13	2.88–3.39
United Kingdom			
General population	1.08	0.23	0.64–1.53
'Health-conscious'	0.76	0.43	0.00–1.60

SEM – standard error of mean; CI – confidence interval.
*Adjusted for energy intake, age, weekday and season of the 24-hour recall assessment.

sub-population of each cohort, with the situation in the underlying populations are limited. However, a fairly good agreement with the results of studies that assessed meat and PM consumption in selected European regions and countries was noted in an earlier report on meat consumption in EPIC using the same database[26]. Valid and comparable dietary data with focus on the intake of different types of PM are, however, rare. Most data derive from food-frequency questionnaires used to study the association between food intake and cancer risk; these data are suitable for categorisation of individuals but do not necessarily give the actual intake level and provide sufficient details on the specific types of PM.

Differences in under- or overreporting among the study regions might influence the quality of our results[28]. Meat intake is affected by underreporting in EPIC Greece and southern Spain[26]. To keep the effect of misreporting as small as possible, all analysed data were adjusted for total energy intake. An effect of seasonal variation on food consumption in the EPIC calibration study[24] seems likely to affect the intake of special types and characteristics of PM as well, e.g. the use of cooking methods like barbecuing or grilling. This aspect has been taken into account by adjusting for season as well as for weekday of the 24-hour recalls.

A number of mechanisms have been proposed as the means by which high intakes of PM may increase the risk of cancer. Humans are exposed to NOC, including nitrosamines, both from the diet (and other environmental sources) and from endogenous synthesis[29]. Preformed NOC in foods have been found almost exclusively in foods containing nitrite or which were exposed to nitrogen oxides. Thus, cured meat and meat products might be the most important contributors to dietary preformed NOC. Additionally, smoking or direct-fire drying increases the NOC concentration in food[2,29]. Haorah *et al.*[30] have shown that the concentration of NOC and their precursors varies widely in foods and even in the same product that was purchased at different times, making it difficult to reliably estimate dietary intake. In addition to this exogenous exposure, there is extensive endogenous nitrate and nitrite generation from inducible and endogenous NO synthases and resulting NOC production in the body. NOC arise from the reaction of nitrite and secondary or tertiary amines in the intestine from *N*-nitrosation of amines which in the colon can be produced by bacterial decarboxylation of amino acids[31]. Bingham *et al.*[31] have shown that haem in red (fresh and processed) but not white meat substantially increases the endogenous burden of NOC. Excess nitrate can also increase endogenous NOC production[32].

The intake of the NOC precursor nitrite as well as the consumption of cured meat were positively associated with cancer risk of the prostate[10] and lower urinary tract[12]. Associations of nitrite, cured meat or NOC intake with gastric cancer are inconsistent[4–6]. Penttila *et al.*[33] estimated that the intake of cured meat products contributes 97% of the total dietary nitrite intake in Finland. In the current investigation, total intake of cured PM is lower in the Mediterranean EPIC centres than in the central and northern European centres. In contrast, the estimated intake of nitrate/nitrite by PM consumption, calculated as $NaNO_2$, is higher in Spanish EPIC cohorts than in the UK and Germany. Although upper limits for the $NaNO_2$ concentration in meat products exist in most European countries, valid data on $NaNO_2$ concentration in ready-to-eat products for intake calculations were available only in these three EPIC countries. The results clearly show marked differences in the amount of nitrite/nitrate added for the production of cured PM as well as differences in factors modulating the formation of nitrite in preserved PM during storage and ripening[14]. In Spain, where salami-type sausages and ham form the majority of intake of cured meat, the amount of nitrate/nitrite added for preservation and probably storage conditions might differ from other countries. Furthermore, the content of additives in food changes over time[34]. Developments in manufacturing practice during the last decades, e.g. addition of ascorbic acid, decreased the amount of nitrate/nitrite added to PM products in most European countries[35]. This raises the question whether information at the food level as an indicator of cured PM consumption is sufficient to describe

Table 4a Mean daily intake (g day^{-1}; adjusted)* of processed meat (PM) subgroups as classified according to their cholesterol, iron or salt content in women across 10 European countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study (24-hour recall)

	Cholesterol-rich		Fe-rich				NaCl-rich	
	Liver-containing PM		Blood-containing PM		Blood- and liver-containing PM		Ham raw & salami-type sausage	
EPIC country and centre	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Greece								
Greece	–	–	–	–	–	–	2.7	0.5
Spain								
Granada	0.8	0.8	1.4	0.5	2.2	1.0	11.6	1.0
Murcia	0.1	0.8	1.0	0.5	1.2	0.9	10.4	1.0
Navarra	0.8	0.8	1.4	0.6	2.2	1.0	16.9	1.0
San Sebastian	0.0	0.9	3.7	0.6	3.7	1.1	11.7	1.1
Asturias	0.8	0.8	1.1	0.5	1.9	0.9	14.8	0.9
Italy								
Ragusa	0.1	1.2	0.0	0.0	0.1	1.4	6.0	1.4
Naples	0.3	0.7	0.1	0.5	0.4	0.8	7.6	0.8
Florence	1.2	0.5	0.1	0.3	1.3	0.6	9.4	0.6
Turin	0.2	0.7	0.0	0.5	0.3	0.8	8.6	0.8
Varese	0.5	0.5	0.0	0.3	0.5	0.6	9.8	0.6
France								
South coast	2.5	0.6	1.3	0.4	3.8	0.7	4.4	0.7
South	3.5	0.4	1.3	0.2	4.7	0.4	3.2	0.4
North-west	3.0	0.5	1.1	0.4	4.1	0.7	2.7	0.7
North-east	3.7	0.3	1.1	0.2	4.8	0.4	3.4	0.4
Germany								
Heidelberg	2.8	0.4	0.4	0.3	3.2	0.5	8.0	0.5
Potsdam	4.1	0.4	1.4	0.3	5.5	0.5	5.9	0.5
The Netherlands								
Bilthoven	3.8	0.4	0.1	0.3	3.9	0.5	2.6	0.5
Utrecht	4.6	0.3	0.7	0.2	5.3	0.4	3.7	0.4
United Kingdom								
General population	1.4	0.6	0.1	0.4	1.5	0.7	1.1	0.7
'Health-conscious'	0.3	1.0	0.0	0.0	0.3	1.2	0.0	0.0
Denmark								
Copenhagen	4.6	0.4	1.1	0.2	5.7	0.4	3.9	0.4
Aarhus	4.7	0.6	0.1	0.4	4.8	0.7	4.3	0.7
Sweden								
Malmö	5.8	0.3	1.1	0.2	6.9	0.4	9.0	0.4
Umeå	4.5	0.3	1.8	0.2	6.4	0.4	9.4	0.4
Norway								
South & East	4.6	0.4	0.2	0.3	4.8	0.5	4.0	0.5
North & West	4.2	0.5	0.3	0.4	4.6	0.6	4.2	0.7

* Results are given as mean and standard error of mean (SEM), adjusted for energy intake and age as well as for weekday and season of the 24-hour recall assessment.

exposure differences for nitrite/nitrate through PM consumption, because this approach assumes a similar $NaNO_2$ content in the ready-to-eat PM products in the different countries which might not be correct.

In a Finnish study, conducted between 1966 and 1972, mean intake of nitrite was 5.3 mg day^{-1}, provided nearly exclusively by cured meats and PM[5,36]. However, another report from Finland calculated an average daily intake of 1.88 mg[33]. In the UK, a daily nitrite intake of 2.4–4.2 mg has been reported[37], which is higher than estimated for the British EPIC cohort. In an Italian case–control study the mean daily nitrite intake was 3.5 mg[6]. Using duplicate 24-hour diet samples, a median daily nitrite intake of 0.1 mg with a range of <0.1–16 mg was found in a small Dutch sample[38]. Comparing analytical data with intake data derived from calculations based on dietary intake estimations, the results obtained in a Polish study showed a limited deviation with 1.67 (analytical) versus 1.18 (calculated) mg nitrite per day and person[39].

The incidence of stomach cancer is higher in the south than in the west and the north of Europe[40]. Evidence suggests that risk of stomach cancer is increased by high intake of some traditionally preserved salted foods, especially meats and pickles, and with salt *per se*[1,41]. Sodium chloride used for salting/curing enhances substantially the generally low NaCl concentration of fresh meat. Whether the intake of NaCl and PM-derived NaCl – the latter being highest in the Spanish EPIC centres, followed by Italian and Dutch centres – is a risk factor for stomach cancer in EPIC has to be examined in forthcoming risk evaluations.

Meat is smoked because of its inactivating effect on enzymes and micro-organisms as well as its effect on taste[42]. However, smoke contains PAH formed by pyrolytic

Table 4b Mean daily intake (g day^{-1}; adjusted)* of processed meat (PM) subgroups as classified according to their cholesterol, iron or salt content in men across eight European countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study (24-hour recall)

	Cholesterol-rich		Fe-rich				NaCl-rich	
	Liver-containing PM		Blood-containing PM		Blood- and liver-containing PM		Ham raw & salami-type sausage	
EPIC country and centre	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Greece								
Greece	–	–	–	–	–	–	4.6	0.8
Spain								
Granada	2.5	1.3	7.3	1.2	9.8	1.8	25.4	1.9
Murcia	0.1	1.2	3.0	1.1	3.1	1.7	20.6	1.7
Navarra	0.6	0.9	2.9	0.8	3.5	1.2	31.4	1.3
San Sebastian	0.0	0.0	2.4	0.8	2.1	1.2	25.8	1.2
Asturias	0.0	0.0	3.8	0.9	3.7	1.3	28.0	1.4
Italy								
Ragusa	0.0	0.0	0.0	0.0	0.0	0.0	8.7	2.1
Florence	1.9	1.1	0.0	0.0	1.8	1.6	15.7	1.6
Turin	0.3	0.7	0.1	0.7	0.4	1.0	14.9	1.0
Varese	0.0	0.0	1.5	0.9	1.1	1.4	15.4	1.5
Germany								
Heidelberg	5.6	0.6	3.2	0.5	8.8	0.8	15.2	0.8
Potsdam	9.1	0.5	3.1	0.5	12.2	0.7	16.7	0.8
The Netherlands								
Bilthoven	6.6	0.6	1.0	0.6	7.5	0.8	6.3	0.9
United Kingdom								
General population	2.3	0.9	0.8	0.8	3.0	1.3	1.3	1.3
'Health-conscious'	0.7	1.8	0.4	1.6	1.1	2.4	1.5	2.5
Denmark								
Copenhagen	14.8	0.5	2.6	0.5	17.4	0.7	7.1	0.7
Aarhus	12.4	0.8	1.0	0.7	13.5	1.1	6.7	1.1
Sweden								
Malmö	9.8	0.5	1.2	0.5	11.0	0.7	14.9	0.8
Umeå	6.2	0.5	4.4	0.5	10.6	0.7	9.2	0.7

* Results are given as mean and standard error of mean (SEM), adjusted for energy intake and age as well as for weekday and season of the 24-hour recall assessment.

processes at high smoking temperatures (400–1000°C). The amount of PAH may vary widely depending greatly on the temperature, the type of wood used, the type of smoking (i.e. direct or indirect, hot or cold) and the use of smoke flavour additives[42]. Lung[43], pancreatic[44] and gastric cancer[45] were positively associated with the consumption of smoked meat. The differences in smoked PM intake described in this study would enable valid risk estimation in EPIC. However, two important limitations have to be kept in mind for such analyses. First, a broad variation in PAH concentration of smoked PM products is to be expected even within a country and for the same product. Second, almost all smoked PM has also been cured. However, the practice of smoking of PM is more prevalent in middle and northern Europe than in the south of Europe, allowing distinguishing between curing and smoking effects and possible interaction between both.

Associations between meat cooking and colon cancer[46,47], lung cancer[7,48], breast cancer[49,50], stomach cancer[51] and prostate cancer[52] were seen. The production of HCA during cooking at high temperatures or PAH during grilling and barbecuing is suspected to be responsible for the carcinogenic effect[15,16]. Accordingly, positive associations between the estimated HCA intake and cancer risk of different sites have been found in some studies but not in others[47,49,52–56].

Interactions between HCM and smoking and curing of meat might further alter cancer risk. For example, carcinogens like benzo(*a*)pyrene arise from grilling over wood or charcoal as well as from smoking. Consequently, grilling smoked sausage could lead to a higher PAH intake than grilling non-smoked sausages. Additionally, the mutagenic activity of broiled meat and fish treated with nitrite is higher than that of broiled meat and fish without nitrite treatment[57]. Frying cured PM, especially bacon, increases the NOC concentration in food[29].

Iron may be associated with increased risk of colorectal cancer via several mechanisms. Haem iron from red meat and thus most PM is associated with increased endogenous NOC formation[17]. *In vitro* data support the hypothesis that inorganic iron in the gut is a risk factor during human carcinogenesis by enhancing oxidative genetic damage in human colon cells[19]. Deneo-Pellegrini *et al.*[58] observed a higher risk of rectal tumours in a case–control study in Uruguay with increasing intake of dietary iron even after adjusting for meat intake. The risk of tumours of the proximal colon was associated with an increasing intake of dietary iron in two US studies[59,60]. Blood- and

Table 5a Mean daily intake (g day^{-1}; adjusted)* of processed meat (PM) as prepared by possibly hazardous cooking methods (HCM; barbecued, fried, grilled) and preservation methods (cured, smoked) in women across 10 European countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study (24-hour recall)

	Total PM								Bacon and ham		Minced PM		Sausages		PM prepared by HCM and			
	All HCM		Barbecued		Fried		Grilled		All HCM		All HCM		All HCM		Cured		Cured and smoked	
EPIC country and centre	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Greece																		
Greece	2.1	0.8	0.5	0.2	1.0	0.7	0.7	0.3	0.3	0.2	0.3	0.4	1.3	0.6	0.7	0.5	0.3	0.4
Spain																		
Granada	3.1	1.7	0.1	0.5	3.0	1.5	0.1	0.7	0.1	0.5	0.9	0.9	0.2	1.3	2.0	1.1	0.1	0.8
Murcia	1.2	1.7	0.1	0.5	1.2	1.5	0.0	0.7	0.2	0.5	0.0	0.9	0.0	1.3	0.6	1.1	0.0	0.8
Navarra	15.5	1.8	0.0	0.5	15.6	1.6	0.0	0.7	6.1	0.5	2.4	0.9	2.9	1.4	12.0	1.2	4.6	0.8
San Sebastian	4.0	1.9	0.0	0.5	4.3	1.7	0.0	0.7	0.6	0.5	0.8	1.0	0.0	1.5	2.9	1.2	0.0	0.9
Asturias	5.1	1.7	0.5	0.5	4.6	1.5	0.0	0.6	1.0	0.4	0.0	0.8	0.8	1.3	4.9	1.1	0.7	0.8
Italy																		
Ragusa	0.8	2.6	0.0	0.7	0.2	2.3	0.7	1.0	0.2	0.7	0.0	1.3	0.1	2.0	1.0	1.6	0.0	1.2
Naples	3.2	1.5	0.0	0.4	2.8	1.3	0.4	0.6	0.5	0.4	0.1	0.7	1.5	1.1	3.1	1.0	0.1	0.7
Florence	1.1	1.1	0.0	0.3	0.2	0.9	0.9	0.4	0.0	0.3	0.0	0.5	0.7	0.8	1.0	0.7	0.1	0.5
Turin	0.9	1.5	0.4	0.4	0.3	1.3	0.2	0.6	0.1	0.4	0.0	0.8	0.2	1.2	0.8	1.0	0.1	0.7
Varese	0.7	1.1	0.0	0.3	0.3	0.9	0.4	0.4	0.2	0.3	0.0	0.5	0.3	0.8	0.6	0.7	0.1	0.5
France																		
South coast	2.5	1.2	0.6	0.3	0.2	1.1	1.8	0.5	0.2	0.3	0.1	0.6	2.1	0.9	2.2	0.8	0.6	0.6
South	3.2	0.8	0.1	0.2	0.9	0.7	2.2	0.3	0.3	0.2	0.1	0.4	2.5	0.6	2.3	0.5	0.6	0.4
North-west	3.9	1.2	1.3	0.3	0.0	1.1	2.6	0.5	1.0	0.3	0.0	0.4	2.9	0.9	3.7	0.8	1.3	0.5
North-east	2.4	0.7	0.3	0.1	0.4	0.6	1.6	0.3	0.4	0.2	0.1	0.3	1.7	0.5	1.5	0.4	0.4	0.3
Germany																		
Heidelberg	9.5	0.9	0.1	0.3	8.0	0.8	1.5	0.4	0.3	0.2	3.8	0.5	5.5	0.7	1.0	0.6	0.5	0.4
Potsdam	11.0	0.9	2.2	0.3	8.1	0.8	0.6	0.4	0.4	0.2	4.1	0.5	6.5	0.7	1.1	0.6	0.5	0.4
The Netherlands																		
Bilthoven	10.3	0.9	0.3	0.3	9.6	0.8	0.4	0.4	2.5	0.2	1.1	0.5	4.6	0.7	3.1	0.6	2.3	0.4
Utrecht	10.6	0.7	0.2	0.2	9.6	0.6	0.8	0.3	2.6	0.2	1.2	0.3	4.9	0.5	4.1	0.4	2.4	0.3
United Kingdom																		
General population	7.8	1.3	0.8	0.4	1.8	1.1	5.3	0.5	3.5	0.3	0.6	0.6	0.3	1.0	3.7	0.8	3.5	0.6
'Health-conscious'	2.1	2.1	0.0	0.6	0.0	1.9	2.1	0.8	1.9	0.6	0.0	1.1	0.0	1.6	1.9	1.4	1.9	1.0
Denmark																		
Copenhagen	4.0	0.8	0.2	0.2	3.4	0.7	0.3	0.3	0.7	0.2	0.1	0.4	3.0	0.6	0.0	0.5	0.0	0.4
Aarhus	3.8	1.3	0.3	0.4	2.6	1.2	0.9	0.5	1.1	0.4	0.0	0.7	2.9	1.0	0.0	0.9	0.0	0.6
Sweden																		
Malmö	10.0	0.7	0.5	0.2	7.5	0.7	2.0	0.3	1.4	0.2	1.6	0.4	6.3	0.6	7.5	0.5	7.3	0.3
Umeå	11.5	0.8	0.3	0.2	9.2	0.7	2.0	0.3	0.9	0.2	3.1	0.4	6.2	0.6	7.0	0.5	6.9	0.3
Norway																		
South & East	16.0	0.9	1.6	0.3	13.9	0.8	0.5	0.3	0.9	0.2	9.9	0.4	5.4	0.7	9.3	0.6	5.4	0.4
North & West	13.2	1.2	0.6	0.3	12.5	1.0	0.2	0.4	0.6	0.3	8.7	0.6	4.0	0.9	8.2	0.8	4.3	0.5

*Results are given as mean and standard error of mean (SEM), adjusted for energy intake and age as well as for weekday and season of the 24-hour recall assessment.

Table 5b Mean daily intake (g day^{-1}; adjusted)* of processed meat (PM) as prepared by possibly hazardous cooking methods (HCM; barbecued, fried, grilled) and preservation methods (cured, smoked) in men across eight European countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study (24-hour recall)

	Total PM								Bacon and ham		Minced PM		Sausages		PM prepared by HCM and			
	All HCM		Barbecued		Fried		Grilled		All HCM		All HCM		All HCM		Cured		Cured and smoked	
EPIC country and centre	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Greece																		
Greece	3.8	1.4	1.1	0.5	1.7	1.2	1.0	0.4	0.3	0.3	0.5	0.6	2.6	1.1	1.1	0.7	0.7	0.5
Spain																		
Granada	17.0	3.4	7.7	1.3	9.4	3.0	0.0	1.0	0.0	0.7	0.0	1.4	0.0	2.8	5.1	1.7	0.0	1.3
Murcia	6.1	3.2	3.4	1.3	2.9	2.8	0.0	1.0	0.0	0.7	0.0	1.3	1.3	2.6	3.0	1.6	0.2	1.2
Navarra	33.3	2.4	0.0	0.9	33.6	2.1	0.0	0.7	8.4	0.5	4.8	1.0	12.5	1.9	16.1	1.2	6.3	0.9
San Sebastian	5.6	2.3	0.0	0.9	6.6	2.0	0.0	0.7	1.4	0.5	2.3	1.0	0.0	1.8	4.0	1.1	0.0	0.9
Asturias	5.6	2.6	0.0	1.0	5.7	2.3	0.0	0.8	1.2	0.5	1.1	1.1	1.5	2.0	3.0	1.2	0.4	0.9
Italy																		
Ragusa	4.4	3.9	0.7	1.5	0.0	3.4	3.9	1.2	0.0	0.8	0.0	1.6	0.5	3.1	4.5	1.9	0.0	1.4
Florence	1.4	3.0	0.0	1.2	0.0	2.7	1.5	0.9	0.1	0.6	0.0	1.3	0.0	2.4	1.7	1.5	0.0	1.1
Turin	2.3	1.9	0.0	0.8	2.1	1.7	0.2	0.6	0.2	0.4	0.0	0.8	0.4	1.5	1.9	0.9	0.0	0.7
Varese	0.0	2.8	0.0	1.1	0.0	2.4	0.1	0.8	0.0	0.6	0.0	1.2	0.0	2.2	0.0	1.3	0.0	1.0
Germany																		
Heidelberg	19.0	1.6	1.0	0.6	15.8	1.4	2.2	0.5	0.8	0.3	5.3	0.7	12.8	1.3	3.5	0.8	0.8	0.6
Potsdam	25.5	1.4	5.1	0.6	18.7	1.3	1.7	0.4	1.0	0.3	9.4	0.6	15.1	1.1	3.6	0.7	1.7	0.5
The Netherlands																		
Bilthoven	14.7	1.6	0.6	0.6	13.0	1.4	1.1	0.5	3.4	0.3	2.1	0.7	7.6	1.3	4.4	0.8	2.9	0.6
United Kingdom																		
General population	15.5	2.5	2.7	1.0	3.8	2.2	9.0	0.8	4.8	0.5	1.2	1.0	1.0	2.0	5.1	1.2	5.0	0.9
'Health-conscious'	5.7	4.7	0.2	1.8	2.0	4.1	3.4	1.4	2.6	1.0	1.3	2.0	0.9	3.8	3.0	2.3	2.7	1.7
Denmark																		
Copenhagen	7.5	1.4	0.4	0.5	6.1	1.2	0.9	0.4	0.7	0.3	0.0	0.6	6.4	1.1	0.0	0.7	0.0	0.5
Aarhus	7.8	2.1	0.0	0.8	6.6	1.9	1.4	0.6	2.7	0.4	0.0	0.9	5.2	1.7	0.0	1.0	0.0	0.8
Sweden																		
Malmö	14.2	1.4	0.7	0.5	12.2	1.2	1.2	0.4	1.3	0.3	3.2	0.6	8.6	1.1	10.0	0.7	9.7	0.5
Umeå	19.9	1.4	0.6	0.5	17.1	1.2	2.2	0.4	1.4	0.3	3.9	0.6	13.1	1.1	12.7	0.7	12.6	0.5

*Results are given as mean and standard error of mean (SEM), adjusted for energy intake and age as well as for weekday and season of the 24-hour recall assessment.

liver-containing PM are rich in iron and seem to be an important source of dietary iron intake at least in some centres of middle and northern Europe (Tables 4a and 4b). Iron may also act as a potent oxidant in foods themselves by forming lipid oxidation products that are absorbed in the human gut[61]. Kato *et al.*[59] noticed a significantly higher risk among subjects with a high fat as well as a high iron intake.

It is known that cholesterol oxidation products are formed during preparation and storage of PM in various amounts[18,20,21] and although a meta-analysis of prospective studies concluded that dietary cholesterol does not significantly contribute to lung cancer risk[62], in a recent nested case–control study plasma 7β-hydroxycholesterol concentrations before onset of disease were significantly associated with lung cancer risk[22]. The plasma concentration of 7β-hydroxycholesterol increased significantly the more meat, including PM, was consumed. Cholesterol oxidation products, rather than cholesterol, in PM may be important in cancer risk[63].

The higher consumption of PM in the cohorts of north and central Europe coincides with higher rates of colorectal cancers in these countries, while rates are considerably lower in southern European countries[40]. This would fit with the results of recent meta-analyses showing that PM consumption is positively associated with colorectal cancer risk[2,3]. The search for causal factors behind this association is, however, an enormous challenge. Although biological explanations are provided for several aspects of PM intake, prospective epidemiological studies are needed to confirm these hypotheses. EPIC offers the opportunity to investigate these questions in a study setting with a large sample size combined with strongly varying dietary habits in between the south and the north of Europe. Calibration of the country-specific dietary questionnaires used in EPIC by means of data shown here using detailed 24-hour recalls will be of important added benefit in investigating the relationship between PM and cancer incidence.

Acknowledgements

We wish to thank all study participants for their co-operation and all the interviewers who participated in the fieldwork studies in each EPIC centre. In addition, the assistance of national experts in meat science and technology to categorise different PM items according to the usual practice of production is kindly acknowledged.

The work described in the paper was carried out with the financial support of the 'Europe Against Cancer' Programme of the European Commission (SANCO); Ligue contre le Cancer (France); Société 3M (France); Mutuelle Générale de l'Education Nationale; Institut National de la Santé et de la Recherche Médicale (INSERM); Institut Gustave Roussy; German Cancer Aid; German Cancer Research Center; German Federal Ministry of Education and Research; Danish Cancer Society; Health Research Fund (FIS) of the Spanish Ministry of Health; the Spanish Regional Governments of Andalucia, Asturia, Basque Country, Murcia and Navarra; Cancer Research UK; Medical Research Council, UK; the Stroke Association, UK; British Heart Foundation; Department of Health, UK; Food Standards Agency, UK; the Wellcome Trust, UK; Greek Ministry of Health; Greek Ministry of Education; Italian Association for Research on Cancer; Italian National Research Council; Dutch Ministry of Public Health, Welfare and Sports; Dutch Prevention Funds; LK Research Funds; Dutch ZON (Zorg Onderzoek Nederland); World Cancer Research Fund; Swedish Cancer Society; Swedish Scientific Council; Regional Government of Skane, Sweden; Norwegian Cancer Society; the Norwegian Research Council.

References

1 World Cancer Research Fund (WCRF)/American Institute for Cancer Research (AICR). *Food, Nutrition and the Prevention of Cancer: A Global Perspective*. Washington, DC: WCRF/AICR, 1997.
2 Norat T, Lukanova A, Ferrari P, Riboli E. Meat consumption and colorectal cancer risk: dose–response meta-analysis of epidemiological studies. *International Journal of Cancer* 2002; **98**(2): 241–56.
3 Sandhu MS, White IR, McPherson K. Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analytical approach. *Cancer Epidemiology, Biomarkers & Prevention* 2001; **10**(5): 439–46.
4 Ngoan LT, Mizoue T, Fujino Y, Tokui N, Yoshimura T. Dietary factors and stomach cancer mortality. *British Journal of Cancer* 2002; **87**(1): 37–42.
5 Knekt P, Jarvinen R, Dich J, Hakulinen T. Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and *N*-nitroso compounds: a follow-up study. *International Journal of Cancer* 1999; **80**(6): 852–6.
6 Palli D, Russo A, Ottini L, Masala G, Saieva C, Amorosi A, *et al*. Red meat, family history, and increased risk of gastric cancer with microsatellite instability. *Cancer Research* 2001; **61**(14): 5415–9.
7 Deneo-Pellegrini H, De Stefani E, Ronco A, Mendilaharsu M, Carzoglio JC. Meat consumption and risk of lung cancer; a case–control study from Uruguay. *Lung Cancer* 1996; **14**(2–3): 195–205.
8 Bosetti C, La Vecchia C, Talamini R, Negri E, Levi F, Dal Maso L, *et al*. Food groups and laryngeal cancer risk: a case–control study from Italy and Switzerland. *International Journal of Cancer* 2002; **100**(3): 355–60.
9 Levi F, Pasche C, Lucchini F, Franceschi S, Monnier P, La Vecchia C. Food groups and oesophageal cancer risk in Vaud, Switzerland. *European Journal of Cancer Prevention* 2000; **9**(4): 257–63.
10 Schuurman AG, van den Brandt PA, Dorant E, Goldbohm RA. Animal products, calcium and protein and prostate cancer risk in The Netherlands Cohort Study. *British Journal of Cancer* 1999; **80**(7): 1107–13.
11 Michaud DS, Augustsson K, Rimm EB, Stampfer MJ, Willett WC, Giovannucci E. A prospective study on intake of animal products and risk of prostate cancer. *Cancer Causes & Control* 2001; **12**(6): 557–67.
12 Wilkens LR, Kadir MM, Kolonel LN, Nomura AM, Hankin JH. Risk factors for lower urinary tract cancer: the role of total

fluid consumption, nitrites and nitrosamines, and selected foods. *Cancer Epidemiology, Biomarkers & Prevention* 1996; **5**(3): 161–6.
13 Blot WJ, Henderson BE, Boice JD Jr. Childhood cancer in relation to cured meat intake: review of the epidemiological evidence. *Nutrition and Cancer* 1999; **34**(1): 111–8.
14 Pogoda JM, Preston-Martin S. Maternal cured meat consumption during pregnancy and risk of paediatric brain tumour in offspring: potentially harmful levels of intake. *Public Health Nutrition* 2001; **4**(2): 183–9.
15 Skog KI, Johansson MA, Jagerstad MI. Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. *Food and Chemical Toxicology* 1998; **36**(9–10): 879–96.
16 Larsson BK, Sahlberg GP, Eriksson AT, Busk LA. Polycyclic aromatic hydrocarbons in grilled food. *Journal of Agricultural and Food Chemistry* 1983; **31**(4): 867–73.
17 Cross AJ, Pollock JR, Bingham SA. Haem, not protein or inorganic iron, is responsible for endogenous intestinal *N*-nitrosation arising from red meat. *Cancer Research* 2003; **63**(10): 2358–60.
18 Echarte M, Ansorena D, Astiasaran I. Fatty acid modifications and cholesterol oxidation in pork loin during frying at different temperatures. *Journal of Food Protection* 2001; **64**(7): 1062–6.
19 Glei M, Latunde-Dada GO, Klinder A, Becker TW, Hermann U, Voigt K, *et al*. Iron-overload induces oxidative DNA damage in the human colon carcinoma cell line HT29 clone 19A. *Mutation Research* 2002; **519**(1–2): 151–61.
20 Osada K, Hoshina S, Nakamura S, Sugano M. Cholesterol oxidation in meat products and its regulation by supplementation of sodium nitrite and apple polyphenol before processing. *Journal of Agricultural and Food Chemistry* 2000; **48**(9): 3823–9.
21 Paniangvait P, King AJ, Jones AD, German BG. Cholesterol oxides in foods of animal origin. *Journal of Food Science* 1995; **60**: 1159–74.
22 Linseisen J, Wolfram G, Miller AB. Plasma 7β-hydroxycholesterol as a possible predictor of lung cancer risk. *Cancer Epidemiology, Biomarkers & Prevention* 2002; **11**(12): 1630–7.
23 Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, *et al*. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutrition* 2002; **5**(6B): 1113–24.
24 Slimani N, Kaaks R, Ferrari P, Casagrande C, Clavel-Chapelon F, Lotze G, *et al*. European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study: rationale, design and population characteristics. *Public Health Nutrition* 2002; **5**(6B): 1125–45.
25 Slimani N, Deharveng G, Charrondiere RU, van Kappel AL, Ocke MC, Welch A, *et al*. Structure of the standardized computerized 24-h diet recall interview used as reference method in the 22 centers participating in the EPIC project. European Prospective Investigation into Cancer and Nutrition. *Computer Methods and Programs in Biomedicine* 1999; **58**(3): 251–66.
26 Linseisen J, Kesse E, Slimani N, Bueno-De-Mesquita HB, Ocke MC, Skeie G, *et al*. Meat consumption in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts: results from 24-hour dietary recalls. *Public Health Nutrition* 2002; **5**(6B): 1243–58.
27 Rohrmann S, Linseisen J, Becker N, Norat T, Sinha R, Skeie G, *et al*. Cooking of meat and fish in Europe – results from the European Prospective Investigation into Cancer and Nutrition (EPIC). *European Journal of Clinical Nutrition* 2002; **56**(12): 1216–30.
28 Ferrari P, Slimani N, Ciampi A, Trichopoulou A, Naska A, Lauria C, *et al*. Evaluation of under- and overreporting of energy intake in the 24-hour diet recalls in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public Health Nutrition* 2002; **5**(6B): 1329–45.
29 Lijinsky W. *N*-Nitroso compounds in the diet. *Mutation Research* 1999; **443**(1–2): 129–38.
30 Haorah J, Zhou L, Wang X, Xu G, Mirvish SS. Determination of total *N*-nitroso compounds and their precursors in frankfurters, fresh meat, dried salted fish, sauces, tobacco, and tobacco smoke particulates. *Journal of Agricultural and Food Chemistry* 2001; **49**(12): 6068–78.
31 Bingham SA, Hughes R, Cross AJ. Effect of white versus red meat on endogenous *N*-nitrosation in the human colon and further evidence of a dose response. *Journal of Nutrition* 2002; **132**(Suppl. 11): 3522S–5S.
32 Rowland IR, Granli T, Bockman OC, Key PE, Massey RC. Endogenous *N*-nitrosation in man assessed by measurement of apparent total *N*-nitroso compounds in faeces. *Carcinogenesis* 1991; **12**(8): 1395–401.
33 Penttila PL, Rasanen L, Kimppa S. Nitrate, nitrite, and *N*-nitroso compounds in Finnish foods and the estimation of the dietary intakes. *Zeitschrift fur Lebensmittel-Untersuchung und -Forschung* 1990; **190**(4): 336–40.
34 Lowik MR. Possible use of food consumption surveys to estimate exposure to additives. *Food Additives and Contaminants* 1996; **13**(4): 427–41.
35 Honikel KO. *The Use of Additives in Meat Products Throughout Europe. Necessity, Customs, Legislation*. Utrecht: ECCEAMST, 1995.
36 Dich J, Jarvinen R, Knekt P, Penttila PL. Dietary intakes of nitrate, nitrite and NDMA in the Finnish Mobile Clinic Health Examination Survey. *Food Additives and Contaminants* 1996; **13**(5): 541–52.
37 Meah MN, Harrison N, Davies A. Nitrate and nitrite in foods and the diet. *Food Additives and Contaminants* 1994; **11**(4): 519–32.
38 Vaessen HA, Schothorst RC. The oral nitrate and nitrite intake in The Netherlands: evaluation of the results obtained by HPIC analysis of duplicate 24-hour diet samples collected in 1994. *Food Additives and Contaminants* 1999; **16**(5): 181–8.
39 Borawska M, Markiewicz R, Witkowska A. Nitrate and nitrite content in daily hospital diets during the winter season – comparison of analytical and calculation methods. *European Journal of Clinical Nutrition* 1998; **52**(7): 489–93.
40 Parkin DM, Whelan SL, Ferlay J, Raymond L, eds. *Cancer Incidence in Five Continents. Vol. II*. Lyon: International Agency for Research on Cancer, 1997.
41 Palli D. Epidemiology of gastric cancer: an evaluation of available evidence. *Journal of Gastroenterology* 2000; **35**(Suppl. 12): 84–9.
42 Simko P. Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* 2002; **770**(1–2): 3–18.
43 Hu J, Mao Y, Dryer D, White K. Canadian Cancer Registries Epidemiology Research Group. Risk factors for lung cancer among Canadian women who have never smoked. *Cancer Detection and Prevention* 2002; **26**(2): 129–38.
44 Ji BT, Chow WH, Gridley G, Mclaughlin JK, Dai Q, Wacholder S, *et al*. Dietary factors and the risk of pancreatic cancer: a case–control study in Shanghai China. *Cancer Epidemiology, Biomarkers & Prevention* 1995; **4**(8): 885–93.
45 Sanchez-Diez A, Hernandez-Mejia R, Cueto-Espinar A. Study of the relation between diet and gastric cancer in a rural area of the Province of Leon, Spain. *European Journal of Epidemiology* 1992; **8**(2): 233–7.
46 Probst-Hensch NM, Sinha R, Longnecker MP, Witte JS, Ingles SA, Frankl HD, *et al*. Meat preparation and colorectal adenomas in a large sigmoidoscopy-based case–control study in California (United States). *Cancer Causes & Control* 1997; **8**(2): 175–83.

47 Butler LM, Sinha R, Millikan RC, Martin CF, Newman B, Gammon MD, *et al*. Heterocyclic amines, meat intake, and association with colon cancer in a population-based study. *American Journal of Epidemiology* 2003; **157**(5): 434–45.
48 Sinha R, Kulldorff M, Curtin J, Brown CC, Alavanja MC, Swanson CA. Fried, well-done red meat and risk of lung cancer in women (United States). *Cancer Causes & Control* 1998; **9**(6): 621–30.
49 Sinha R, Gustafson DR, Kulldorff M, Wen WQ, Cerhan JR, Zheng W. 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, a carcinogen in high-temperature-cooked meat, and breast cancer risk. *Journal of the National Cancer Institute* 2000; **92**(16): 1352–4.
50 Zheng W, Gustafson DR, Sinha R, Cerhan JR, Moore D, Hong CP, *et al*. Well-done meat intake and the risk of breast cancer. *Journal of the National Cancer Institute* 1998; **90**(22): 1724–9.
51 Ward MH, Sinha R, Heineman EF, Rothman N, Markin R, Weisenburger DD, *et al*. Risk of adenocarcinoma of the stomach and esophagus with meat cooking method and doneness preference. *International Journal of Cancer* 1997; **71**(1): 14–9.
52 Norrish AE, Ferguson LR, Knize MG, Felton JS, Sharpe SJ, Jackson RT. Heterocyclic amine content of cooked meat and risk of prostate cancer. *Journal of the National Cancer Institute* 1999; **91**(23): 2038–44.
53 Cross AJ, Peters U, Hayes RB, Andriole GL, Reding D, Sinha R. Heterocyclic amines formed in meat cooked at high temperatures may increase prostate cancer risk. Paper presented at the *95th Annual Meeting of the American Association for Cancer Research*, Orlando, FL, 27–31 March 2004; 45.
54 Le Marchand L, Hankin JH, Pierce LM, Sinha R, Nerurkar PV, Franke AA, *et al*. Well-done red meat, metabolic phenotypes and colorectal cancer in Hawaii. *Mutation Research* 2002; **506–507**: 205–14.
55 Sinha R, Kulldorff M, Swanson CA, Curtin J, Brownson RC, Alavanja MC. Dietary heterocyclic amines and the risk of lung cancer among Missouri women. *Cancer Research* 2000; **60**(14): 3753–6.
56 Augustsson K, Skog K, Jagerstad M, Dickman PW, Steineck G. Dietary heterocyclic amines and cancer of the colon, rectum, bladder, and kidney: a population-based study. *Lancet* 1999; **353**(9154): 703–7.
57 Kangsadalampai K, Butryee C, Manoonphol K. Direct mutagenicity of the polycylic aromatic hydrocarbon-containing fraction of smoked and charcoal-broiled foods treated with nitrite in acid solution. *Food and Chemical Toxicology* 1997; **35**(2): 213–8.
58 Deneo-Pellegrini H, De Stefani E, Boffetta P, Ronco A, Mendilaharsu M. Dietary iron and cancer of the rectum: a case–control study in Uruguay. *European Journal of Cancer Prevention* 1999; **8**(6): 501–8.
59 Kato I, Dnistrian AM, Schwartz M, Toniolo P, Koenig K, Shore RE, *et al*. Iron intake, body iron stores and colorectal cancer risk in women: a nested case–control study. *International Journal of Cancer* 1999; **80**(5): 693–8.
60 Wurzelmann JI, Silver A, Schreinemachers DM, Sandler RS, Everson RB. Iron intake and the risk of colorectal cancer. *Cancer Epidemiology, Biomarkers & Prevention* 1996; **5**(7): 503–7.
61 Sesink AL, Termont DS, Kleibeuker JH, Van der Meer R. Red meat and colon cancer: the cytotoxic and hyperproliferative effects of dietary heme. *Cancer Research* 1999; **59**(22): 5704–9.
62 Smith-Warner SA, Ritz J, Hunter DJ, Albanes D, Beeson WL, van den Brandt PA, *et al*. Dietary fat and risk of lung cancer in a pooled analysis of prospective studies. *Cancer Epidemiology, Biomarkers & Prevention* 2002; **11**(10 Pt 1): 987–92.
63 Schroepfer GJ Jr. Oxysterols: modulators of cholesterol metabolism and other processes. *Physiological Review* 2000; **80**(1): 361–554.

3. Méthodes de cuisson de la viande et du poisson dans des populations de l'Europe-résultats de l'Etude Prospective Européenne sur la Nutrition et le Cancer (EPIC).

Basé sur:

Cooking of meat and fish in Europe-results from the European Prospective Investigation into Cancer and Nutrition (EPIC). Eur J Clin Nutr. 2002 ;56 :1216-30

Il a été suggéré que les agents responsables de l'association entre la viande rouge et le cancer colorectal sont les amines hétérocycliques (HCA), des substances potentiellement cancérogènes formées dans la viande et le poisson pendant leur cuisson par réaction de Maillard à partir de la créatinine, des acides aminés et des sucres (144). L'effet cancérogène des différents membres de la famille des HCAs a été démontrée dans plusieurs modèles animaux (145) mais il n'a pas été démontrée chez les humains. D'autre part, des hydrocarbures polycycliques aromatisés (PAHs) dont certains ont une activité cancérogène importante, ont été détectés dans les viandes et les poissons cuits en barbecue, en particulier quand le combustible utilisé est le bois. Les PAHs sont des polluants qui se retrouvent dans plusieurs aliments, y compris les légumes, et dans la fumée du tabac. Leur ubiquité rend la mesure de l'exposition aux PAHs par voie alimentaire extrêmement difficile.

Aujourd'hui, il semble que la mesure indirecte la plus appropriée pour estimer l'exposition aux HCAs et PAHs par voie alimentaire dans les études épidémiologiques est la méthode de cuisson (131). Les valeurs de consommation de la viande et du poisson selon méthode de cuisson ainsi que leur fréquence d'utilisation n'a pas été systématiquement analysée en Europe. Nous avons donc réalisé une étude descriptive comprenant les populations de la cohorte EPIC de neuf pays de l'Europe.

Pour cette étude, nous avons utilisé les données des rappels de 24 heures collectées sur un échantillon représentatif de la population de la cohorte EPIC à l'aide du logiciel EPIC-Soft,

spécialement conçu pour obtenir une information standardisée sur la consommation d'aliments ainsi que sur leurs méthodes de préparation (146). Pour les méthodes de cuisson, en particulier, 29 descripteurs de cuisson ont été intégrés dans le logiciel (Tableau 1).

En total, 60850 événements de consommation de viande et de poisson pour 35644 sujets dans l'étude ont été analysés (Tableau 2). Les méthodes de cuisson par friture, en bouillon, au four, par grillade, la cuisson à l'étuvée et la cuisson par friture rapide « *stir-frying* » représentent 62% des méthodes utilisés dans la population étudiée (Tableau 2, Figure 1). La fréquence d'utilisation des méthodes de cuisson à haute température - grillade et friture – supposées être en relation avec le contenu des HCAs dans la viande est plus bas (11%) dans les centres du nord de l'Italie et plus haut dans les centres du Pays-Bas (45.6%). Une autre méthode de cuisson à haute température, le barbecue, est peu utilisée en générale dans les populations EPIC, excepté en Grèce, où 8% de la viande et du poisson consommés étaient cuits au barbecue (Tableau 3, Figure 2).

En ce qui concerne la viande rouge cuite par friture –bœuf, veau, agneau, porc- les apports étaient plus élevés dans les populations du nord de l'Europe, ainsi que du nord de l'Espagne et à Naples (Italie) [Figure 3]. Chez les femmes françaises des centres EPIC situées au nord de la France, une proportion importante de la viande rouge a été cuite au four, alors que dans les centres du sud de la France, la plupart de la viande rouge était cuite par « *stir frying* ». Les volailles ont été cuites au four dans la plupart de centres, sauf en Allemagne, au Danemark et au Pays-Bas, où la friture est la méthode la plus utilisée (Tableaux 4 et 5).

Les méthodes de cuisson qui conduisent à la formation de quantités élevées de HCAs et PHAs sont en général moins utilisées pour cuire le poisson que pour cuire les viandes. Dans tous les centres, le poisson est préparé pané et frit ou bouilli, sauf dans les centres du nord de l'Espagne où une partie importante du poisson est cuit par friture.

Pour l'interprétation des nos résultats, il faut tenir compte que les erreurs de mesure de l'alimentation ne sont pas de la même valeur ni de la même direction entre les centres. Par exemple, la sous-estimation de la consommation de viande semble plus importante en Grèce et dans les centres du sud de l'Espagne (147) (148). D'autre part, l'étude EPIC a comme avantage que la définition des méthodes de cuisson a été standardisée préalablement à la collection de données, ce qui confère un degré important de comparabilité à nos données.

Notre étude est la première qui décrit les apports de viande et de poisson selon la méthode de cuisson dans des populations européennes. Nos données montrent que les habitudes alimentaires varient selon le pays et parfois à l'intérieur d'un même pays. Les résultats les plus remarquables sont l'utilisation élevée de la friture comme méthode de cuisson dans les pays du nord de l'Europe, en contraste avec la plus faible utilisation des méthodes « nocives» au sud de l'Europe.

Il existe des disparités géographiques des taux d'incidence de cancers en Europe, caractérisés par un gradient nord-sud. Par exemple, les taux de cancer colorectal et du sein sont plus élevées au nord de l'Europe, alors que les taux de cancer de l'estomac sont plus élevés dans les populations du sud de l'Europe (5). Il reste à déterminer si les méthodes de cuisson de la viande jouent un rôle sur cette disparité.

Les résultats de cette étude seront utilisés pour développer un nouveau questionnaire alimentaire relatif aux méthodes de cuisson et qui devra être implémenté dans une deuxième mesure de l'alimentation prévue dans l'étude EPIC.

European Journal of Clinical Nutrition (2002) 56, 1216–1230
 0954–3007/02 $25.00
www.nature.com/ejcn

ORIGINAL COMMUNICATION

Cooking of meat and fish in Europe — results from the European Prospective Investigation into Cancer and Nutrition (EPIC)

S Rohrmann[1]*, J Linseisen[1], N Becker[1], T Norat[2], R Sinha[3], G Skeie[4], E Lund[4], C Martínez[5], A Barricarte[6], I Mattisson[7], G Berglund[8], A Welch[9], G Davey[10], K Overvad[11], A Tjønneland[12], F Clavel-Chapelon[13], E Kesse[13], G Lotze[1], K Klipstein-Grobusch[14], E Vasilopoulou[15], E Polychronopoulos[15], V Pala[16], E Celentano[17], HB Bueno-de-Mesquita[18], PHM Peeters[19], E Riboli[2] and N Slimani[2]

[1]*Division of Clinical Epidemiology, Deutsches Krebsforschungszentrum, Heidelberg, Germany;* [2]*Unit of Nutrition and Cancer, International Agency for Research on Cancer, Lyon, France;* [3]*Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, USA;* [4]*Institute of Community Medicine, University of Tromsø, Tromsø, Norway;* [5]*Granada Cancer Registry, Escuela Andaluza de Salud Pública, Granada, Spain;* [6]*Department of Health Sciences, Navarra Public University, Pamplona, Spain;* [7]*Department of Medicine, Surgery and Orthopaedics, Lund University, Malmö, Sweden;* [8]*Department of Public Health and Clinical Medicine, Umea University, Umea, Sweden;* [9]*Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK;* [10]*Cancer Epidemiology Unit, Imperial Cancer Research Fund, Oxford, UK;* [11]*Department of Epidemiology and Social Medicine, University of Aarhus, Denmark;* [12]*Danish Cancer Society, Copenhagen, Denmark;* [13]*Institut Gustave Roussy, INSERM, Villejuif, France;* [14]*Department of Epidemiology, German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany;* [15]*Department of Hygiene and Epidemiology, University of Athens, Athens, Greece;* [16]*Epidemiology Unit, Italian National Cancer Institute, Milan, Italy;* [17]*Epidemiology Unit, Italian National Cancer Institute, Naples, Italy;* [18]*Department of Chronic Diseases Epidemiology, National Institute for Public Health and the Environment, Bilthoven, The Netherlands; and* [19]*Julius Center for Patient Oriented Research, University Medical Center, Utrecht, The Netherlands*

Objectives: There is epidemiologic evidence that the consumption of fried, grilled or barbecued meat and fish that are well-done or browned may be associated with an increased cancer risk. These high-temperature cooking methods are thought to be surrogates for mutagens and carcinogens produced in meat and fish, eg heterocyclic amines or polycyclic hydrocarbons. Since data on food cooking methods are scarce, the aim of this study was to describe the variation in meat and fish cooking methods in different parts of Europe.

Design: Using a standardized 24 h recall from a sub-sample of the EPIC cohort (35 644 persons, 35 – 75 y old), mean daily intake of meat and fish prepared by different cooking methods and the relative contribution of the cooking methods to the overall cooking of meat and fish was calculated.

Results: Whereas frying was more often noted in northern Europe, roasting and stir frying were more often used in the south. Concerning high-temperature cooking methods, their frequency of application varies between 15% in the EPIC cohort of North-Italy and 49% in the cohort of The Netherlands. Average consumption of fried, grilled and barbecued meat and fish ranges from a low of 12 g/day in the centres in southern Spain to a high of 91 g/day in northern Spain.

***Correspondence: S Rohrmann, Johns Hopkins Bloomberg School of Public Health, Department of Epidemiology, 615 North Wolfe Street (Rm/E6133), Baltimore, MD 21205, USA.**
E-mail: srohrman@jhsph.edu
Guarantor: **S Rohrmann.**
Contributors: **ER initiated the EPIC study. SR performed data evaluation and statistical analyses, conceptualized and drafted the manuscript. JL contributed to data evaluation, statistical analyses, the concept, and writing of the paper. NB, TN, RS and NS contributed to the concept and writing of the paper. NS initiated the general concept and design of EPIC-SOFT and co-ordinated the international calibration study. The other authors were responsible for the field work and data collection and contributed to writing the paper.**
Received 3 September 2001; revised 20 March 2002; accepted 22 March 2002

Conclusion: High variation in both the kind of meat/fish consumed as well as its cooking methods is observed within EPIC. In order to use this variation for the evaluation of the impact of cooking methods on cancer risk, a questionnaire on meat and fish cooking methods is being developed and could be applied in the whole EPIC cohort.
European Journal of Clinical Nutrition (2002) **56,** 1216–1230. doi:10.1038/sj.ejcn.1601494

Keywords: EPIC; meat; fish; cooking methods; 24 h recall

Introduction

During recent decades, a possible relationship between the methods of meat and fish preparation and the risk of cancer development of different sites has been observed in epidemiological studies (Sinha *et al*, 1998,1999,2000a,b,2001; Deitz *et al*, 2000; Norrish *et al*, 1999; Zheng *et al*, 1999; De Stefani *et al*, 1997a,b,1998a,b; Probst-Hensch *et al*, 1997; Ward *et al*, 1997; Schiffman & Felton, 1990; Steineck *et al*, 1990). It has been suggested that heterocyclic amines (HCA) may explain at least some of the observed effect. HCA are formed during high temperature cooking of meat and fish from creatinine, amino acids, and sugar (Skog *et al*, 1998). These compounds can react together during cooking at high temperatures in Maillard reactions (Jägerstad *et al*, 1983). The carcinogenicity of different members of the HCA family has been proven in animal models (Adamson *et al*, 1990; Shirai *et al*, 1995). However, recent epidemiological studies concerning HCA intake gave conflicting results. While case–control studies conducted in Uruguay and in the USA showed positive correlations between the intake of HCA and the risk of breast (Sinha *et al*, 2000a; De Stefani *et al*, 1997b), colon (Sinha *et al*, 2001; De Stefani *et al*, 1997a), lung (Sinha *et al*, 2000b), and gastric cancer (De Stefani *et al*, 1998a), other groups were not able to detect any association between the intake of HCA and the cancer risk of different sites (Norrish *et al*, 1999; Augustsson *et al*, 1999b; Lyon & Mahoney, 1988).

Polycyclic aromatic hydrocarbons (PAH) have also been found in barbecued meat and fish in variable amounts, depending on the type of heat source used. Highest amounts arise when foods were barbecued over wood or cones, but lower when barbecued over charcoal (Larsson *et al*, 1983). An association between barbecued, but not fried, meat and stomach cancer suggests that dietary exposure to PAH may be involved in gastric carcinogenesis (World Cancer Research Fund, 1997). Since PAH are ubiquitous environmental contaminants and a variety of foods, including oils, grains and vegetables, contribute to PAH intake, it is not easy to calculate their intake nor to estimate the relative contribution of food cooking methods applied to meat and fish (Guillén *et al*, 1997; Rothman *et al*, 1993).

At present, assessing the use of cooking methods might be a more appropriate method in epidemiologic research than estimating the intake of single carcinogens like HCA or PAH, since reliable estimation of HCA or PAH is a difficult task. The main reason is that no standardized databases that include a large number of different foods analysed for their HCA or PAH concentration exist which could be used for the calculation of the intake. Several studies have focussed on meat and fish cooking methods. High-temperature cooking techniques, such as grilling, frying and barbecuing, are more likely to be associated with carcinogen production (Skog *et al*, 1998). Several reports, focussing on meat preparation by these high-temperature cooking methods, were able to show an increased risk of lung cancer (Sinha *et al*, 1998; Doneo-Pellegrini *et al*, 1996), breast cancer (Sinha *et al*, 2001; Zheng *et al*, 1998), prostate cancer (Norrish *et al*, 1999), colorectal tumors (Sinha *et al*, 1999; Probst-Hensch *et al*, 1997; Ward *et al*, 1997; Gerhardsson de Verdier *et al*, 1991). Apart from the cooking methods, cancer risk was shown to be influenced by the degree of browning of the cooked meat or fish item (Sinha *et al*, 1999; Zheng *et al*, 1999; Probst-Hensch *et al*, 1997; Ward *et al*, 1997). For example, Sinha *et al* (1999) computed a higher risk for colorectal adenomas when red meat was consumed well-done or very well-done in contrast to medium or rare red meat, but in two earlier studies no association between cancer risk and cooking method was observed (Muscat & Wynder, 1994; Lyon & Mahoney, 1988).

A systematic analysis of differences in meat and fish preparation methods used within Europe has not been reported so far. In this report, we describe the differences in meat and fish cooking techniques in different countries and regions in the European Prospective Investigation into Cancer and Nutrition (EPIC), obtained by means of 24 h recalls where detailed information on cooking methods was available.

Material and methods

EPIC is a multi-centre study conducted in 10 European countries with 27 study centres and about half a million participants. It focuses on the relation between diet, nutritional and metabolic characteristics, various lifestyle factors and the risk of cancer (Riboli & Kaaks, 1997). The recruited study participants were not a representative sample of the participating countries. Furthermore, no strict criteria of selection were used. For example, in Greece or Germany, participants were recruited from the general population, whereas in France only female teachers were included in the cohort. During the recruitment phase of EPIC, information about individual usual food habits and lifestyle was

obtained from each participant (Riboli, 1992). Individual long-term dietary intakes were obtained using a country-specific validated dietary assessment instrument. In order to calibrate individual estimates obtained with these country-specific methods, a single 24 h recall was obtained in a representative subgroup of about 10% of all EPIC cohorts.

A computer program, EPIC-SOFT, was developed to ensure that 24 h recalls were conducted in a standardized manner in each country (Slimani *et al*, 1999). The 24 h recall obtained information about type and quantity of foods consumed as well as details on cooking methods. Details on portion sizes were assessed using different methods such as household measurements, standard units and a picture book. In order to standardize information on the consumed food items across countries, systematic questions used for describing a given food were asked, eg cooking method, brand name or fat content. These characteristics of foods were described by so-called 'facets' (Slimani *et al*, 2000). For each facet a set of 'descriptors' was defined, allowing specifying the facet more precisely. The facet 'cooking method' was used to assess *the preparation of meat and fish items just before consumption*. In the case of 'cooking method' 29 common possible answers (descriptors) were foreseen to cover the wide within- and between-centre variations in food cooking methods (Table 1). When a person reported eating a special meat or fish item, questions (facets) on cooking methods were automatically prompted on the screen with several possible pre-defined answers (descriptors). If a person was not able to answer the question precisely, 'not specific' cooking methods could be chosen (Slimani *et al*, 2000). The cooking method 'fried' was used as a generic descriptor in EPIC-SOFT. In its original meaning it describes foods cooked in hot fat until done, and other descriptors were used to describe foods that were deep fried or stir fried. Although a certain degree of misclassification cannot be excluded, we believe that this kind of misclassification is small and frying is used in most cases in its original meaning since well-trained staff performed all interviews.

Meat and fish data were analysed by country or, as for France, Italy and Spain, by region. We decided to divide these countries into regions because of varying meat and fish cooking habits. Italy and Spain have up to five study centres, located in different regions (Riboli & Kaaks, 1997). For Italy three regions were defined: northern Italy (Florence, Varese, Turin), Naples and Ragusa. Spain was divided into southern Spain (Granada, Murcia) and northern Spain (Oviedo, Pamplona, San Sebastian). France, Norway and Greece recruited participants from all regions of these countries. For statistical analysis in this paper, we included French participants on basis of two regions: southern France and northern France. Norwegian participants were originally assigned to the centres northwest and southeast, but they were analysed by country in this study. Greek data were analysed by country too. The British participants were divided into two groups,

Table 1 Definition of cooking methods in EPIC

Cooking method[a]	*Definition*
Baked	Cooked by dry heat in an oven, covered or uncovered, no additional fat used for cooking
Barbecued	Cooked on grill bars over burning charcoal or wood
Battered and baked	Covered by batter (flour, milk, and egg mixture) and baked
Battered and fried	Covered by batter (flour, milk, and egg mixture) and fried
Boiled	Cooked in boiling liquid
Breaded and baked	Covered by an outer layer of breadcrumbs and baked
Breaded and fried	Covered by an outer layer of breadcrumbs and fried
Breaded and griddled	Covered by an outer layer of breadcrumbs and griddled
Coated and fried	Covered by an outer layer and fried: includes battered and fried, breaded and fried, in flour and fried
Deep fried	Cooked in a hot fat or oil by immersing the food entirely
Fried	Generic descriptor for cooked in heated fat, usually over a direct source of heat
Griddled	Cooked on a heated flat metal surface over a source of direct heat; a little fat or oil may be used to grease the metal surface
Grilled	Cooked rapidly without moisture, on grill bars under or over intense direct heat, no fat used
In flour and fried	Covered by an outer layer of flour and fried
Microwaved	Cooked or reheated in a microwave over; no fat used
Poached	Cooked by dropping in boiling liquid
Reheated	Made hot; no liquid nor fat is added
Roasted	Cooked by dry heat in an oven or over a fire
Shallow fried	Cooked in a shallow layer of heated fat
Steamed	Cooked by steam, in pressure cooker or cooked suspended above boiling water
Stewed	Cooked by boiling or simmering in liquid contained in an enclosed vessel; the food is cooked over a low heat for a long period of time
Stir fried/sauteed	Cooked by frying food over high heat, by stirring constantly to avoid sticking
Toasted	Cooked with direct heat until the surface of the food is browned
Cooked NS (not specified)	Generic descriptor, used in the case the subject does not know the answer
Cooking method not applicable[b]	Generic descriptor for food on which no cooking method can be applied
Raw[b]	Generic descriptor for food on which no cooking method was applied

[a]Cooking method is defined as the preparation of meat and fish items just before consumption.
[b]These descriptors do not exclude cooking outside house or preparation in another way.

the general population and health-conscious persons. The group of UK health-conscious persons ($n = 311$) was excluded from the present analysis because of their low meat consumption.

The present report deals with data concerning the consumption of prepared meat and fish (which means meat and fish with information on cooking method). In total, 60850 consumption events with information on meat and fish cooking methods from 35644 participants were used. Food items mentioned in these data sets were divided into several food classes as already defined by EPIC-SOFT.

A general view is given for the frequency of the most important cooking methods per region and, in a second step, all cooking methods per food group were analysed by region. Furthermore, mean daily intake of meat and fish prepared by different cooking methods was calculated. Mean adjusted intake was computed by using the ANACOVA procedure to adjust for age and by applying weights to ANACOVA estimates to consider the categorical variables weekday (two categories) and season (four categories). For all analyses SAS Version 6.12 (SAS Institute Inc., Cary, NC, USA) was used.

Results

Table 2 shows the distribution of study participants by sex and age. Men and women were not equally distributed because some countries and centres decided not to recruit men for different reasons (France, Norway, Naples). Age distribution differed in the participating countries as EPIC cohorts are convenience samples and no strict criteria of selection were used.

Summing up the answers of all countries, the most frequently consumed food groups of meat and fish were processed meat (including all kinds of sausages, ham, bacon, processed meat cuts, processed minced meat), fish, pork, beef, chicken, crustacean and molluscs, minced meat, lamb and turkey (in decreasing order). These food groups counted for 94.3% of all items specified and each was mentioned more than 1000 times in the 24 h recalls. They are included in the following analyses.

In Table 3, the specific cooking methods used in each region are listed. The most important were frying, boiling, baking, stewing, roasting, stir frying, grilling, as well as breading and frying. These eight cooking methods amounted to 62% of the preparation methods of meat and fish. The variation of cooking methods between the regions is large, for example frying was used for the preparation of 45.2% of meat and fish in the EPIC cohort of The Netherlands but only for 1.6% in Greek participants. Stir frying varied between 0.9% in the cohort of Norway and 20.1% in the cohort of southern France.

In Figure 1, high-temperature cooking methods that are often investigated in studies because of their possible influence on cancer risk (barbecuing, grilling, frying) are shown for the different study regions. The frequency of these three cooking methods ranged from a low of 11.5% in northern Italian centres to a high of 46.5% in the Dutch cohort. Although barbecuing was a rarely used cooking method in most EPIC regions, more than 8% of meat and fish was barbecued in Greece.

Cooking methods were not specified for 5080 meat and fish consumption events (8.3%; Table 3), varying from 0.3% in the Greek cohort to 22.0% in the UK. Even more often indicated were the descriptors 'raw' and 'cooking method not applicable', which were regrouped in this analysis and were both included in 'no cooking method applied'. This means that in 7.8 to 39.5% of the consumption events, a food was not cooked before consumption or was eaten raw; the first applies especially to processed meat items (see below).

Table 2 Study participants completing the 24-hour recall in EPIC by sex and age groups

	Women					*Men*				
Region/country[a]	*35–44*	*45–54*	*55–64*	*65–74*	*Total*	*35–44*	*45–54*	*55–64*	*65–74*	*Total*
Northern France[b]		1091	1064	475	2630					
Southern France		824	814	370	2008					
Northern Italy	181	681	958	151	1971	101	411	662	102	1276
Ragusa	38	52	41	7	138	16	73	72	7	168
Naples	29	174	174	26	403					
Northern Spain	148	320	316	55	839	136	548	515	121	1320
Southern Spain	104	226	224	50	604	33	119	243	62	457
Great Britain	64	203	174	130	571	39	116	123	126	404
Netherlands	342	1071	1084	463	2960	268	418	330	8	1024
Greece	169	390	449	366	1374	117	244	376	575	1312
Germany	552	629	941	29	2151	276	709	1197	86	2268
Sweden	256	892	1274	863	3285	113	517	1239	896	2765
Denmark		771	1144	80	1995		752	1107	64	1923
Norway	320	1200	278		1798					
Total	2203	8515	8926	3056	*22727*	1099	3907	5864	2047	*12917*

[a]Participants of EPIC recruited in each country or region are not a representative subsample of the population. The participants of the calibration study are a representative subsample of each cohort.
[b]The regions/countries include study centres as given in the figure captions.

Looking at the relative contribution of different cooking methods of meat and fish subgroups, 34.3% of the beef consumed in the EPIC centres of southern France, but only 20% in northern France or less than 1% in the Danish cohort, was stir fried (Table 4). A high variation in the use of frying with respect to all meat and fish groups can be observed. In southern regions of Europe, processed meat was more often grilled or roasted, whereas people in the north preferred frying, boiling or baking. In case of fish, crustacean and processed meat, a high percentage was consumed raw or no cooking method was applied, because the foods had already been cooked or processed when they were bought.

Mean daily intake of fried red meat, including beef, veal, lamb and pork, was high in the northern European countries as well as among the EPIC participants in northern Spain and Naples, where it ranged from 13.1 to 38.0 g in women and from 21.6 to 59.7 g in men, respectively (Table 5). In contrast, a high portion of red meat in the cohort of northern France was consumed baked, in the southern French cohort stir-fried, or stewed in Greece, Ragusa and northern Italy. Higher amounts of grilled red meat were eaten in the southern than in the northern EPIC countries (note that mean adjusted intake of barbecued red meat in Ragusa amounted to 14.8 g/day among women and that this estimate was based on the consumption of only four women; in fact unadjusted intake was 3.7 g/day).

White meat (including chicken and turkey) was predominantly consumed baked. In the centres of Germany, Denmark and The Netherlands most white meat was eaten fried. Regarding processed meat, there was no dominant cooking method with respect to the consumed amounts of these foods. Baking, boiling as well as frying were common. In the EPIC cohorts of some countries, high portions of processed meat were eaten without cooking at home (which means that no cooking method was applied before the foods were consumed).

In case of fish and crustaceans, highest amounts were consumed boiled or coated and fried. High-temperature cooking methods were less important regarding the preparation of these foods, except for the cohort of northern Spain, where men consumed 25.3 g and women 14.6 g fried fish and crustaceans per day. However, one must keep in mind that a high proportion of these foods was described by 'no cooking method applicable' or 'raw' and might have been prepared

Table 3 Use of different cooking methods (percentage of consumption events) to prepare meat and fish by region/country in EPIC assessed by 24 h recalls in 22 727 women and 12 917 men

	Percentage consumption events of specified CM														
	Northern France[a]	*Southern France*	*Northern Italy*	*Ragusa*	*Naples*	*Northern Spain*	*Southern Spain*	*Great Britain*	*Netherlands*	*Greece*	*Germany*	*Sweden*	*Denmark*	*Norway*	*Total*
Specified cooking method (*n*)[b]	3601	2527	5677	443	570	5861	2809	1258	5720	2905	7279	9052	5560	2508	55 768
Fried	5.4	4.2	5.0	4.1	19.8	25.5	9.8	8.0	45.2	1.6	19.8	27.4	38.1	26.9	21.6
Boiled	14.2	13.2	8.7	8.3	13.4	12.1	14.0	5.2	4.7	8.7	25.1	15.3	17.7	23.2	14.2
Baked	10.8	11.6	4.5	7.3	2.4	4.6	3.7	16.9	1.8	2.9	3.1	13.3	14.9	6.8	7.4
Stewed	7.8	5.5	13.2	19.0	11.7	11.8	9.2	7.2	2.3	26.3	4.3			2.4	6.5
Roasted	15.0	9.1	7.0	9.5	6.1	4.4	12.2	8.7	0.4	17.8	0.8	5.9	2.0	2.2	5.8
Stir fried	12.3	20.1	3.8	1.2	1.3	6.9	9.8	6.2	3.1	4.7	2.5	4.4	0.9	0.9	5.1
Grilled	8.5	13.8	6.3	10.2	3.7			15.2	3.2	8.0	3.3	4.5	4.1	4.5	4.7
Breaded and fried	0.5	1.2	3.4	6.6	2.2	3.3	1.0	1.2	0.7	1.0	3.7	1.6	4.4	0.2	2.2
Barbecued	3.5	1.4	0.2	2.4	1.1	1.0	0.9	2.1	0.9	8.8	1.3	2.4	0.6	2.1	1.8
Battered and fried	0.1	0.1	0.6		0.6	6.5	2.7	2.7	0.2	0.5	0.1	2.3	0.4	0.5	1.5
In flour and fried	1.8	1.4	1.3	1.2	1.8	0.4	2.2	0.2	0.1	10.2	0.1	0.2	0.7	0.3	1.2
Reheated	2.0	1.0	0.4			0.2	0.3	0.8	3.2		3.4	0.2	0.1	1.1	1.2
Griddled	0.3	0.2	4.3	3.9	5.9	2.3	0.4	1.2	0.2	0.6	1.3		0.1	1.0	1.1
Deep fried	0.1	0.1	0.8	2.0	1.7	1.1	3.9	0.5	1.8		0.1	0.4	0.5	0.1	0.8
Microwaved	2.0	1.6	0.2		0.4	0.2	0.3	2.7	0.5		0.3	0.6	0.2	0.3	0.6
Steamed	0.9	1.3	0.4	0.2		0.4	0.9	1.3	0.2		0.1		0.9	2.5	0.5
Shallow fried	0.4	0.1	0.2	0.7	0.2	1.3	2.5	0.9	0.6	1.1	0.3			0.1	0.5
Poached	0.1	0.3				0.3		1.1	0.2			0.1		1.7	0.2
Breaded and baked	0.1		0.2	0.2	0.7			2.0	0.1		0.1		0.1	0.1	0.1
No cooking method applied[c]	14.1	13.9	39.5	22.9	27.2	17.8	26.2	15.6	30.6	7.8	30.1	21.1	14.4	23.1	23.0
Cooking method not specified (*n*)	*398*	*356*	*289*	*15*	*1*	*229*	*53*	*355*	*362*	*10*	*148*	*1778*	*736*	*350*	*5080*

[a]The regions/countries include following study centres as given in the figure captions.
[b]*n* = number of consumption events.
[c]No cooking method applied: includes 'raw' and 'cooking method not applicable'.

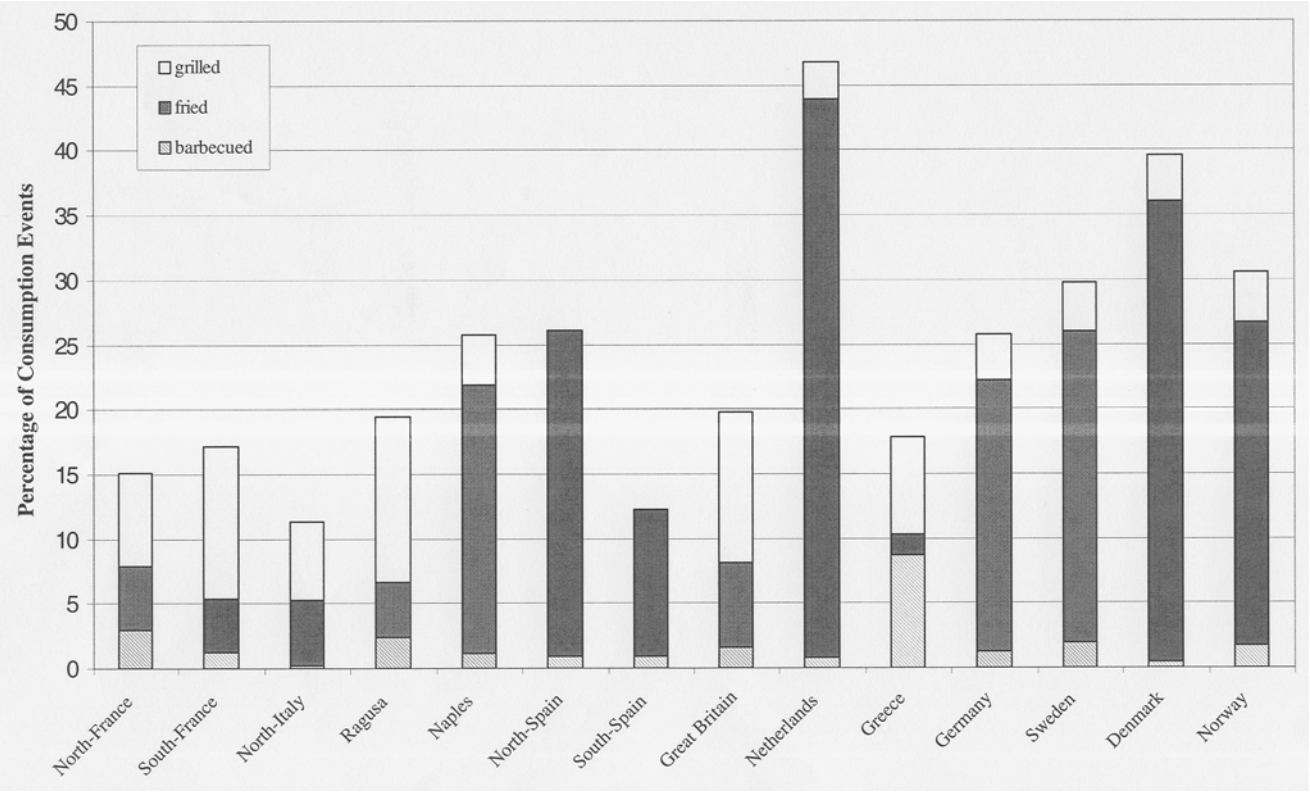


Figure 1 Frequency (percentage of consumption events) of barbecued, fried and grilled meat and fish by region/country in EPIC assessed by 24 h recalls in 22 727 women and 12 917 men. The regions/countries include the following study centres: *northern France* — north east, north west; *southern France* — south, south coast; *northern Italy* — Varese, Turin, Florence; *northern Spain* — Oviedo, Pamplona, San Sebastian; *southern Spain* — Granada, Murcia; *Great Britain* — general population; *Netherlands* — Bilthoven, Utrecht; *Germany* — Heidelberg, Potsdam; *Sweden* — Malmo, Umea; *Denmark* — Copenhagen, Aarhus; *Norway* — northwest, southeast. The cooking method 'fried' does not include deep fried and stir fried. Mean adjusted for age, season and week day.

outside the home. For these foods no information about the method of preparation outside home is available and no justified assumptions can be made.

With respect to high-temperature cooking methods (Figure 2), mean daily intake of fried, grilled and barbecued meat and fish varied between 11 g (southern Spain) and 55 g (Netherlands) for women and between 20 g (northern Italy) and 91 g (northern Spain) for men. In the northern parts of Europe as well as in the cohort of northern Spain, these high-temperature cooking methods were more often used than in the centres in France, Greece, Italy and the UK. The intake of grilled meat and fish was higher in French, Greek, Italian and British EPIC cohorts than in the north of Europe, but the consumption of fried meat and fish was lower. No grilled meat was consumed in the EPIC cohorts of Spain. Whereas the intake of fried and barbecued meat and fish was highest in northern Spain for men, the highest consumption of red meat prepared by these high-temperature cooking techniques (Figure 3) was observed in The Netherlands (women, 39.4 g/day; men, 59.7 g/day).

Discussion

This is the first study to look at meat and fish cooking methods in different Western European countries. For this study we used data of the 24 h recall that was applied in a representative sub-sample of the EPIC cohort. Detailed information how a food was consumed was assessed by means of the program EPIC-SOFT. Information on cooking methods of meat and fish was assessed by means of 29 descriptors specifying the method by which the food item was cooked by the participant just before consumption. Consequently, neither cooking methods used for preservation nor cooking nor preparation methods applied before the food has been bought were covered by this facet.

For a varying proportion of meat and fish items (ranging from 0.3% in the cohort of Greece to 22.0% in cohort in the UK) the participants were not able to recall the cooking method of the consumed food item. In these cases a non-specific cooking method was used for coding the reported answer. No information about a specific recall bias for certain cooking methods is available. However, it seems to be that

Table 4 Frequency of cooking methods (number and percentage of consumption events) most often applied to prepare meat and fish by region/country in EPIC assessed by 24 h recalls in 22 727 women and 12 917 men

Cooking method	*Northern France*[a]	*Southern France*	*Northern Italy*	*Ragusa (Italy)*	*Naples (Italy)*	*Northern Spain*	*Southern Spain*	*Great Britain*	*Netherlands*	*Greece*	*Germany*	*Sweden*	*Denmark*	*Norway*
Beef (*n*)	516	359	765	104	43	35	1	129	891	74	435	858	971	269
Baked/roasted (%)[b]	23.4	18.9	12.8	7.7	7.0	25.7	100.0	30.2	0.9	24.3	0.7	19.8	17.8	10.4
Barbecued (%)	6.6	0.8	0.1	1.9		14.3		2.3	0.2		0.5	1.9	0.7	3.0
Boiled (%)	6.6	5.0	9.9	9.6	4.7	5.7		7.8	2.7		26.9	4.2	4.1	8.2
Fried (%)[c]	12.0	4.5	8.4	7.7	41.9	14.3		8.5	82.2		40.0	39.6	65.9	55.8
Stir fried (%)	20.0	34.3	7.2	1.0	4.7			11.6	1.7		2.1	23.0	0.5	2.2
Grilled (%)	17.8	27.0	13.9	21.2	9.3	2.9		6.2	0.6		6.0	10.1	5.6	
Stewed (%)	9.7	7.5	23.7	21.2	11.6	11.4		28.7	8.6	74.3	21.4	0.1		16.4
Coated and fried (%)[d]	0.4		7.3	19.2	2.3	8.6			0.1			0.2	0.5	
Other cooking method (%)[e]	3.5	1.9	16.8	10.6	18.6	17.1		4.6	3.0	1.4	2.5	1.0	4.8	4.1
Veal (*n*)	172	141	326	9	59	698	104	0	47	590	38	42	125	8
Baked/roasted (%)[b]	37.2	23.4	16.3			12.2	31.7			26.9	2.6	21.4	32.0	12.5
Barbecued (%)	2.9	0.7			1.7	2.0				2.4		2.4	0.8	
Boiled (%)	5.8	7.1	11.0		10.2	10.0	17.3		8.5	4.1		33.3	4.0	25.0
Fried (%)[c]	12.8	5.0	8.3	11.1	35.6	39.5	4.8		74.5		36.8	26.2	53.6	25.0
Stir fried (%)	13.4	34.0	10.1	11.1	3.4	3.0	14.4			15.1			0.8	
Grilled (%)	2.9	7.1	4.9		11.9					3.2	5.3		2.4	
Stewed (%)	20.9	14.9	26.1	33.3	15.3	11.5	29.8		10.6	47.1	34.2			
Coated and fried (%)[d]	2.3	6.4	9.8	11.1	8.5	14.2	1.9		2.1	0.2	21.1	16.7	6.4	37.5
Other cooking method (%)[e]	1.7	1.4	13.5	33.3	13.6	7.5			4.2	1.0				
Pork (*n*)	294	172	203	19	30	272	248	97	863	294	1080	1422	983	168
Baked/roasted (%)[b]	50.3	33.1	30.0	10.5		12.1	23.0	45.4	0.6	16.0	5.5	18.8	30.2	22.6
Barbecued (%)	5.8	2.3	0.5	15.8	3.3	1.5	1.6	4.1	1.3	32.0	3.7	5.1	0.5	8.9
Boiled (%)	8.5	7.0	0.5	5.3	13.3	3.7	4.4	3.1	3.5	1.4	13.9	12.0	6.1	11.9
Fried (%)[c]	3.4	2.9	5.4	5.3	40.0	51.1	22.2	4.1	76.0	5.1	37.7	41.1	47.9	36.9
Stir fried (%)	12.6	27.9	9.4			8.1	19.4	9.3	7.9	4.1	0.6	6.3	1.3	4.8
Grilled (%)	7.5	13.4	19.2	5.3	6.7			20.6	4.3	11.9	5.1	7.2	4.0	5.4
Stewed (%)	7.8	7.6	18.2	47.4	6.7	11.0	19.8	5.2	0.9	26.5	12.4	0.1		1.8
Coated and fried (%)[d]		0.6	3.4		6.7	6.6	3.2	2.1	1.6	2.0	13.5	6.9	8.6	1.8
Other cooking method (%)[e]	4.1	5.2	13.3	10.5	23.3	5.9	6.5	6.2	3.9	1.0	7.7	2.5	1.3	6.0
Lamb (*n*)	176	140	22	4	5	154	47	67	66	169	26	52	72	71
Baked/roasted (%)[b]	45.5	46.4	40.9	50.0	20.0	30.5	51.1	55.2	3.0	50.9	7.7	19.2	56.9	25.4
Barbecued (%)	6.8	2.9		25.0	20.0	9.7	12.8	1.5	4.5	15.4	3.8	11.5	2.8	1.4
Boiled (%)	0.6	1.4				3.2	8.5	4.5	3.0	4.1	7.7	21.2	5.6	54.9
Fried (%)[c]	9.7	0.7	9.1		20.0	21.4	8.5	7.5	69.7	0.6	65.4	36.5	27.8	14.1
Stir fried (%)	10.8	20.7	4.5			1.9	8.5	10.4	1.5	1.8		11.5		
Grilled (%)	10.2	20.0	18.2					10.4	10.6	5.3	11.5		1.4	1.4
Stewed (%)	14.8	6.4	13.6	25.0	20.0	23.4	4.3	6.0	6.1	21.9				1.4
Coated and fried (%)[d]			13.6			3.9								
Other cooking method (%)[e]	1.7	1.4			20.0	5.8	6.4	4.5	1.5		3.8		5.6	1.4
Minced meat (*n*)	3	7	86	8	23	12	16	2	248	15	154	597	287	34
Baked/roasted (%)[b]		28.6	25.6	50.0	26.1	25.0	56.3	50.0	4.0	20.0	39.6	15.2	4.5	11.8
Barbecued (%)									0.4		0.6	3.5		2.9
Boiled (%)	33.3		2.3		8.7	8.3	18.8		2.4		3.9	4.5	2.8	23.5
Fried (%)[c]		28.6	24.4		21.7	16.7	6.3		73.0	13.3	31.8	75.0	88.9	52.9
Stir fried (%)			8.1			33.3	12.5		4.4	26.7	13.0	1.0		
Grilled (%)	33.3		15.1		4.3				0.4		3.2	0.2		2.9
Stewed (%)	33.3		2.3	25.0	13.0	8.3	6.3	50.0	0.4	40.0	3.9			2.9
Coated and fried (%)[d]		14.3	4.7			8.3					1.9	0.3	3.8	
Other cooking method (%)[e]		28.6	17.5	25.0	26.0				14.9		1.9	0.2		2.9
Processed meat (*n*)	551	386	2190	127	198	1915	852	409	2190	202	4293	2965	874	957
Baked/roasted (%)[b]	15.1	12.2	5.6	19.7	8.6	3.7	8.8	11.7	2.3	18.8	2.4	14.5	9.3	5.7
Barbecued (%)	4.5	2.1	0.2	1.6		0.2	1.1	2.7	0.8	6.4	1.1	1.3	0.8	2.1
Boiled (%)	17.6	19.2	2.3	1.6	3.5	16.3	12.4	3.9	1.5	7.9	31.4	12.6	43.8	29.3
Fried (%)[c]	3.4	6.2	2.2	3.1	19.7	23.1	7.4	10.3	19.9	1.0	12.4	24.0	28.8	30.8
Stir fried (%)	9.3	11.9	1.4	0.8	0.5	6.1	4.1	4.4	2.8	1.5	3.2	0.8		
Grilled (%)	18.5	23.8	1.1	6.3	1.5			29.8	2.0	6.9	1.0	6.2	8.7	1.4
Stewed (%)	7.3	7.8	2.8	4.7	2.0	13.8	11.7	0.2	0.6	1.0	0.3			0.1
Coated and fried (%)[d]	0.2	0.3		0.8		2.2	0.9		0.2		0.1	0.2	0.8	0.2

Table 4 continued.

Table 4 cont.

Cooking method	*Northern France*[a]	*Southern France*	*Northern Italy*	*Ragusa (Italy)*	*Naples (Italy)*	*Northern Spain*	*Southern Spain*	*Great Britain*	*Netherlands*	*Greece*	*Germany*	*Sweden*	*Denmark*	*Norway*
No CM applied (%)[f]	16.7	13.5	81.3	56.7	61.1	31.2	46.6	27.6	58.7	41.1	41.5	38.3	6.9	22.3
Other cooking method (%)	7.4	3.1	3.0	4.7	3.0	3.2	6.9	9.3	11.2	15.3	6.7	2.0	0.9	8.2
Chicken (*n*)	309	227	459	35	32	499	314	207	529	414	300	518	440	138
Baked/roasted (%)[b]	60.8	51.5	20.0	37.1	25.0	28.9	26.1	46.9	2.5	27.3	6.3	58.3	24.5	6.5
Barbecued (%)	1.9	1.8	0.7	2.9	3.1	0.6	0.6	2.4	1.7	11.8	0.3	9.1	0.7	2.2
Boiled (%)	5.8	3.5	15.7	5.7	18.8	8.2	21.3	2.9	11.9	19.3	30.0	8.1	46.4	5.1
Fried (%)[c]	3.2	0.9	7.8	2.9	21.9	30.9	17.5	6.8	60.5		22.7	17.0	18.2	23.9
Stir fried (%)	10.0	23.3	5.7	5.7		6.0	18.8	7.7	1.7		0.3	5.0	2.5	2.9
Grilled (%)	3.2	10.1	14.6	5.7	6.3	0.2		2.9	12.7	13.8	27.3	0.8	5.7	57.2
Stewed (%)	11.0	4.8	15.7	22.9	3.1	8.6	11.5	16.9	1.7	25.4	1.3			0.7
Coated and fried (%)[d]	1.0		12.9	5.7	6.3	12.6	1.6	7.7	1.7	0.7	6.3	0.6	0.5	
Other cooking method (%)[e]	2.9	4.0	7.0	11.5	15.6	4.0	2.5	3.7	5.7	1.7	5.3	1.2	1.6	1.4
Turkey (*n*)	78	55	187	6	20	27	27	42	58	29	178	37	213	15
Baked/roasted (%)[b]	33.0	29.2	20.3	16.7	30.0	14.8	18.5	47.6	3.4	17.2	5.6	51.4	13.6	20.0
Barbecued (%)	1.7					3.7			5.2			2.7	0.9	
Boiled (%)	3.5	1.4	3.2	16.7	5.0		22.2	7.1	6.9	17.2	1.7	13.5	5.6	20.0
Fried (%)[c]	7.8	2.8	12.3		10.0	29.6	48.1	2.4	70.7		56.7	16.2	57.3	13.3
Stir fried (%)	25.2	36.1	4.8					11.9	3.4		0.6		6.1	
Grilled (%)	8.7	11.1	10.7	16.7				4.8	3.4	20.7	6.2		4.7	6.7
Stewed (%)	10.4	4.2	22.5	50.0	35.0	33.3	7.4	2.4		24.1	4.5			6.7
Coated and fried (%)[d]	4.3	8.3	18.2			18.5	3.7			10.3	16.9	8.1	9.9	13.3
Other cooking method (%)[e]	5.2	7.0	8.0		20.0			23.8	6.9	10.1	7.9	8.1	1.8	20.0
Fish (*n*)	832	559	696	67	105	1458	725	208	554	742	504	1602	984	474
Baked/roasted (%)[b]	12.1	7.9	14.1	20.9	4.8	6.9	15.3	8.2	5.4	12.0	2.2	17.0	6.4	11.0
Barbecued (%)	1.2	1.4	0.3	1.5	1.9	0.4	0.4	0.5	0.2	3.9	0.8	0.4	0.2	0.2
Boiled (%)	20.2	17.5	13.1	11.9	27.6	3.6	8.3	3.8	5.8	7.3	9.7	16.6	13.1	17.7
Fried (%)[c]	2.8	5.5	2.3	1.5	1.9	22.9	5.0	6.3	10.3	0.5	6.5	8.9	3.2	11.4
Stir fried (%)	5.4	6.3	1.7		1.9	2.0	1.2	0.5		1.2		0.6	0.1	0.2
Grilled (%)	1.8	4.1	3.6	7.5				8.7	2.2	10.0	1.4	0.6		0.2
Stewed (%)	1.7	0.9	14.8	14.9	21.0	11.2	2.1	1.4	1.8	10.9	6.7			1.1
Coated and fried (%)[d]	7.2	7.0	10.3	10.4	14.3	18.9	14.9	14.9	4.0	38.1	12.9	14.5	13.7	2.7
No cooking method applied (%)[f]	34.9	37.0	32.3	25.4	23.8	25.4	38.9	26.4	63.4	15.8	54.0	39.0	56.8	42.8
Other cooking method (%)	12.7	12.3	7.5	6.0	2.9	8.6	13.9	29.3	7.0	0.3	5.8	2.4	6.5	12.7
Crustaceans (*n*)	161	116	279	31	30	529	345	41	156	171	63	496	164	200
Baked/roasted (%)[b]	4.5	4.5	1.8			1.5	8.1	12.2	3.8		14.3	16.3	11.0	1.5
Barbecued (%)	0.7					0.4	0.3			7.6				
Boiled (%)	38.4	40.7	42.3	32.3	53.3	34.2	29.0	34.1	41.0	26.3	22.2	73.8	37.2	38.0
Fried (%)[c]	1.1	2.3	4.3	3.2	3.3	6.0	9.0	14.6	19.2	10.5	9.5	0.6	7.3	0.5
Stir fried (%)	22.0	23.7	2.9			30.6	26.4	9.8	2.6	4.1		3.0		1.5
Grilled (%)	0.4	2.8	5.0	9.7	3.3				1.9	1.2	1.6			
Stewed (%)	6.3	2.8	35.5	45.2	33.3	5.5	2.9			36.3	1.6			
Coated and fried (%)[d]	0.4	1.7	3.2	3.2		11.0	7.0		3.2	10.5	6.3		4.9	
No cooking method applied (%)[f]	23.1	18.6	0.7	3.2		4.0	7.0	12.2	19.2	3.5	38.1	3.8	33.5	55.0
Other cooking method (%)	3.0	2.8	4.3	3.2	6.7	6.8	10.4	17.1	9.0		6.3	2.4	6.1	3.5

[a]The regions/countries include the centres as given in the figure captions.
[b]% = Percentage of specified cooking methods.
[c]Fried does not include deep fried and stir fried.
[d]Coated and fried: includes breaded and fried, battered and fried, in flour and fried.
[e]Other cooking method includes 'no cooking method applied'.
[f]No cooking method applied: includes 'raw' and 'cooking method not applicable'.

eating in restaurants or canteens may be the main reason for missing specification of cooking methods, since unspecific cooking methods were more often used for meat and fish not consumed at home (data not shown). It can be assumed that these foods, especially in canteens, were prepared according to the distribution of the specific cooking methods in each country and region.

Furthermore, for an even greater proportion of meat and fish items (mean = 23%) no cooking method was applied before consumption. The descriptors 'raw' and 'cooking

Table 5 Mean daily intake (g/day) of red meat, white meat, processed meat, and fish by cooking method, sex and region/country in EPIC assessed by 24 h recalls in 22 727 women and 12 917 men

	Northern France		Southern France		Northern Italy		Ragusa		Naples		Northern Spain		Southern Spain		Great Britain		Nether-lands		Greece		Germany		Sweden		Denmark		Norway	
Cooking method	*Mean*[b]	*s.e.*[c]	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*
Women																												
Red meat																												
baked/roasted	16.8	0.7	11.0	0.8	6.6	0.8	1.4	2.9	4.4	1.8	8.9	1.1	12.4	1.3	11.8	1.4	0.5	0.6	12.4	1.1	2.6	0.8	7.5	0.6	10.8	0.8	6.3	0.8
barbecued	3.7	0.3	0.8	0.4	0.0	0.4	14.8	1.5	0.6	0.9	2.3	0.6	0.2	0.7	0.5	0.7	0.2	0.3	4.9	0.6	1.1	0.4	2.2	0.3	1.0	0.4	2.0	0.4
boiled	2.5	0.4	1.7	0.4	3.7	0.4	2.0	1.7	3.0	1.0	3.0	0.6	1.6	0.8	0.9	0.8	0.9	0.4	1.6	0.6	3.9	0.4	2.7	0.3	2.2	0.4	5.8	0.5
fried[d]	5.4	0.8	1.9	0.9	2.2	0.9	0.5	3.5	14.1	2.1	16.3	1.3	4.2	1.6	1.9	1.6	38.0	0.8	1.2	1.3	15.1	0.9	18.8	0.7	28.6	0.9	13.1	1.0
stir fried	7.4	0.4	13.0	0.5	3.9	0.5	0.0	1.7	0.2	1.1	0.7	0.7	2.5	0.8	2.0	0.8	0.7	0.4	2.3	0.6	0.3	0.5	2.5	0.4	0.3	0.5	0.7	0.5
grilled	6.8	0.5	11.0	0.5	7.2	0.5	5.6	2.0	7.5	1.2	0.2	0.8	0.0	0.9	4.8	0.9	1.2	0.4	2.7	0.8	1.5	0.5	2.2	0.4	2.0	0.5	0.3	0.6
stewed	4.8	0.4	3.5	0.5	7.6	0.4	12.8	1.7	5.3	1.0	4.9	0.6	3.9	0.8	1.7	0.8	1.5	0.4	14.8	0.6	3.3	0.4	0.1	0.3	0.0	0.4	1.0	0.5
coated and fried[e]	0.2	0.4	1.0	0.4	3.2	0.4	9.3	1.6	2.5	1.0	7.1	0.6	1.2	0.7	0.2	0.7	0.5	0.3	0.7	0.6	5.0	0.4	1.9	0.3	3.7	0.4	0.4	0.4
no cooking method applied[f]	0.4	0.1	0.5	0.2	0.8	0.2	0.4	0.6	0.4	0.4	0.1	0.2	0.0	0.3	0.2	0.3	0.5	0.1	0.1	0.2	0.6	0.2	0.3	0.1	1.3	0.2	0.4	0.2
other cooking method	0.8	0.3	0.6	0.3	4.1	0.3	2.8	1.2	7.7	0.7	2.8	0.5	1.3	0.6	1.3	0.6	1.4	0.3	0.7	0.5	1.5	0.3	0.1	0.2	0.2	0.3	0.7	0.3
White meat																												
baked/roasted	10.3	0.6	8.3	0.7	4.3	0.7	2.5	2.5	3.7	1.5	9.9	1.0	10.7	1.2	13.6	1.2	0.3	0.5	3.2	0.9	1.5	0.7	6.0	0.5	5.1	0.7	1.0	0.7
barbecued	0.4	0.2	0.3	0.2	0.0	0.2	0.0	0.7	0.6	0.4	0.3	0.3	0.2	0.3	0.7	0.3	0.3	0.2	2.3	0.3	0.0	0.2	0.8	0.1	0.2	0.2	0.1	0.2
boiled	0.8	0.3	0.4	0.4	3.6	0.4	1.3	1.4	3.5	0.8	1.3	0.5	4.1	0.6	0.5	0.6	0.7	0.3	3.4	0.5	1.3	0.4	0.7	0.3	3.1	0.4	0.8	0.4
fried[d]	0.9	0.4	0.2	0.5	1.6	0.5	1.0	1.9	2.4	1.1	6.4	0.7	3.2	0.9	1.8	0.9	9.5	0.4	0.0	0.7	4.8	0.5	1.7	0.4	6.3	0.5	2.8	0.5
stir fried	2.2	0.2	4.4	0.3	1.2	0.3	0.0	1.0	0.0	0.6	1.1	0.4	2.9	0.5	1.7	0.5	0.2	0.2	0.0	0.4	0.1	0.3	0.5	0.2	0.3	0.3	0.2	0.3
grilled	0.8	0.4	2.3	0.5	4.1	0.5	0.5	1.8	1.1	1.1	0.1	0.7	0.0	0.8	0.8	0.9	2.1	0.4	3.2	0.7	4.3	0.5	0.1	0.4	1.3	0.5	8.0	0.5
stewed	1.9	0.3	0.8	0.3	4.3	0.3	2.9	1.2	3.5	0.7	3.5	0.5	1.7	0.5	1.5	0.6	0.1	0.3	4.1	0.4	0.5	0.3	0.0	0.2	0.0	0.3	0.1	0.3
coated and fried[e]	0.6	0.3	0.4	0.3	3.6	0.3	2.5	1.1	0.6	0.7	3.3	0.4	0.4	0.5	1.0	0.5	0.4	0.2	1.3	0.4	1.6	0.3	0.2	0.2	0.9	0.3	0.0	0.3
no cooking method applied[f]	0.2	0.1	0.4	0.1	0.0	0.1	0.4	0.3	0.0	0.2	0.0	0.1	0.0	0.2	0.7	0.2	0.2	0.1	0.1	0.1	0.3	0.1	0.0	0.1	0.0	0.1	0.1	0.1
other cooking method	0.4	0.2	0.4	0.2	1.5	0.2	0.0	0.8	2.5	0.5	0.3	0.3	0.9	0.4	1.1	0.4	0.5	0.2	0.2	0.3	0.6	0.2	0.2	0.2	0.6	0.2	0.2	0.2
Processed meat																												
baked/roasted	2.6	0.3	1.7	0.3	0.7	0.3	5.4	1.3	0.5	0.8	1.5	0.5	2.1	0.6	2.9	0.6	0.5	0.3	0.4	0.5	0.5	0.3	5.7	0.3	0.5	0.3	1.7	0.4
barbecued	0.8	0.2	0.4	0.2	0.1	0.2	0.0	0.7	0.0	0.4	0.2	0.3	0.0	0.3	0.8	0.3	0.3	0.2	0.5	0.3	0.1	0.2	0.5	0.1	0.4	0.2	1.4	0.2
boiled	2.4	0.4	2.6	0.5	1.5	0.5	0.6	1.9	1.2	1.1	3.6	0.7	1.6	0.9	0.7	0.9	0.4	0.4	0.7	0.7	7.9	0.5	5.3	0.4	5.7	0.5	12.4	0.5
fried[d]	0.8	0.5	1.2	0.6	0.1	0.6	0.0	2.2	3.2	1.4	5.5	0.8	1.3	1.0	1.4	1.0	7.8	0.5	0.1	0.8	7.9	0.6	9.6	0.5	4.2	0.6	15.7	0.6
stir fried	1.1	0.1	0.9	0.1	0.1	0.1	0.0	0.5	0.5	0.3	0.9	0.2	0.7	0.2	0.3	0.3	0.3	0.1	0.0	0.2	0.6	0.1	0.4	0.1	0.0	0.1	0.0	0.2
grilled	2.3	0.2	2.7	0.3	0.7	0.3	0.7	1.0	0.4	0.6	0.0	0.4	0.0	0.5	6.2	0.5	0.6	0.2	1.0	0.4	0.3	0.3	2.3	0.2	0.7	0.3	0.4	0.3
stewed	0.5	0.1	0.2	0.1	0.6	0.1	0.8	0.4	0.1	0.2	2.0	0.1	0.5	0.2	0.3	0.2	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1
coated and fried[e]	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.3	0.0	0.2	0.5	0.1	0.7	0.2	0.0	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1
no cooking method applied[f]	1.4	0.4	1.5	0.5	17.3	0.5	9.5	1.7	9.8	1.1	10.1	0.7	9.9	0.8	3.7	0.8	10.0	0.4	2.6	0.6	13.1	0.5	5.7	0.4	0.3	0.5	2.9	0.5
other cooking method	1.2	0.3	0.6	0.4	1.0	0.4	1.0	1.5	2.7	0.9	0.7	0.6	3.7	0.7	1.9	0.7	4.1	0.3	1.3	0.5	6.7	0.4	1.0	0.3	0.3	0.4	3.8	0.4
Fish and crustaceans																												
baked/roasted	5.5	0.4	2.9	0.5	2.7	0.5	5.2	1.9	1.3	1.2	5.8	0.7	11.3	0.9	2.2	0.9	0.9	0.4	4.9	0.7	0.3	0.5	4.7	0.4	1.1	0.5	3.6	0.5
barbecued	0.6	0.2	0.5	0.2	0.0	0.2	0.0	0.7	0.8	0.4	0.5	0.3	0.2	0.3	0.1	0.3	0.0	0.2	3.6	0.3	0.0	0.2	0.1	0.1	0.3	0.2	0.0	0.2
boiled	11.2	0.6	9.2	0.7	4.2	0.7	8.0	2.7	13.8	1.7	5.0	1.0	8.4	1.3	2.2	1.3	1.4	0.6	4.0	1.0	1.9	0.7	7.2	0.6	3.4	0.7	11.9	0.8
fried[d]	1.2	0.4	2.3	0.5	0.7	0.5	0.5	1.9	0.3	1.1	14.6	0.7	1.5	0.8	1.3	0.9	1.9	0.4	0.9	0.7	1.6	0.5	2.3	0.4	0.8	0.5	6.6	0.5
stir fried	3.1	0.2	3.7	0.3	0.8	0.3	0.0	1.1	0.0	0.6	2.5	0.4	1.2	0.5	0.3	0.5	0.0	0.2	1.0	0.4	0.0	0.3	0.2	0.2	0.0	0.3	0.2	0.3
grilled	1.0	0.3	1.6	0.3	1.5	0.3	6.2	1.1	0.5	0.7	0.0	0.4	0.0	0.5	2.3	0.5	0.5	0.2	5.7	0.4	0.2	0.3	0.4	0.2	0.0	0.3	0.1	0.3
stewed	0.9	0.3	0.4	0.4	4.3	0.4	7.6	1.4	6.2	0.9	8.5	0.5	2.2	0.7	0.3	0.7	0.5	0.3	6.8	0.5	1.8	0.4	0.0	0.3	0.0	0.4	0.6	0.4
coated and fried[e]	3.6	0.6	3.1	0.7	3.6	0.7	5.9	2.7	2.5	1.7	19.0	1.0	12.8	1.3	5.1	1.3	1.2	0.6	20.0	1.0	3.0	0.7	4.8	0.6	4.7	0.7	1.7	0.8
no cooking method applied[f]	7.5	0.5	6.5	0.6	2.5	0.6	3.4	2.2	5	1.3	4.1	0.8	9.1	1.0	3.4	1.0	5.4	0.5	2.6	0.8	5.3	0.6	6.8	0.4	10.5	0.6	6.1	0.6
other cooking method	5.7	0.5	5.5	0.6	2.3	0.5	2.0	2.1	1.7	1.3	7.4	0.8	9.3	0.9	9.4	1.0	1.4	0.4	0.3	0.8	1.7	0.5	0.8	0.4	2.2	0.5	6.1	0.6

Table 5 continued.

Table 5 Cont.

Cooking method	Northern France		Southern France		Northern Italy		Ragusa		Naples		Northern Spain		Southern Spain		Great Britain		Nether-lands		Greece		Germany		Sweden		Denmark		Norway	
	Mean[b]	s.e.[c]	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Men																												
Red meat																												
baked/roasted					8.6	1.2	5.2	3.6			15.2	1.2	17.2	2.0	18.2	2.1	0.0	1.4	17.1	1.4	2.8	0.9	12.1	0.9	14.6	1.0		
barbecued					0.0	0.7	1.2	2.0			3.5	0.6	2.3	1.1	1.7	1.2	0.3	0.8	7.8	0.7	1.9	0.5	3.4	0.5	0.9	0.6		
boiled					5.1	0.8	6.5	2.2			5.1	0.7	2.4	1.2	2.1	1.3	1.2	0.9	2.1	0.8	7.6	0.6	3.4	0.5	3.6	0.6		
fried[d]					4.8	1.8	7.1	5.3			40.9	1.7	7.0	3.0	1.9	3.1	59.7	2.1	1.1	2.0	21.6	1.4	27.7	1.3	45.2	1.5		
stir fried					2.0	0.4	0.2	1.2			1.0	0.4	4.9	0.7	1.9	0.7	1.3	0.5	4.6	0.5	0.6	0.3	3.8	0.3	0.6	0.4		
grilled					5.1	0.8	12.0	2.2			0.0	0.7	0.0	1.2	6.2	1.3	0.0	0.9	5.7	0.8	3.7	0.6	3.6	0.5	3.5	0.6		
stewed					13.0	0.9	17.5	2.6			9.8	0.9	8.6	1.5	1.0	4.6	2.8	1.1	22.8	1.0	6.8	0.7	0.3	0.6	0.0	0.7		
coated and fried[e]					4.8	0.9	9.8	2.6			9.3	0.8	2.2	1.4	0.3	1.5	0.0	1.0	0.6	1.0	6.9	0.7	4.1	0.6	4.5	0.7		
no cooking method applied[f]					1.6	0.3	0.2	0.9			0.0	0.3	0.0	0.5	0.5	0.6	0.9	0.4	0.1	0.4	0.8	0.2	0.4	0.2	2.0	0.3		
other cooking method					9.5	0.7	2.2	2.0			9.1	0.7	2.6	1.1	0.8	1.2	2.5	0.8	0.4	0.8	1.8	0.5	0.3	0.5	0.2	0.6		
White meat																												
baked/roasted					6.4	0.9	16.2	2.7			10.3	0.9	14.1	1.5	13.2	1.6	0.5	1.1	4.0	1.0	1.0	0.7	5.7	0.6	4.8	0.8		
barbecued					0.3	0.2	0.9	0.6			0.2	0.2	0.0	0.4	0.5	0.4	0.0	0.3	1.4	0.2	0.0	0.2	1.0	0.2	0.1	0.2		
boiled					3.6	0.5	0.7	1.6			2.0	0.5	4.3	0.9	1.5	0.9	0.6	0.6	3.8	0.6	1.7	0.4	0.6	0.4	3.3	0.4		
fried[d]					3.3	0.8	0.0	2.4			10.9	0.8	3.9	1.3	0.7	1.4	12.3	1.0	0.1	0.9	5.4	0.6	2.1	0.6	6.6	0.7		
stir fried					1.3	0.3	0.5	0.7			0.4	0.2	3.8	0.4	1.3	0.4	0.3	0.3	0.0	0.2	0.2	0.2	0.9	0.2				
grilled					2.9	0.6	1.6	1.7			0.0	0.6	0.0	1.0	1.2	1.0	2.4	0.7	3.5	0.7	4.9	0.5	0.3	0.4	1.3	0.5		
stewed					5.3	0.6	13.6	1.6			3.3	0.5	4.8	0.9	2.9	1.0	1.6	0.6	6.0	0.6	0.3	0.4	0.0	0.4	0.0	0.5		
coated and fried[e]					4.1	0.6	0.6	1.6			5.2	0.5	0.5	0.9	1.8	1.0	0.0	0.6	0.2	0.6	2.1	0.4	0.2	0.4	0.6	0.5		
no cooking method applied[f]					0.0	0.1	0.0	0.2			0.0	0.1	0.0	0.1	0.5	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1		
other cooking method					2.9	0.4	2.2	1.1			2.1	0.3	0.6	0.6	1.8	0.6	0.4	0.4	0.2	0.4	0.8	0.3	0.1	0.3	0.3	0.3		
Processed meat																												
baked/roasted					1.3	0.4	4.1	1.2			0.8	0.4	2.9	0.7	3.1	0.7	0.0	0.5	0.5	0.5	0.8	0.3	5.5	0.3	0.6	0.3		
barbecued					0.1	0.3	1.1	0.9			0.0	0.3	2.2	0.5	2.8	0.6	0.8	0.4	1.0	0.4	0.7	0.2	0.5	0.2	0.4	0.3		
boiled					1.1	0.9	0.2	2.7			8.8	0.9	3.7	1.5	1.7	1.6	2.0	1.1	0.3	1.0	19.8	0.7	8.6	0.6	7.6	0.7		
fried[d]					1.5	1.1	0.2	3.2			9.2	1.0	2.2	1.8	3.9	1.9	12.8	1.3	0.2	1.2	14.9	0.8	15.8	0.8	7.8	0.9		
stir fried					0.6	0.2	0.0	0.6			1.5	0.2	0.8	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.9	0.2	0.4	0.1	0.0	0.2		
grilled					0.5	0.4	4.8	1.1			0.0	0.4	0.0	0.6	10.3	0.7	0.9	0.5	1.0	0.4	0.8	0.3	2.1	0.3	1.3	0.3		
stewed					1.2	0.3	0.4	0.9			6.5	0.3	3.8	0.5	0.0	0.5	0.4	0.4	0.0	0.3	0.3	0.2	0.0	0.2	0.0	0.3		
coated and fried[e]					0.0	0.1	0.0	0.4			1.4	0.1	0.3	0.2	0.0	0.2	0.0	0.2	0.0	0.1	0.0	0.1	0.1	0.1	0.2	0.1		
no cooking method applied[f]					25.4	0.9	12.4	2.7			10.9	0.9	14.3	1.5	4.8	1.6	21.2	1.1	4.8	1.0	20.7	0.7	10.8	0.6	0.6	0.8		
other cooking method					2.0	0.6	0.2	1.7			1.1	0.6	3.3	1.0	5.3	1.0	7.4	0.7	1.6	0.7	6.3	0.5	2.0	0.4	0.4	0.5		
Fish and crustaceans																												
baked/roasted					5.2	0.9	6.9	2.6			7.7	0.9	14.0	1.5	2.3	1.6	0.5	1.1	9.3	1.0	0.4	0.7	5.7	0.6	1.8	0.7		
barbecued					0.2	0.3	0.9	1.0			0.4	0.3	0.6	0.6	0.1	0.6	0.0	0.4	4.7	0.4	0.2	0.3	0.5	0.2	0.0	0.3		
boiled					6.4	0.9	11.9	2.5			5.5	0.8	7.3	1.4	0.9	1.5	2.0	1.0	6.6	1.0	1.7	0.7	7.8	0.6	4.2	0.7		
fried[d]					1.1	1.0	0.6	2.8			25.3	0.9	7.1	1.6	2.3	1.6	3.8	1.1	0.6	1.1	1.4	0.7	4.6	0.7	1.9	0.8		
stir fried					0.5	0.4	0.0	1.0			5.0	0.3	3.1	0.6	0.2	0.6	0.0	0.4	0.9	0.4	0.0	0.3	0.2	0.3	0.0	0.3		
grilled					0.8	0.4	1.3	1.1			0.1	0.3	0.0	0.6	3.0	0.6	0.4	0.4	6.1	0.4	0.4	0.3	0.0	0.3	0.0	0.3		
stewed					8.0	0.7	7.6	2.1			13.3	0.7	1.5	1.2	0.3	1.2	0.1	0.8	7.4	0.8	1.2	0.6	0.1	0.5	0.0	0.6		
coated and fried[e]					5.1	1.4	3.9	4.0			25.9	1.3	17.7	2.2	5.9	2.4	1.4	1.6	29.6	1.5	3.7	1.1	7.2	1.0	5.3	1.1		
no cooking method applied[f]					3.3	0.8	1.5	2.3			8.9	0.8	10.9	1.3	5.4	1.4	6.7	0.9	6.9	0.9	6.5	0.6	6.7	0.6	11.6	0.7		
other cooking method					2.8	0.7	1.3	2.1			9.5	0.7	14.2	1.2	8.4	1.2	1.4	0.8	0.3	0.8	0.7	0.5	1.1	0.5	2.2	0.6		

[a]The regions/countries include the study centres as given in the figure captions. [b]Mean adjusted for age, season and week day. [c]s.e., standard error of adjusted mean. [d]Fried does not include deep fried and stir fried. [e]Coated and fried includes breaded and fried, battered and fried, in flour and fried. [f]No cooking method applied includes 'raw' and 'cooking method not applicable'.

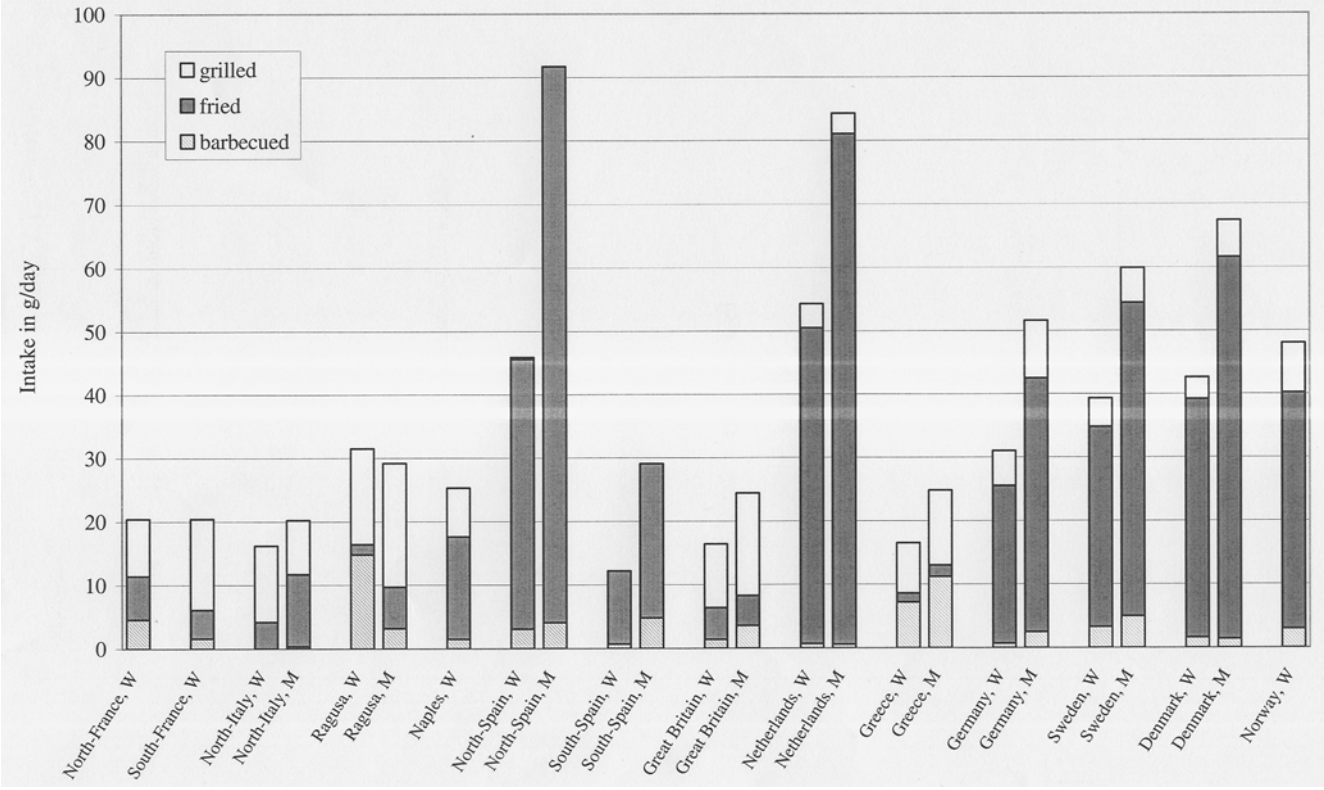


Figure 2 Mean intake of barbecued, fried, and grilled meat and fish by region/country in EPIC assessed by 24 h recalls in 22 727 women and 12 917 men. The regions/countries include the following study centres: *northern France* — northeast, northwest; *southern France* — south, south coast; *northern Italy* — Varese, Turin, Florence; *northern Spain* — Oviedo, Pamplona, San Sebastian; *southern Spain* — Granada, Murcia; *Great Britain* — general population; *Netherlands* — Bilthoven, Utrecht; *Germany* — Heidelberg, Potsdam; *Sweden* — Malmo, Umea; *Denmark* — Copenhagen, Aarhus; *Norway* — northwest, southeast. W = women; M = men. The cooking method 'fried' does not include deep fried and stir fried. Mean adjusted for age, season and week day.

method not applicable' were mostly used for processed meat, fish and crustaceans. Since these descriptors do not exclude cooking outside the home or preparation in another way, no assumptions can be made about the way in which these foods were consumed. In particular mean daily intake of processed meat to which no cooking method was applied is as high as 17 g in some countries. Furthermore, for a subgroup of meat and fish items, especially for processed meat, the cooking method was not assessed because these foods were usually not cooked before consumption, eg salami-type sausages. These foods were not included in the data set used for the present analysis. As a consequence, the intake of meat given in Tables 4 and 5 is lower than the intake of meat estimated by the 24 h recalls. The difference is larger for processed meat than for red and white meat.

Differences in under- or over-reporting among the study regions might influence the data. Meat intake is affected by under-reporting in Greece and southern Spain. With adjustment for energy intake, total meat intake results increased distinctly in these EPIC centres (Linseisen *et al*, 2002). The topic of underreporting in the EPIC calibration study will be discussed in a special paper (Ferrari *et al*, 2002).

To calculate the mean intake of cooked meat and fish, adjustment for different variables was made. Mean adjusted intake was computed by using the ANACOVA procedure to adjust for age and to apply weights to the ANACOVA estimates when considering the categorical variables weekday and season. Weighing factors for weekday and season were included because the 24 h recalls were not equally distributed concerning the different seasons and weekdays. This is important especially for foods prepared by cooking methods which were applied only during a certain season, eg barbecuing during the summer. If more interviews were made in the winter than in the summer, the intake of barbecued meat and fish would have been underestimated without adjustment for season.

The data from this study suggests that dietary habits concerning meat and fish consumption differ greatly by

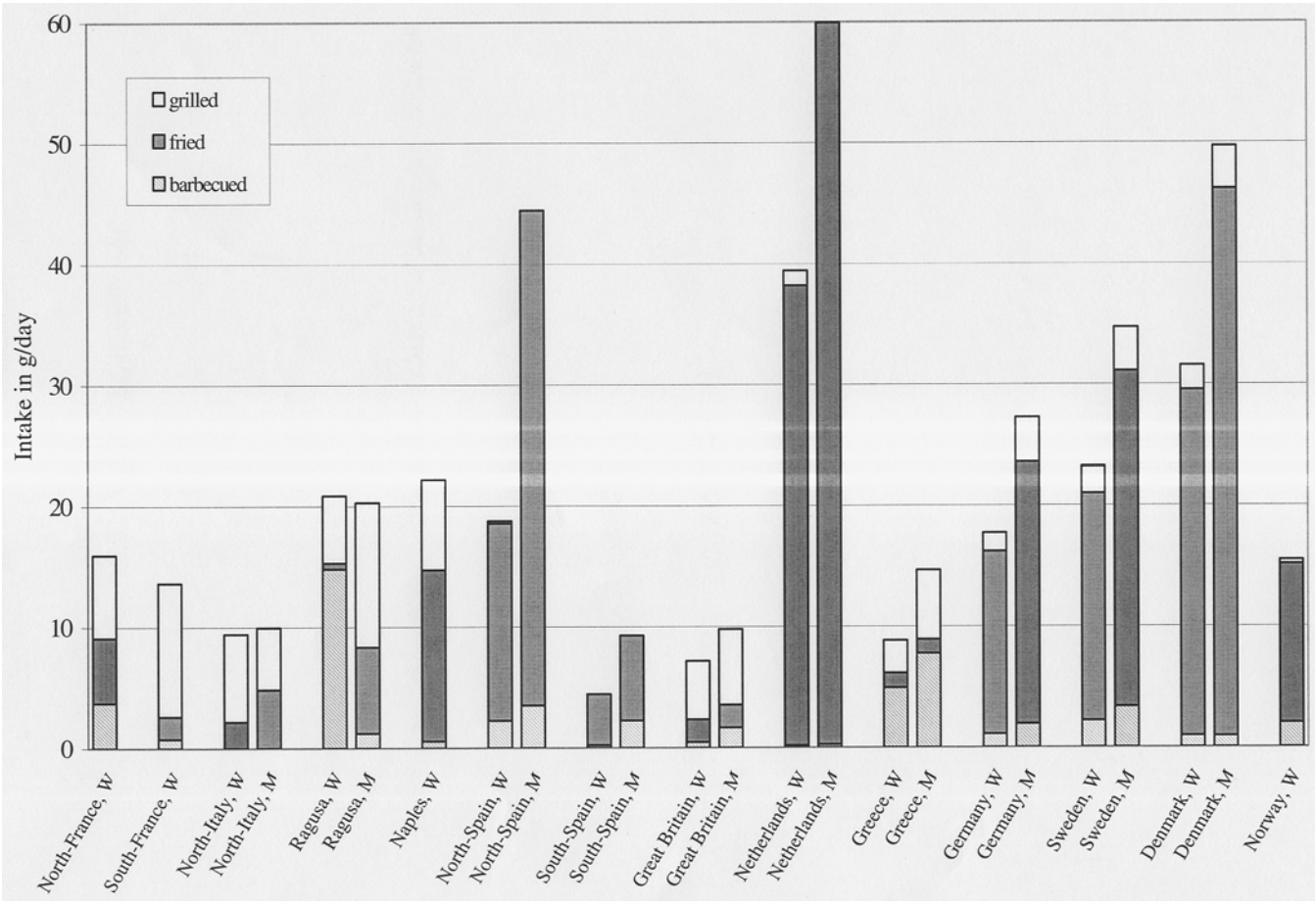


Figure 3 Mean intake of barbecued, fried and grilled red meat by region/country in EPIC assessed by 24 h recalls in 22 727 women and 12 917 men. The regions/countries include the following study centres: *northern France* — northeast, northwest; *southern France* — south, south coast; *northern Italy*: Varese, Turin, Florence; *northern Spain*: Oviedo, Pamplona, San Sebastian; *southern Spain*: Granada, Murcia; *Great Britain* — general population; *Netherlands* — Bilthoven, Utrecht; *Germany* — Heidelberg, Potsdam; *Sweden* — Malmo, Umea; *Denmark* — Copenhagen, Aarhus; *Norway* — northwest, southeast. W = women; M = men. The cooking method 'fried' does not include deep fried and stir fried. Mean adjusted for age, season, and week day.

country and region. In general, frying is more common in EPIC cohorts of northern European countries and less in the south, whereas roasting and stewing are more frequently used in the south than in the north. This indicates characteristics in meat and fish preparation patterns across Europe. Comparable data concerning the application of cooking methods are quite rare. A study in Sweden aimed to look at cooking methods of meat and fish in an elderly population (124 men and women between 50 and 75 y old) in Stockholm (Augustsson *et al*, 1999a). Their results indicated a dominant role of frying (61% of consumed meat and fish). Twenty percent of meat and fish was baked/roasted and 15% boiled. They asked only for the frequency of four cooking methods (grilling, frying, boiling and baking/roasting). Taking into account only these four cooking methods in the present study, frying contributes 42.2% to the preparation of meat and fish in the Swedish study centres and baking/roasting 28.2%. This confirms the results of Augustsson *et al* (1999a).

Cooking methods differ by cooking temperature, the use of direct heat (flame) and the use of fat. This influences the production of possibly carcinogenic compounds like HCA and PAH. Steaming, boiling and stewing expose food to temperatures mostly below or around 100°C. Baking, microwaving and roasting produces temperatures up to 200°C, but foods are not exposed directly to a hot surface. In contrast, grilling, barbecuing, frying and stir frying expose foods to a hot surface or to direct flame for a longer or shorter period of time (Augustsson *et al*, 1999a; Skog *et al*, 1998; World Cancer Research Fund, 1997). However, since the contact with the hot surface is only for a short time period, the production of HCA is lower during stir frying compared with the other cooking methods (World Cancer Research Fund, 1997).

Most studies dealing with cooking methods put their main focus on high-temperature cooking methods, where most carcinogenic substances are produced (Skog *et al*, 1995,1998; Knize *et al*, 1996; Johansson & Jägerstad, 1994). Extracting high-temperature methods applied in the present

study, it turns out that the variation between countries or regions is enormously high, the intake in the study centres in north Spain being at least four times higher than that in northern Italy. High-temperature cooking methods are used less often in the EPIC cohorts in France, northern Italy, Greece, and southern Spain; the amount of meat and fish eaten fried, grilled or barbecued is low as well in these countries compared to the cohorts in the northern part of Europe. Most interesting is northern Spain. In men, more than 90 g of meat and fish are eaten fried and barbecued, which is the highest intake in EPIC, but both methods account for only 26% of meat and fish cooking in northern Spain. This indicates that exclusively qualitative information, eg frequency of consumption, has to be supported by a quantitative estimate of the exposure. A specially designed questionnaire can help to get both kinds of information. Keating and Bogen (2001) analysed data of about 20 000 participants of the Continuing Survey of Food Intakes by Individuals for cooking methods used to prepare meat and fish. Additionally, they made assumptions about the doneness level of the consumed foods and calculated the HCA intake based on these assumptions. In EPIC, however, it can be assumed that the preferred doneness level differs quite strongly and therefore it seems to be more appropriate to use a specially designed questionnaire to assess the HCA intake.

Incidence rates of cancer differ quite substantially in Europe, especially between northern and southern countries. Age-adjusted cancer incidence rates for colorectal and breast cancer are higher in the northern and western countries of Europe than in the south, whereas the incidence of stomach cancer is higher in the south than in the west and the north of Europe (Parkin *et al*, 1997). An influence of different cooking techniques on cancer incidences might be possible. The report of the World Cancer Research Fund (1997) concludes that cooking meat at high temperatures possibly increases the risk of colorectal cancer and grilling and barbecuing possibly increase the risk of stomach cancer. For frying results are conflicting. Several studies have explored these relationships with contradicting results concerning cancer risk. The general problem of these international studies is the different definition of cooking methods (Sinha & Rothman, 1999). For example, barbecuing has different meanings worldwide and grilling can mean heating on an open fire as well as heating on a hot surface (World Cancer Research Fund, 1997). In this study, the same definitions (and coding) of cooking methods across countries were used which excludes this kind of bias at least partly.

Some case–control studies observed a higher cancer risk for persons eating more red meat. A recently published study from Italy (Tavani *et al*, 2000) showed higher risks for persons eating red meat ≥ 7 times per week compared to those eating red meat ≤ 3 times per week for different cancer sites, including colon, rectum and stomach. In contrast, Kampman *et al* (1999) were not able to observe an association between the consumption of red meat and the risk of colon cancer. Not only the quantity of red meat intake is of interest but also the cooking of it. Associations between the consumption of fried, grilled, barbecued or pan-fried red meat and the risk of colon cancer (Schiffman & Felton, 1990), colorectal adenomas (Sinha *et al*, 1999), breast cancer (Zheng *et al*, 1999) and stomach cancer (Ward *et al*, 1997) were found, but there are several investigations that were not able to detect any association (Delfino *et al*, 2000; Gertig *et al*, 1999; Ambrosone *et al*, 1998). In the present study, the preparation of red meat shows a great variability across the participating countries and regions. Whereas in the EPIC centres in northern Spain, the Netherlands, Germany, Sweden, Denmark and Norway, frying is the common way to prepare it, other cooking methods are preferred in the south. Considerable amounts of red meat are consumed boiled and stewed in the cohorts of Italy, southern Spain and Greece, underlining that high-temperature cooking methods were more often used in northern Europe.

In addition to the cooking method, the degree of browning that individuals prefer when eating prepared meat and fish is a further important characteristic. On this basis, Sinha *et al* (1999) showed an increased risk of colorectal adenomas for the consumption of red meat in general as well as of well-done and very well-done red meat and of grilled red meat in a case–control study. This correlation was also apparent in study conducted in California (Ward *et al*, 1997). In a case–control study conducted by Zheng *et al* (1999), women who ate bacon, beef steak, and hamburgers well-done had a significantly higher breast cancer risk than women who preferred rare or medium meat. However, two earlier studies found no evidence for an association between cooking method, degree of doneness and cancer risk (Muscat & Wynder, 1994; Lyon & Mahoney, 1988). In the present study no data on the degree of browning or doneness were obtained.

EPIC offers the opportunity to compare data on cooking methods in Europe, because cooking methods used in the program EPIC-Soft were defined for all participating countries by the coordinating centre in Lyon and these definitions were used equally in each country. The results of this study will be used to develop a questionnaire on cooking methods of meat and fish that could be applied in all EPIC countries. It will allow the assessment of the applied food preparation methods from all EPIC participants. Apart from the cooking methods, this questionnaire will, in contrast to the program EPIC-SOFT, also be able to assess the degree of doneness and browning of prepared meat and fish. By means of such data, the heterogeneity within EPIC provides a fairly good chance to proceed in our knowledge of the relationship between food preparation methods and cancer risk.

Acknowledgements
This work is part of the EPIC project supported by the 'Europe Against Cancer' Programme of the European Commission.

References

Adamson RH, Thorgeirsson UP, Snyderwine EG, Thorgeirsson SS, Reeves J, Dalgard DW, Takayama S & Sugimura T (1990): Carcinogenicity of 2-amino-3-methylimidazo[4,5-*f*]quinoline in nonhuman primates: induction of tumors in three macaques. *Jpn. J. Cancer Res.* **81**, 10–14.

Ambrosone CB, Freudenheim JL, Sinha R, Graham S, Marshall JR, Vena JE, Laughon R, Nemoto R & Shields PG (1998): Breast cancer risk, meat consumption and N-acetyltransferase (NAT2) genetic polymorphisms. *Int. J. Cancer* **75**, 825–839.

Augustsson K, Lindblad J, Övervik E & Steineck G (1999a): A population-based dietary inventory of cooked meat and assessment of the daily intake of food mutagens. *Food Additives Contamin.* **16**, 215–225.

Augustsson K, Skog K, Jägerstad M, Dickman PW & Steineck G (1999b): Dietary heterocyclic amines and cancer of the colon, rectum, bladder, and kidney: a population-based study. *Lancet* **353**, 703–707.

Deitz AC, Zheng W, Leff MA, Gross M, Wen W-Q, Doll MA, Xiao GH, Folsom AR & Hein DW (2000): *N*-Acetyltransferase 2 genetic polymorphisms, well-done meat intake, and breast cancer risk among postmenopausal women. *Cancer Epidemiol. Biomark. Prev.* **9**, 905–910.

Delfino RJ, Sinha R, Smith C. West J, White E, Lin HJ, Liao S-Y, Gim JSY, Ma HL, Butler J & Anton-Culver H (2000): Breast cancer, heterocyclic amines from meat and *N*-acetyltransferase 2 genotype. *Carcinogenesis* **21**, 607–615.

De Stefani E, Doneo-Pellegrini H, Mendilaharsu M & Ronco A (1997a): Meat intake, heterocyclic amines and risk of colorectal cancer: a case-control study in Uruguay. *Int. J. Oncol.* **10**, 573–580.

De Stefani E, Ronco A, Mendilaharsu M, Guidobono M & Doneo-Pellegrini H (1997b): Meat intake, heterocyclic amines, and risk of breast cancer: a case–control study in Uruguay. *Cancer Epidemiol. Biomark. Prev.* **6**, 573–581.

De Stefani E, Boffetta P, Mendilaharsu M, Carzoglio J & Deneo-Pellegrini H (1998a): Dietary nitrosamines, heterocyclic amines, and risk of gastric cancer: a case–control study in Uruguay. *Nutr. Cancer* **30**, 158–162.

De Stefani E, Ronco A, Mendilaharsu M & Doneo-Pellegrini H (1998b): Case–control study on the role of heterocyclic amines in the etiology of upper aerodigestive cancers in Uruguay. *Nutr. Cancer* **32**, 43–48.

Doneo-Pellegrini H, De Stefani E, Ronco A, Mendilaharsu M & Carzoglio JC (1996): Meat consumption and risk of lung cancer; a case-control study from Uruguay. *Lung Cancer* **14**, 195–205.

Ferrari P, Slimani N, Ciampi A, Trichopoulos D, Naska A, Lauria C, Veglia F, Bueno-de-Mesquita HB, Ocké MC, Brustad M, Braaten T, Tormo MJ, Amiano P, Mattisson I, Johansson G, Welch A, Davey G, Overvad K, Tjønneland A, Clavel-Chapelon F, Thiebaut A, Linseisen J, Boeing H, Hemon B & Riboli E (2002): Evaluation of misresporting in the 24-hour diet recalls in EPIC. *Pub. Health Nutr.* (in press).

Gerhardsson de Verdier M, Hagman U, Peters UK, Steineck G & Övervik E (1991): Meat, cooking methods and colorectal cancer: a case-referent study in Stockholm. *Int. J. Cancer* **49**, 520–525.

Gertig DM, Hankinson SE, Hough H, Spiegelman D, Colditz GA, Willett WC, Kelsey KT & Hunter DJ (1999): *N*-Acetyltransferase 2 genotypes, meat intake and breast cancer risk. *Int. J. Cancer* **80**, 13–17.

Guillén MD, Sopelana P & Partearroyo (1997): Food as a source of polycyclic aromatic carcinogens. *Rev. Environ. Health* **13**, 133–146.

Jägerstad M, Laser Reuterswärd A, Öste R, Dalquist A, Grivas S, Olsson K & Nyhammar T (1983): Creatine and Maillard reaction products as precursors of mutagenic compounds formed in fried beef. *ACS Symp. Ser.* **215**, 507–519.

Johansson MAE & Jägerstad M (1994): Occurrence of mutagenic/carcinogenic heterocyclic amines in meat and fish products, including pan residues, prepared under domestic conditions. *Carcinogenesis* **15**, 1511–1518.

Kampman E, Slattery ML, Bigler J, Leppert M, Samowitz W, Caan BJ & Potter JD (1999): Meat consumption, genetic susceptibility, and colon cancer risk: a United States multicenter case–control study. *Cancer Epidemiol. Biomark. Prev.* **8**, 14–24.

Keating GA & Bogen KT (2001): Methods to estimate heterocyclic aromatic amine concentrations in cooked meats in the US diet. *Food Chem. Toxicol.* **11**, 29–43.

Knize MG, Sinha R, Salmon CP, Metha SS, Dewhirst KP & Felton JS (1996): Formation of heterocyclic amine mutagens/carcinogens during home and commercial cooking of muscle food. *J. Muscle Foods* **7**, 271–279.

Larsson BK, Sahlberg GP, Eriksson AT & Busk LÅ (1983): Polycyclic aromatic hydrocarbons in grilled food. *J. Agric. Food Chem.* **31**, 867–873.

Linseisen J, Kesse E, Slimani N, Bueno-de-Mesquita HB, Ocké MC, Skeie G, Kumle M, Iraeta MD, Gómez PM, Janzon L, Stattin P, Welch A, Spencer EA, Overvad K, Tjønneland A, Clavel-Clapelon F, Miller AB, Klipstein-Grobusch K, Lagiou P, Kalapothaki V, Masala G, Giurdanella MC, Norat T & Riboli E (2002): Meat consumption in the EPIC cohorts — results from the 24-hour dietary recalls. *Pub. Health Nutr.* (in press).

Lyon JL & Mahoney AW (1988): Fried foods and the risk of colon cancer. *Am. J. Epidemiol.* **128**, 1000–1006.

Muscat JE & Wynder EL (1994): The consumption of well-done red meat and the risk of colorectal cancer. *Am. J. Public Health* **84**, 856–858.

Norrish AE, Ferguson LR, Knize MG, Felton JS, Sharpe SJ & Jackson RT (1999): Heterocyclic amine content of cooked meat and risk of prostate cancer. *J. Natl Cancer Inst.* **91**, 2038–2044.

Parkin DM, Whelan SL, Ferlay J, Raymond L & Young J (1997): *Cancer Incidence in Five Continents*, Vol. VII. IARC Scientific Publications no. 143. Lyon: International Agency for Research on Cancer.

Probst-Hensch NM, Sinha R, Longnecker MP, Witte JS, Ingles SA, Frankl HD, Lee ER & Haile RW (1997): Meat preparation and colorectal adenomas in a large sigmoidoscopy-based case–control study in California (United States). *Cancer Causes Control* **8**, 175–183.

Riboli E (1992): Nutrition and cancer: background and rationale of the European Prospective Investigation into Cancer and Nutrition (EPIC). *Ann. Oncol.* **3**, 783–791.

Riboli E & Kaaks R (1997): The EPIC Project: rationale and study design. *Int. J. Epidemiol.* **26**(Suppl 1), S6–S14.

Rothman N, Correa-Villasenor A, Ford DP, Poirier MC, Haas R, Hansen JA, O'Toole T & Strickland PT (1993): Contribution of occupation and diet to white blood cell polycyclic aromatic hydrocarbon–DNA adducts in Wildland firefighters. *Cancer Epidemiol. Biomark. Prev.* **2**, 341–347.

Schiffman MH & Felton JS (1990): Re: 'Fried foods and the risk of colon cancer'. *Am. J. Epidemiol.* **131**, 376–378.

Shirai T, Tamano S, Sano M, Masui T, Hasegawa R & Ito N (1995): Carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in rats: dose–response studies. *Princess Takamatsu Symposia* **23**, 232–239.

Sinha R & Rothman N (1999): Role of well-done, grilled red meat, heterocyclic amines (HCAs) in the etiology of human cancer. *Cancer Lett.* **143**, 189–194.

Sinha R, Kulldorff M, Curtin J, Brown CC, Alavanja MCR & Swanson C A (1998): Fried, well-done red meat and risk of lung cancer in women (United States). *Cancer Causes Control* **9**, 621–630.

Sinha R, Chow WH, Kulldorff M, Denobile J, Butler J, Garcia-Closas M, Weil R, Hoover RN & Rothman N (1999): Well-done, grilled red meat increases the risk of colorectal adenomas. *Cancer Res.* **59**, 4320–4324.

Sinha R, Gustafson DR, Kulldorf M, Wen W-Q, Cerhan JR & Zheng W (2000a): 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, a carcinogen in high-temperature-cooked meat, and breast cancer risk. *J. Natl Cancer Inst.* **92**, 1352–1354.

Sinha R, Kulldorff M, Swanson CA, Curtin J, Brownson RC & Alavanja MCR (2000b): Dietary heterocyclic amines and the risk of lung cancer among Missouri women. *Cancer Res.* **60**, 3753–3757.

Sinha R, Kulldorff M, Chow W-H, Denobile J & Rothman N (2001): Dietary intake of heterocyclic amines, meat-derived mutagenic activity, and risk of colorectal adenomas. *Cancer Epidemiol. Biomark. Prev.* **10**, 559–562.

Skog K, Steineck G, Augustsson K & Jägerstad M (1995): Effect of cooking temperature on the formation of heterocyclic amines in fried meat products and pan residues. *Carcinogenesis* **16**, 861–867.

Skog KI, Johansson MAE & Jägerstad MI (1998): Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. *Food Chem. Toxicol.* **36**, 879–896.

Slimani N, Deharveng G, Charrondière RU, van Kappel AL, Ocké MC, Welch A, Lagiou A, van Liere M, Agudo A, Pala V, Brandstetter B, Andren C, Stripp C, van Staveren WA & Riboli E (1999): Structure of the standardised computeried 24-h recall interview used as reference method in the 22 centers participating in the EPIC project. *Comp. Meth. Progr. Biomed.* **58**, 251–266.

Slimani N, Ferrari P, Ocké MC, Welch A, Boeing H, van Liere A, Pala V, Amiano P, Lagiou A, Mattisson I, Stripp C, Engset D, Charrondière R, Buzzard M, van Staveren W & Riboli E (2000): Standardization of the 24-hour diet recall calibration method used in the European Prospective Investigation into Cancer and Nutrition (EPIC): general concepts and preliminary results. *Eur. J. Clin. Nutr.* **54**, 900–917.

Steineck G, Hagman U, Gerhardsson de Verdier M & Norell SE (1990): Vitamin A supplements, fried foods, fat and urothelial cancer. A case-referent study in Stockholm in 1985–87. *Int. J. Cancer* **45**, 1006–1011.

Tavani A, La Vecchia C, Gallus S, Lagiou P, Trichopoulos D, Levi F & Negri E (2000): Red meat intake and cancer risk. *Int. J. Cancer* **86**, 425–429.

Ward MH, Sinha R, Heineman EF, Rothman N, Markin R, Weisenburger DD, Correa P & Zahm SH (1997): Risk of adenocarcinoma of the stomach and esophagus with meat cooking method and doneness preference. *Int. J. Cancer* **71**, 14–19.

World Cancer Research Fund (1997): Food, nutrition and the prevention of cancer: a global perspective. Washington, DC: American Institute for Cancer Research.

Zheng W, Gustafson DR, Sinha R, Cerhan JR, Moore D, Hong C-P, Anderson KE, Kushi LH, Sellers TA & Folsom AR (1998): Well-done meat intake and the risk of breast cancer. *J. Natl Cancer Inst.* **90**, 1724–1729.

Zheng W, Deitz AC, Campbell DR, Wen W-Q, Cerhan JR, Sellers TA, Folsom AR & Hein DW (1999): *N*-Acetyltransferase 1 genetic polymorphism, cigarette smoking, well-done meat intake, and breast cancer risk. *Cancer Epidemol. Biomark. Prev.* **8**, 233–239.

CHAPITRE IV. LA VIANDE, LE POISSON ET LE RISQUE DE CANCER COLORECTAL : L'ETUDE PROSPECTIVE EUROPEENNE SUR LE CANCER ET LA NUTRITION (EPIC).

Basé sur: Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. J Natl Cancer Inst. 2005 ; 97 :906-16.

Reply to the letter of Dr. Batty and the letter of Dr. Gonder and Dr. Worm. J Natl Cancer Inst 2005

De nombreuses études de laboratoire, chez les animaux et des études épidémiologiques indiquent que la consommation élevée de viande rouge pourrait augmenter le risque de cancer colorectal, comme l'ont montré l'évaluation systématique des données scientifiques disponibles jusqu'au 1997 (50;69) et deux méta-analyses des études épidémiologiques de type cas-témoins et de cohorte (149;150). La consommation élevée de poisson pourrait diminuer le risque de ce cancer, mais les données sont moins convaincantes que pour la viande rouge.

Les études de cohorte publiées ultérieurement aux évaluations systématiques précédentes n'ont pas donné des preuves conclusives sur la relation entre la viande rouge et le risque de cancer colorectal (82;151). En particulier, dans l'analyse plus récente des cohortes américaines des infirmières (*Nurses' Health Study*) et des professionnels de la santé (*Health Professionals Follow-up Study*) (105), la relation entre la viande rouge et le cancer colorectal est plus faible que celle rapportée dans les analyses précédentes à ces deux études.

Nous avons examiné cette relation dans la population de l'Etude Prospective Européenne sur la Nutrition et le Cancer (EPIC), une cohorte intégrée par un demi-million d'hommes et de femmes des dix pays de l'Europe, avec des habitudes alimentaires et de style de vie diverses (152). Puisque les sujets qui suivent des régimes alimentaires riches en viande rouge ont

tendance à consommer très peu de fibres et de poissons, nous avons aussi déterminé le risque de cancer colorectal associé aux niveaux de consommations différents de viande rouge, pour des niveaux différents de consommation de fibres alimentaires et de poisson.

Les groupes alimentaires considérés dans cette analyse ont été la viande rouge (bœuf, veau, porc, agneau), la viande traitée (en général, viande du porc et du bœuf traité avec des procédures telles que salaison, fumaison, marinade, séchage, cuisson), les volailles et le poisson, frais ou traité. La consommation habituelle individuelle de viande et de poisson a été mesurée à l'aide des questionnaires alimentaires spécifiques pour chaque pays. La validité des méthodes utilisées a été établie par des études de validation, utilisant des biomarqueurs dans les urines et dans le sang (153).

Nous avons suivi prospectivement 478 040 hommes et femmes des dix pays européens, sans antécédents de maladie cancéreuse au moment de leur inclusion dans l'étude (Tableau 1). Le suivi a été fait de manière passive avec des Registres de Cancer et des Registres de Mortalité sauf en Grèce, France et Allemagne, où plusieurs méthodes de suivi actif ont été utilisées. Après un suivi moyen de 4.8 ans, 1329 cas de cancer colorectal ont été diagnostiqués dans la population (Tableau 2).

La relation entre la consommation de viande rouge, volailles et du poisson et le risque de cancer colorectal, a été analysée à l'aide du modèle des risques proportionnels, ajusté selon l'âge, le sexe, les apports caloriques, la taille, le poids, l'activité physique, le tabagisme, la consommation de fibres alimentaires et du folate, et la consommation d'alcool. Tous les modèles ont été stratifiés par centre.

Une étude de calibration sur 36 994 participants a été mise en place pour corriger partiellement l'effet de l'erreur de mesure de l'alimentation sur l'estimation des associations (*hazard ratios*) et leurs intervalles de confiance (IC) respectifs.

Dans la population EPIC, le risque de cancer colorectal est positivement associé aux niveaux de consommation de viande rouge et des viandes traitées (charcuterie) [Tableau 3]. Le *hazard ratio* associé à une consommation de viande rouge supérieure à 160 grammes par jour comparé à une consommation inférieure à 20 grammes par jour a été de 1.35 (95% IC=0.96-1.88), avec une tendance linéaire statistiquement significative (P=0.03). Le risque de cancer colorectal a été inversement lié à la consommation de poisson. Le *hazard ratio* a été estimé à 0.69 (IC=0.54-0.88) pour une consommation de poisson supérieure à 80 grammes par jour comparée à moins de 10 grammes par jour, avec une tendance linéaire statistiquement significative (P<0.001) [Figure 2]. La consommation de volailles est sans relation avec le risque de cancer colorectal.

La relation du risque de cancer colorectal pour la viande rouge et pour le poisson était plus marquée pour les tumeurs situées dans la partie gauche du côlon et pour le rectum, et de magnitude similaire pour les hommes et les femmes.

Les données suggèrent que l'association pourrait être plus forte avec la viande rouge traitée qu'avec la viande rouge non traitée. Les mécanismes biologiques qui pourraient induire une telle différence ne sont pas bien compris. Des sels de nitrites peuvent être ajoutés aux viandes pendant leur traitement, mais les quantités sont limitées, réduisant la plausibilité de cette hypothèse.

La correction de l'erreur de mesure a renforcé les estimations des associations (Tableau 4). Le *hazard ratio* pour chaque 100 grammes d'augmentation de la consommation de viande rouge était 1.25 avant calibration et 1.55 après calibration. Pour le poisson, le hazard ratio pour chaque 100 grammes d'augmentation de la consommation de poisson a été 0.70 et 0.46 avant et après calibration respectivement (Figure 2).

Pour tester si l'association observée avec la viande rouge est attribuable au remplacement de la viande rouge par le poisson, nous avons réalisé des analyses classant les sujets par niveaux de consommation de viande rouge et de poisson simultanément (Figure 3). Nous n'avons pas observé d'interaction significative. Nous avons aussi fait des analyses classant les sujets selon leur consommation de viande rouge et de fibre. Il n'y a pas d'augmentation du risque de cancer colorectal chez les sujets avec régime alimentaire simultanément riche en fibres et viande rouge, comparés avec les sujets qui ont un régime riche en fibre et faible en viande rouge. Le test d'interaction a été à la limite de la significativité statistique (P=0.06). Ces résultats laissent penser que la fibre alimentaire pourrait interférer avec l'effet nocif de la viande rouge mais les mécanismes doivent encore être explorés.

Dans la population étudiée, le risque absolu de développer un cancer colorectal au bout de dix ans de suivi d'un sujet âgé de 50 ans au moment du recrutement est 1.71% si le sujet a une consommation habituelle de viande rouge supérieure à 160 grammes, alors que le risque est 1.28% si la consommation de viande rouge est inférieure à 20 grammes par jour. Par rapport au poisson, le risque pour un sujet de 50 ans au recrutement, au bout de dix ans de suivi est 1.86% si la consommation de poisson est inférieure à 10 grammes par jour, et de 1.28% si celle-ci est supérieure à 80 grammes par jour.

Il a été estimé à 70% la fraction du risque de cancer colorectal évitable par la modification du style de vie dans les populations de pays occidentaux, y compris la réduction de la consommation de viande rouge. Nos données confirment l'hypothèse que la viande rouge joue un rôle sur le risque de cancer colorectal et suggère que le poisson pourrait avoir un effet protecteur.

Meat, Fish, and Colorectal Cancer Risk: The European Prospective Investigation into Cancer and Nutrition

Teresa Norat, Sheila Bingham, Pietro Ferrari, Nadia Slimani, Mazda Jenab, Mathieu Mazuir, Kim Overvad, Anja Olsen, Anne Tjønneland, Francoise Clavel, Marie-Christine Boutron-Ruault, Emmanuelle Kesse, Heiner Boeing, Manuela M. Bergmann, Alexandra Nieters, Jakob Linseisen, Antonia Trichopoulou, Dimitrios Trichopoulos, Yannis Tountas, Franco Berrino, Domenico Palli, Salvatore Panico, Rosario Tumino, Paolo Vineis, H. Bas Bueno-de-Mesquita, Petra H. M. Peeters, Dagrun Engeset, Eiliv Lund, Guri Skeie, Eva Ardanaz, Carlos González, Carmen Navarro, J. Ramón Quirós, María-José Sanchez, Göran Berglund, Irene Mattisson, Göran Hallmans, Richard Palmqvist, Nicholas E. Day, Kay-Tee Khaw, Timothy J. Key, Miguel San Joaquin, Bertrand Hémon, Rodolfo Saracci, Rudolf Kaaks, Elio Riboli

Background: **Current evidence suggests that high red meat intake is associated with increased colorectal cancer risk. High fish intake may be associated with a decreased risk, but the existing evidence is less convincing.** ***Methods:*** **We prospectively followed 478 040 men and women from 10 European countries who were free of cancer at enrollment between 1992 and 1998. Information on diet and lifestyle was collected at baseline. After a mean follow-up of 4.8 years, 1329 incident colorectal cancers were documented. We examined the relationship between intakes of red and processed meat, poultry, and fish and colorectal cancer risk using a proportional hazards model adjusted for age, sex, energy (nonfat and fat sources), height, weight, work-related physical activity, smoking status, dietary fiber and folate, and alcohol consumption, stratified by center. A calibration substudy based on 36 994 subjects was used to correct hazard ratios (HRs) and 95% confidence intervals (CIs) for diet measurement errors. All statistical tests were two-sided.** ***Results:*** **Colorectal cancer risk was positively associated with intake of red and processed meat (highest [>160 g/day] versus lowest [<20 g/day] intake, HR = 1.35, 95% CI = 0.96 to 1.88; P_{trend} = .03) and inversely associated with intake of fish (>80 g/day versus <10 g/day, HR = 0.69, 95 % CI = 0.54 to 0.88; P_{trend}<.001), but was not related to poultry intake. Correcting for measurement error strengthened the associations between colorectal cancer and red and processed meat intake (per 100-g increase HR = 1.25, 95% CI =1.09 to 1.41, P_{trend} = .001 and HR = 1.55, 95% CI = 1.19 to 2.02, P_{trend} = .001 before and after calibration, respectively) and for fish (per 100 g increase HR = 0.70, 95% CI = 0.57 to 0.87, P_{trend}<.001 and HR = 0.46, 95% CI = 0.27 to 0.77, P_{trend} = .003; before and after correction, respectively). In this study population, the absolute risk of development of colorectal cancer within 10 years for a study subject aged 50 years was 1.71% for the highest category of red and processed meat intake and 1.28% for the lowest category of intake and was 1.86% for subjects in the lowest category of fish intake and 1.28% for subjects in the highest category of fish intake.** ***Conclusions:*** **Our data confirm that colorectal cancer risk is positively associated with high consumption of red and processed meat and support an inverse association with fish intake. [J Natl Cancer Inst 2005;97:906–16]**

The finding that a high intake of red meat but not of chicken or fish might be associated with increased colon cancer risk was first reported in prospective studies by Willett et al. in 1990 *(1)*, from an analysis of 150 colorectal cancer patients in the Nurses' Health Study. Later, results from a systematic review of observational and experimental studies *(2)* and two meta-analyses *(3,4)* also supported the initial finding. However, the association

Affiliations of authors: From the Nutrition and Hormones Group, International Agency for Research on Cancer, Lyon, France (TN, PF, NS, MJ, MM, BH, RS, RK, ER); Medical Research Council Dunn Human Nutrition Unit, Cambridge, UK (SB); Department of Clinical Epidemiology, Aalborg Hospital and Aarhus University Hospital, Department of Epidemiology and Social Medicine, University of Aarhus, Denmark (KO); Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark (AO, A. Tjønneland); Institut National de la Santé et de la Recherche Médicale (INSERM) U521, Institut Gustave Roussy, Villejuif, France (FC, M-CB-R, EK); German Institute of Human Nutrition, Postdam-Rehbücke, Germany (HB, MMB); Division of Clinical Epidemiology, Deutches Krebsforschungszentrum, Heidelberg, Germany (AN, JL); Department of Hygiene and Epidemiology, Medical School, University of Athens, Greece (A. Trichopoulou, DT, YT); Epidemiology Unit, Istituto Tumori, Milan, Italy (FB); Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Center, Scientific Institute of Tuscany, Florence, Italy (DP); Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy (SP); Cancer Registry, Azienda Ospedaliera Civile MP, Arezzo, Ragusa, Italy (RT); University of Torino, Italy and Imperial College London, UK (PV); Center for Nutrition and Health, National Institute for Public Health and the Environment, Bilthoven, The Netherlands (HBB); Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, The Netherlands (PHMP); Institute of Community Medicine, University of Tromso, Norway (DE, EL, GS); Department of Epidemiology, Catalan Institute of Oncology, Barcelona, Spain (CG); Epidemiology Department, Health Council of Murcia, Spain (CN); Public Health Institute of Navarra, Pamplona, Spain (EA); Public Health and Health Planning Directorate, Asturias, Spain (JRQ); Andalusian School of Public Health, Granada, Spain (M-JS); Malmö Diet and Cancer Study, Lund University, Malmö, Sweden (GB, IM); Department of Nutritional Research (GH) and Department of Medical Biosciences, Pathology (RP), University of Umeå, Sweden; Strangeways Research Laboratory, Cambridge, UK (NED); the Clinical Gerontology Unit, University of Cambridge, UK (K-TK); Cancer Research UK Epidemiology Unit, University of Oxford, UK (TJK, MSJ).

Correspondence to: Elio Riboli, MD, MPH, International Agency for Research on Cancer, 150 Cours Albert Thomas, 69372 Lyon cedex 08, France (e-mail: riboli@iarc.fr).

See "Notes" following "References."

DOI: 10.1093/jnci/dji164

between colon cancer and red meat consumption was slightly weaker in a longer follow-up of the Nurses' Health Study *(5)* than in the previous analysis *(1)* and, in the combined analysis with the Health Professionals Follow-up Study, a statistically significant trend with processed meat but not with beef, pork, or lamb as a main dish was observed *(5)*. Results from another prospective study of American women showed no evidence of an association between meat and colorectal cancer *(6)*. More recently, results of the Cancer Prevention Study II Nutrition Cohort *(7)* showed that prolonged high consumption of red and processed meat might be associated with an increased risk of cancer of the distal portion of the large intestine; however, the increase was not statistically significant.

The evidence of an inverse association between colon cancer risk and fish intake is less consistent than the evidence of a positive association with red meat *(2)*. An inverse association has been observed in several prospective studies *(1,8–16)*, but the association was statistically significant in only two of them *(12,16)*. Fish intake was not associated with colorectal cancer risk in the most recently published prospective studies *(17–19)*.

No association with intake of poultry and colon cancer has been observed in almost all of the cohort studies *(8–10, 12–16)* that have examined this relationship. One study reported a statistically significant inverse trend *(1)*.

To examine whether associations exist between intakes of red and processed meat, of poultry, and of fish and colorectal cancer risk, we prospectively followed a large Western European population that includes half a million subjects from 10 European countries: the European Prospective Investigation into Cancer and Nutrition (EPIC) *(20)*. People who eat diets rich in meat also tend to eat less fiber and less fish *(21)*, and a statistically significant inverse association between dietary fiber consumption and colorectal cancer risk in this cohort has been reported elsewhere *(22)*. We therefore also investigated the risk of colorectal cancer associated with intakes of red and processed meat in individuals with different levels of intake of fish and fiber.

Subjects and Methods

Study Cohort

EPIC is a prospective study that was designed to investigate the relationships among diet, lifestyle, genetic and environmental factors, and the incidence of different forms of cancer. The study has been described in detail previously *(20,23)*. EPIC includes 366 521 women and 153 457 men, most aged 35–70 years at enrollment between 1992 and 1998, who were recruited in 23 centers in 10 European countries (Table 1). The study subjects were recruited from the general population and resided in defined areas in each country with some exceptions (women who were members of a health insurance scheme for state school employees in France and women attending breast cancer screening in Utrecht, The Netherlands; components of the Italian and Spanish cohorts included members of local blood donor associations). A large number of subjects who did not eat meat were enrolled in the Oxford "Health conscious" cohort. Eligible participants gave written informed consent and completed questionnaires on their diet, lifestyle, and medical history. Approval for this study was obtained from the ethical review boards of the International Agency for Research on Cancer and from all local institutions where subjects had been recruited for the EPIC study.

For this analysis, we excluded 22 432 cohort members with prevalent cancer at enrollment other than nonmelanoma skin cancer, 10 208 members who were in the lowest and highest 1% of the distribution of the ratio of reported total energy intake to energy requirement *(24)*, and 9298 members with missing questionnaire data or missing dates of diagnosis or follow-up. The number of subjects included in this analysis was 478 040.

Diet and Lifestyle Questionnaires

Diet over the 12 months before enrollment was measured between 1992 and 1998 by country-specific validated questionnaires. Most centers adopted a self-administered dietary questionnaire of 88 to 266 food items. In Greece, all centers in Spain, and Ragusa, Italy, the questionnaire was administered at a personal interview. In Malmö, Sweden, a questionnaire method combined with a food record was used. Data on height and weight, alcohol use, smoking status, occupational physical activity, and previous illnesses were also collected. Descriptions of the questionnaires used can be found on websites of the participating cohorts *(20)*. The validity of methods used was established in prior studies using 24-hour urine and blood samples as sources of biomarkers *(25)*.

For this analysis, meats were grouped into red meat, processed meat, and poultry. Red meat included all fresh, minced, and frozen beef, veal, pork, and lamb. Processed meats were mostly pork and beef that were preserved by methods other than freezing, such as salting (with and without nitrites), smoking, marinating, air drying, or heating (i.e., ham, bacon, sausages, blood sausages, meat cuts, "liver paté," salami, bologna, tinned meat, luncheon meat, corned beef, and others). Lamb and poultry are rarely processed into these types of meats in Europe. Poultry included all fresh, frozen, and minced chicken, and, in some cohorts, turkey. Fish included fresh, canned, salted, and smoked fish.

Identification of Colorectal Cancer Case Patients

The follow-up was based on population cancer registries, except in France, Germany, and Greece, where a combination of methods, including health insurance records, cancer and pathology registries, and active follow-up of study subjects and their next-of-kin was used. Mortality data were collected from either the cancer or mortality registries at the regional or national level.

Follow-up began at the date of enrollment and ended at either the date of diagnosis of colorectal cancer, death, or last complete follow-up. By October 30, 2002, for the centers using record linkage with cancer registry data, complete follow-up was available until December 31, 1998 (Bilthoven, Naples, Ragusa, and Turin), June 30, 1999 (Aarhus and Copenhagen), December 31, 1999 (Murcia and Varese), December 31, 2000 (Asturias, Granada, Navarra, San Sebastian, Florence, Norfolk, Oxford, Utrecht, and Norway), June 30, 2001 (Umea), December 31, 2001 (Malmö), and for the centers using active follow-up, the last contact dates were July 30, 2002 (France) July 15, 2002 (Greece), September 4, 2002 (Heidelberg), and September 20, 2002 (Postdam). Mortality data were coded using the 10th revision of the International Classification of Diseases, Injuries and Causes of Death, and cancer incidence following the International Classification of Diseases for Oncology, 2nd version. We included all incident cases of colon (C18) and rectal cancer. Cancer of the rectum included tumors occurring at the rectosigmoid

Table 1. Description of the centers participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study*

Center, country	Enrollment age, y	No. of colorectal cancer patients		Person–years		Mean (standard error) of daily intake (g/day)					
						Red and processed meat		Fish		Poultry	
		Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
Aarhus, Denmark	50–65	27	29	25 807	26 720	119.2 (0.3)	68.9 (0.2)	43.9 (0.02)	31.3 (0.1)	14.5 (0.05)	17.8 (0.1)
Copenhagen, Denmark	50–65	67	54	60 476	68 822	124.9 (0.2)	68.6 (0.1)	45.2 (0.1)	37.5 (0.1)	18.5 (0.04)	15.3 (0.05)
France	43–68	—	174	—	436 848	—	76.0 (0.1)	—	39.2 (0.04)	—	24.2 (0.1)
Heidelberg, Germany	40–65	33	15	42 033	45 772	143.2 (0.4)	75.2 (0.2)	17.0 (0.1)	14.0 (0.1)	19.4 (0.8)	15.8 (0.04)
Potsdam, Germany	35–65	39	27	49 560	71 605	140.9 (0.4)	73.5 (0.2)	23.6 (0.1)	19.3 (0.1)	16.7 (0.8)	11.9 (0.03)
Greece	30–76	13	11	37 985	54 628	64.0 (0.2)	34.6 (0.1)	52.1 (0.2)	31.2 (0.1)	15.3 (0.4)	12.4 (0.03)
Florence, Italy	35–65	17	30	16 598	49 991	102.4 (0.5)	64.1 (0.2)	33.2 (0.2)	21.0 (0.1)	41.0 (0.2)	24.5 (0.05)
Turin, Italy	35–65	18	4	18 739	12 430	96.3 (0.3)	60.6 (0.3)	32.0 (0.2)	20.3 (0.1)	40.4 (0.1)	24.0 (0.1)
Varese, Italy	35–72	6	20	7586	42 682	99.5 (0.5)	64.4 (0.2)	31.0 (0.3)	19.4 (0.1)	39.1 (0.2)	23.4 (0.05)
Naples, Italy	35–68	—	3	—	18 332	—	59.8 (0.2)	—	26.5 (0.1)	—	24.0 (0.1)
Ragusa, Italy	35–65	2	1	10 268	10 050	89.2 (0.4)	56.8 (0.3)	27.4 (0.2)	17.6 (0.1)	39.5 (0.2)	24.0 (0.1)
Bilthoven, Netherlands	21–64	13	9	35 278	41 618	141.2 (0.5)	81.2 (0.3)	17.0 (0.1)	11.6 (0.1)	19.0 (0.1)	13.6 (0.1)
Utrecht, Netherlands	49–70	—	75	—	84 003	—	78.8 (0.2)	—	14.0 (0.05)	—	13.2 (0.05)
Norway	41–56	—	20	—	57 409	—	77.8 (0.1)	—	48.6 (0.1)	—	12.8 (0.03)
Granada, Spain	35–65	7	9	9838	30 165	127.8 (0.8)	65.7 (0.2)	96.6 (0.7)	60.0 (0.2)	33.1 (0.2)	25.0 (0.1)
Murcia, Spain	35–65	7	10	14 117	29 926	134.3 (0.7)	72.0 (0.3)	90.3 (0.1)	59.5 (0.2)	36.3 (0.2)	28.5 (0.1)
Asturias, Spain	35–65	11	11	19 679	34 291	134.9 (0.5)	71.3 (0.2)	96.1 (0.5)	62.8 (0.2)	37.7 (0.2)	27.8 (0.1)
Navarra, Spain	35–64	14	10	25 195	26 081	147.3 (0.5)	79.5 (0.3)	91.4 (0.5)	59.4 (0.2)	38.5 (0.2)	28.0 (0.1)
San Sebastian, Spain	35–65	20	11	22 676	23 782	141.3 (0.5)	71.1 (0.3)	99.9 (0.5)	64.7 (0.2)	40.0 (0.2)	28.6 (0.1)
Malmo, Sweden	45–73	94	100	78 719	105 514	123.1 (0.2)	78.5 (0.1)	42.0 (0.1)	33.1 (0.05)	11.5 (0.04)	11.2 (0.02)
Umea, Sweden	30–60	44	34	90 463	92 742	121.7 (0.2)	77.5 (0.1)	33.5 (0.1)	25.6 (0.03)	7.7 (0.03)	8.1 (0.02)
Norfolk, U.K.	41–76	82	68	51 031	61 102	78.8 (0.3)	46.2 (0.1)	31.7 (0.1)	28.7 (0.03)	26.4 (0.2)	22.2 (0.1)
Oxford, U.K.	21–83	8	14	5041	16 705	79.9 (0.6)	49.4 (0.2)	33.0 (0.1)	28.2 (0.05)	28.6 (0.4)	23.2 (0.2)
Oxford, Health Conscious, U.K.	21–83	20	48	50 317	166 460	24.4 (0.2)	14.0 (0.1)	13.6 (0.2)	12.9 (0.02)	10.7 (0.2)	9.6 (0.05)

*Enrollment ages are the 1st–99th percentile. Calibrated mean daily intakes of red meat, fish, and poultry are shown. Center- and sex-specific calibration models were obtained by regression of the main dietary questionnaires on the 24-hour diet recall values. Models included energy from nonfat sources, energy from fat sources, weight, height, age at recruitment, day of the week, and season of the year on which the 24-hour diet recall was collected.

junction (C19) and at the rectum (C20). Anal canal tumors were excluded. Right colon tumors included tumors of the caecum, appendix, ascending colon, hepatic flexure, transverse colon, and splenic flexure (codes C18.0–18.5 of the International Statistical Classification of Diseases for Oncology, version 2). Left colon tumors included those in the descending and sigmoid colon (C18.6–18.7).

Statistical Methods

Analyses were conducted using Cox regression. We tested the proportional hazard assumption for red meat, fish, and poultry intake variables in relation to colorectal cancer using the likelihood ratio test, comparing models with and without product terms for the meat and fish variables and follow-up time (years). Data were stratified by center to control for differences in questionnaire design, follow-up procedures, and other center effects. The five Italian centers were combined for analysis, as were the five Spanish centers. The Norfolk and Oxford general U.K. populations were combined. Age was used as the primary time variable, and sex was included as a covariate. The analysis focused on food groups of meats and fish available in all EPIC cohorts: red meat, processed meat, poultry, and fish *(26,27)*. Dietary intakes were analyzed as continuous variables and in five categories using cut points based on the progressive doubling of intake levels. The same cut points were applied to red meat, processed meat, and fish, with the aim of estimating relative risks for comparable levels of intake. Categorical variables were scored from 1 to 5, according to the interval in which an observation lay. Trend tests were calculated on these scores. Categorical relative risks were calculated from the hazard ratio.

The results were adjusted for estimated energy intake, which was divided into energy from fat and energy from nonfat sources to control partly for error in estimated intakes of foods. To control for body size and obesity, we adjusted for weight and height. Further adjustment included smoking (never, former, and current smoker), alcohol intake (grams per day), dietary fiber (grams per day), and occupational physical activity (no activity, sedentary, standing, manual, and heavy manual). In some models, meat and fish intakes were adjusted for each other. The results were adjusted for dietary folate and use of multivitamin supplements at baseline in 409 135 control subjects and 1176 case patients for whom information on dietary folate was available in the dataset. Separate analyses were done for men and women. Analyses of women were adjusted for use of hormonal replacement therapy. No important differences between the sexes emerged, and only the results for both sexes combined are presented in this report. Subsequent analyses were performed after the exclusion of case patients who were diagnosed during the first 2 years of follow-up.

Calibration of the Dietary Data

A second dietary measurement was taken from an 8% random sample of the cohort (36 994 participants) using a very detailed computerized 24-hour diet recall method *(28)* to calibrate dietary measurements across countries and to correct for systematic over- or underestimation of dietary intakes *(29–31)*. The 24-hour diet recall values of these 36 994 cohort participants were regressed on the main dietary questionnaire values for red and processed meat, poultry, and fish. Zero consumption values in the main dietary questionnaires were excluded in the regression calibration models (5%–13% of the participants depending on the food variable). Energy from nonfat sources, energy from fat sources, weight, height, age at recruitment, day of the week, and season of the year on which the 24-hour recall was collected were included as covariates. Energy from nonfat sources and from fat sources were calibrated following the same approach. Center- and sex-specific calibration models were used to obtain individual predicted values of dietary exposure for all participants.

Cox regression models were then applied using the predicted values for each individual on a continuous scale. The standard error of the de-attenuated coefficient was calculated with bootstrap sampling in the calibration and disease models, consecutively. The P_{trend} values for the de-attenuated coefficient were calculated by dividing the de-attenuated coefficient by the bootstrap-derived standard error and approximating the standard normal distribution *(31)*.

The Wald statistic was used to test for homogeneity of risks of the left-sided and right-sided colon tumors *(32)*. To assess heterogeneity of de-attenuated risk estimates across centers, we included center as main effect and interaction terms in Cox models. Heterogeneity was explored by meta-regression using the Genmod procedure. All analyses were performed using SAS Statistical Software, version 8 (SAS Institute, Cary, NC), and all statistical tests were two-sided. For all analyses, P values $<.05$ were considered statistically significant.

Results

A total of 478 040 participants contributed 2 279 075 person-years in a mean follow-up of 4.8 years since 1992. During follow-up, 1329 participants were diagnosed with colorectal cancer. Of these cancers, 95% were histologically verified; 855 tumors were located in the colon and 474 in the rectum. The number of colorectal cancer subjects, person-years, and the mean calibrated intakes of meat and fish by center are shown in Table 1. Baseline characteristics of the participants are also given in Table 2.

Increasing red and processed meat intake was statistically significantly associated with increasing risk of colorectal cancer (hazard ratio [HR] for highest versus lowest intake level = 1.57, 95% confidence interval [CI] = 1.13 to 2.17, P_{trend} = .001) in analysis adjusted for sex and energy intake (Table 3). This increase in risk was somewhat reduced after adjustment for other covariates (HR = 1.35, 95% CI = 0.96 to 1.88, P_{trend} = .03). The association with cancers of the left side of the colon and the rectum was somewhat stronger than that with cancers of the right side of the colon, but the difference was not statistically significant ($P_{heterogeneity}$ = .29). In separate analyses, intake of red meat was positively but not statistically significantly associated with colorectal cancer (HR for highest versus lowest intake = 1.17, 95% CI = 0.92 to 1.49, P_{trend} = .08), whereas intake of processed meat was statistically significantly associated with increased colorectal cancer risk (HR for highest versus lowest intake = 1.42, 95% CI = 1.09 to 1.86, P_{trend} = .02). The results for red meat were similar for colon and rectum and for right and left side of the colon ($P_{heterogeneity}$ = .72). Hazard ratios for processed meat intakes were somewhat higher for tumors of the left side of the colon and tumors of the rectum as compared with tumors of the right side of the colon, but the differences were not statistically significant ($P_{heterogeneity}$ = .87).

In analyses of subgroups of red meats, colorectal cancer risk was statistically significantly associated with intake of pork

Table 2. Baseline characteristics according to colorectal cancer status at the end of follow-up in the European Prospective Investigation into Cancer and Nutrition (EPIC)*

Characteristic	Men		Women	
	Cases (n = 542)	Noncases (n = 141 445)	Cases (n = 787)	Noncases (n = 335 265)
Age, y	59.6 (7.4)	52.2 (10.1)	58.7 (7.9)	50.8 (9.8)
Weight, kg	83.3 (12.6)	81.3 (12)	67.6 (12.1)	66.1 (11.8)
Height, cm	174.2 (6.8)	174.8 (7.4)	161.8 (6.3)	162.3 (6.8)
Fiber, g/day	21.8 (8.2)	24.1 (9.4)	21.6 (7.5)	22.3 (7.7)
Folate, μg/day†	299 (105)	318 (116)	300 (129)	296 (129)
Smoking, % in each category†				
Nonsmokers	27	33	57	56
Former smokers	48	37	24	23
Smokers	24	29	17	20
Physical activity at work, % in each category‡				
No work activity	42	23	50	30
Sedentary	26	34	16	22
Standing	16	21	24	28
Manual, heavy manual	15	19	6	7

*Mean (standard deviation) or percentage in each group.

†Folate values from 1176 case patients and 409 135 cohort participants.

‡Percentages do not add to 100% due to missing values.

(for highest versus lowest intake, HR = 1.18, 95% CI = 0.95 to 1.48, P_{trend} = .02) and lamb (HR = 1.22, 95% CI = 0.96 to 1.55, P_{trend} = .03) but not with beef/veal (HR = 1.03, 95% CI = 0.86 to 1.24, P_{trend} = .76). In analyses in which intake of each meat was mutually adjusted for intake of the other meats, only the trend for increased colorectal cancer risk with increased pork intake remained statistically significant (P_{trend} = .03). Intakes of ham (for highest versus lowest intake, HR = 1.12, 95% CI = 0.90 to 1.37, P_{trend} = .44), of bacon (HR = 0.96, 95% CI = 0.79 to 1.17, P_{trend} = .34), and of other processed meats (mainly sausages) (HR = 1.05, 95% CI = 0.84 to 1.32, P_{trend} =.22) were not independently related to colorectal cancer risk.

Intake of fish was statistically significantly inversely associated with colorectal cancer risk (for highest versus lowest intake HR = 0.69, 95% CI = 0.54 to 0.88, P_{trend}<.001). The trend for an inverse association was statistically significant for cancers of the left side of the colon (P_{trend} = .02) and the rectum (P_{trend}<.001), but not for cancers of the right side of the colon (Table 3). Intake of poultry was not statistically significantly associated with colorectal cancer risk. The inverse association with fish and the positive association with red and processed meat intake persisted when fish, poultry, and red and processed meat were all included as continuous variables in the same model (P_{trend}<.001 for fish and P_{trend} = .02 for red and processed meat). In this study population, the absolute risk of developing colorectal cancer within 10 years for a study subject aged 50 years was 1.71% for the highest category of red and processed meat intake and 1.28% for the lowest category of intake, was 1.86% for subjects in the lowest category of fish intake, and was 1.28% for subjects in the highest category of fish intake.

When we adjusted for dietary folate intake in a subset of the cohort including only participants for whom the information on folate intake was available in the core dataset (1176 colorectal cancer case patients and 407 959 participants free of colorectal cancer), the results were not substantially modified. For this subset, the hazard ratio for the highest intake of red and processed meat versus lowest intake was 1.27 (P_{trend} = .12) before adjustment for folate and 1.25 (P_{trend} = .15) after adjustment. For the highest versus the lowest intake of fish, the hazard ratios were 0.68 (P_{trend}<.001) before and 0.67 (P_{trend}<.001) after adjustment for folate.

We tested the consistency of these results after the exclusion of the case patients diagnosed during the first 2 years of follow-up, because these case patients might have modified their diet during the prediagnostic disease phase that preceded enrollment. The hazard ratios for the group with the highest consumption of red and processed meat were 1.35 (95% CI = 0.96 to 1.88) before and 1.35 (95% CI = 0.90 to 2.03) after exclusion (1329 and 861 colorectal cancer case patients, respectively); for fish the hazard ratios were 0.69 before and 0.70 after the exclusions.

Calibration of the data for systematic and random dietary intake measurement errors strengthened the observed associations between red and processed meat and fish intake and colorectal cancer risk. The multivariable hazard ratio per 100-g increase in intake of red and processed meat was 1.25 (95% CI = 1.09 to 1.41, P_{trend} = .001) before calibration and 1.55 (95% CI = 1.19 to 2.02, P_{trend} = .001) after calibration. In corrected models, the association between intake of processed meat and colon cancer risk (HR per 100-g increase =1.68, 95% CI = 0.87 to 3.27) was stronger than the association between intake of red meat (HR = 1.36, 95% CI = 0.74 to 2.50), but neither association was statistically significant. The corrected estimates for rectal cancer were similar to those for colon cancer (Table 4). The hazard ratios per 100-g increase in fish intake were 0.70 (95% CI = 0.57 to 0.87, P_{trend}<.001) and 0.46 (95% CI = 0.27 to 0.77, P_{trend} = .003) before and after correction. The association was statistically significant and similar for both colon and rectal cancers. Uncorrected and corrected hazard ratios across all ranges of red and processed meat and fish consumed are shown (Fig. 1).

Calibrated hazard ratios were estimated for each center with more than 50 colorectal cancer case patients (Fig. 2). The association of red and processed meat intake with colorectal cancer was consistent across centers ($P_{heterogeneity}$ = .82). However, the association with fish intake was not consistent across centers ($P_{heterogeneity}$ = .03). In meta-regression analyses, none of the following variables independently explained the heterogeneity: geographic region (Nordic countries, United Kingdom, Central Europe, or South of Europe), mean fish intake in each cohort *(27)*, and proportion of consumed fish that was grilled, fried, or barbecued, as estimated from 24-hour dietary recall *(33)*. In addition, when mean fatty fish intake from 24-hour dietary recall *(27)* was included in the models instead of mean total fish intake, the results were unchanged.

To examine whether the displacement of red and processed meat intake by fish could partially explain the inverse association of fish intake with colorectal cancer risk, we conducted cross-classified analyses by sex-defined tertiles of fish and red and processed meat intake (Spearman correlation coefficient r, between intake levels of fish and red and processed meat after adjustment for age, sex, center, energy intake, height, and weight = .04 in men and .07 in women). No interaction between fish and meat was observed ($P_{interaction}$ = .82). The risk increase associated with high consumption of red and processed meat versus low consumption (>129 g/day in men and >85 g/day in women versus <30 g/day in men and <13 g/day in women) was 12%–20%, independent of the levels of fish consumption (Fig. 3). The risk increase associated with low versus high fish consumption (<14 g/day in both men and women versus >50 g/day in men and women) was approximately 40%, independent of the levels of red and processed meat intake.

Table 3. Multivariable hazard ratios of colorectal cancer and 95% confidence intervals for categories of consumption of red meat, processed meat, poultry, and fish, according to anatomic location for participants in the European Prospective Investigation into Cancer and Nutrition (EPIC)

Food group, g/day	*N**	Colorectal cancer† (*N* = 1329)	Colorectal cancer‡ (*N* = 1329)	Colon cancer‡			Rectal cancer‡ (*N* = 474)
				All colon (*N* = 855)	Right-side (*N* = 351)	Left-side (*N* = 391)	
Red and processed meat							
<10	90	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)
20–40	133	1.04 (0.79 to 1.38)	0.98 (0.74 to 1.30)	0.98 (0.70 to 1.37)	0.89 (0.55 to 1.45)	1.34 (0.75 to 2.39)	0.97 (0.57 to 1.64)
40–80	466	1.33 (1.04 to 1.71)	1.22 (0.95 to 1.56)	1.05 (0.78 to 1.42)	0.99 (0.64 to 1.51)	1.34 (0.79 to 2.29)	1.66 (1.06 to 2.62)
80–160	524	1.39 (1.07 to 1.80)	1.23 (0.94 to 1.60)	1.17 (0.86 to 1.60)	1.09 (0.70 to 1.72)	1.55 (0.89 to 2.69)	1.40 (0.87 to 2.25)
≥160	116	1.57 (1.13 to 2.17)	1.35 (0.96 to 1.88)	1.17 (0.78 to 1.77)	1.03 (0.56 to 1.91)	1.51 (0.76 to 3.02)	1.75 (0.98 to 3.10)
P_{trend}		.001	.03	.15	.47	.14	.06
Red meat							
<10	132	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)
10–20	138	1.04 (0.81 to 1.33)	1.00 (0.78 to 1.28)	1.04 (0.77 to 1.41)	1.13 (0.70 to 1.84)	1.07 (0.68 to 1.68)	0.93 (0.60 to 1.44)
20–40	323	1.09 (0.88 to 1.35)	1.03 (0.83 to 1.28)	1.02 (0.78 to 1.32)	1.00 (0.65 to 1.54)	1.10 (0.65 to 1.63)	1.07 (0.74 to 1.55)
40–80	486	1.24 (1.01 to 1.54)	1.16 (0.94 to 1.43)	1.16 (0.90 to 1.51)	1.36 (0.90 to 2.07)	1.11 (0.75 to 1.64)	1.16 (0.80 to 1.66)
≥80	250	1.28 (1.1 to 1.64)	1.17 (0.92 to 1.49)	1.20 (0.88 to 1.61)	1.18 (0.73 to 1.91)	1.24 (0.80 to 1.94)	1.13 (0.74 to 1.71)
P_{trend}		.008	.08	.14	.22	.38	.32
Processed meat							
<10	232	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)
10–20	256	1.15 (0.95 to 1.38)	1.10 (0.91 to 1.32)	1.08 (0.86 to 1.36)	1.04 (0.73 to 1.49)	1.30 (0.92 to 1.83)	1.13 (0.81 to 1.58)
20–40	402	1.19 (1.00 to 1.43)	1.12 (0.94 to 1.35)	1.06 (0.85 to 1.32)	0.95 (0.67 to 1.34)	1.32 (0.94 to 1.85)	1.27 (0.93 to 1.74)
40–80	318	1.23 (1.01 to 1.50)	1.14 (0.94 to 1.40)	1.21 (0.95 to 1.54)	1.17 (0.80 to 1.70)	1.45 (1.00 to 2.11)	1.05 (0.74 to 1.50)
≥80	121	1.54 (1.18 to 2.02)	1.42 (1.09 to 1.86)	1.30 (0.92 to 1.84)	1.19 (0.70 to 2.01)	1.48 (0.87 to 2.53)	1.62 (1.04 to 2.50)
P_{trend}		.005	.02	.12	.45	.08	.20
Fish							
<10	247	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)
10–20	233	0.90 (0.75 to 1.08)	0.88 (0.74 to 1.06)	0.94 (0.74 to 1.18)	0.97 (0.69 to 1.38)	0.81 (0.57 to 1.14)	0.80 (0.59 to 1.09)
20–40	428	0.88 (0.74 to 1.05)	0.86 (0.72 to 1.02)	0.89 (0.72 to 1.11)	0.76 (0.54 to 1.08)	0.96 (0.70 to 1.31)	0.80 (0.60 to 1.06)
40–80	303	0.70 (0.57 to 0.84)	0.67 (0.56 to 0.82)	0.69 (0.54 to 0.88)	0.83 (0.57 to 1.20)	0.61 (0.42 to 0.88)	0.64 (0.47 to 0.88)
≥80	118	0.71 (0.55 to 0.91)	0.69 (0.54 to 0.88)	0.82 (0.60 to 1.11)	0.85 (0.53 to 1.37)	0.70 (0.44 to 1.11)	0.49 (0.32 to 0.76)
P_{trend}		<.001	<.001	.01	.30	.02	<.001
Poultry							
<5	368	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)
5–10	216	0.92 (0.77 to 1.10)	0.90 (0.75 to 1.08)	0.92 (0.74 to 1.14)	0.83 (0.59 to 1.16)	1.00 (0.72 to 1.38)	0.87 (0.64 to 1.18)
10–20	319	0.90 (0.77 to 1.06)	0.88 (0.75 to 1.03)	0.83 (0.67 to 1.01)	0.72 (0.52 to 0.99)	0.88 (0.65 to 1.18)	0.97 (0.75 to 1.27)
20–40	235	0.87 (0.73 to 1.04)	0.85 (0.71 to 1.01)	0.86 (0.69 to 1.07)	0.87 (0.62 to 1.21)	0.85 (0.61 to 1.18)	0.83 (0.62 to 1.12)
≥40	191	0.94 (0.78 to 1.15)	0.92 (0.76 to 1.12)	0.89 (0.70 to 1.13)	0.78 (0.53 to 1.16)	0.87 (0.60 to 1.25)	0.99 (0.71 to 1.37)
P_{trend}		.29	.18	.19	.21	.27	.65

*Distribution of colorectal cancer cases by category of meat intake.

†Cox regression using age, sex, energy from nonfat sources (continuous variable), energy from fat sources (continuous variable), and stratified for center.

‡Cox regression using age, sex, energy from nonfat sources (continuous variable), energy from fat sources (continuous variable), height (tertiles defined for each sex and center), weight (tertiles defined for each sex and center), occupational physical activity (no activity, sedentary, standing, manual, or heavy manual), smoking status (never, former, or current smoker), dietary fiber (g/day), alcohol intake (g/day), and stratified for center.

Table 4. Multivariable hazard ratios (HRs, per 100 g) and 95% confidence intervals (CIs) of colorectal cancer for observed and calibrated intakes of red meat, processed meat, fish, and poultry by anatomic location for participants in the European Prospective Investigation into Cancer and Nutrition (EPIC)*

Food group	Cancer site	Observed		Calibrated	
		HR (95% CI), per 100 g	P_{trend}	HR (95% CI), per 100 g	P_{trend}
Red and processed meat	Colorectum	1.25 (1.09 to 1.41)	.001	1.55 (1.19 to 2.02)	.001
	Colon	1.26 (1.07 to 1.48)	.006	1.49 (1.03 to 2.16)	.04
	Rectum	1.22 (0.99 to 1.51)	.06	1.65 (1.05 to 2.62)	.03
Red meat	Colorectum	1.21 (1.02 to 1.43)	.03	1.49 (0.91 to 2.43)	.11
	Colon	1.20 (0.96 to 1.48)	.10	1.36 (0.74 to 2.50)	.32
	Rectum	1.23 (0.94 to 1.62)	.14	1.75 (0.93 to 3.30)	.08
Processed meat	Colorectum	1.32 (1.07 to 1.63)	.009	1.70 (1.05 to 2.76)	.03
	Colon	1.39 (1.06 to 1.82)	.01	1.68 (0.87 to 3.27)	.12
	Rectum	1.22 (0.87 to 1.71)	.25	1.70 (0.83 to 3.47)	.14
Fish	Colorectum	0.70 (0.57 to 0.87)	<.001	0.46 (0.27 to 0.77)	.003
	Colon	0.76 (0.59 to 0.99)	.04	0.49 (0.26 to 0.93)	.03
	Rectum	0.61 (0.43 to 0.87)	.006	0.41 (0.17 to 0.97)	.04
Poultry	Colorectum	0.92 (0.68 to 1.25)	.61	0.85 (0.43 to 1.70)	.65
	Colon	0.92 (0.63 to 1.35)	.68	0.76 (0.29 to 2.03)	.59
	Rectum	0.92 (0.56 to 1.53)	.77	1.04 (0.34 to 3.23)	.94

*Cox regression with age as primary time variable. Covariates are sex, energy from fat, energy from -nonfat sources except alcohol, height (tertiles defined by sex and center), weight (tertiles defined by sex and center), current alcohol intake (g/day), occupational physical activity, smoking status (never, former, or current smoker), and fiber intake. Stratification by center.

Subjects with high red meat and low fish intake were at 63% increased risk of colorectal cancer (HR = 1.63, 95% CI = 1.22 to 2.16), compared with subjects with low red meat and high fish intake.

We also used cross-classified analysis to investigate whether low fiber intake could partially explain the increase in colorectal cancer risk in high consumers of red and processed meat (Spearman correlation coefficient between fiber and red and processed meat after adjustment for age, sex, center, energy intake, height, and weight = −.18 in men and −.21 in women). The increase in colorectal cancer risk associated with high intake of red and processed meat was more apparent in the group of participants in the categories of low (<17 g/day) and medium (17 to 26 g/day in women and 17 to 28 g/day in men) fiber intake than in the high (>26 g/day in women and >28 g/day in men) intake group ($P_{interaction}$ = .06). The hazard ratio in the cohort participants with high intake of red and processed meat was 1.09 (95% CI = 0.83 to 1.42) for the group with high intake of fiber, 1.20 (95% CI = 0.93 to 1.56) for the group with medium intake of fiber, and 1.50 (95% CI = 1.15 to 1.97) for the group with low intake of fiber compared with the group with low intake of red and processed meat and high intake of fiber. A statistically significant risk increase was also observed for the group of subjects with low intake of fiber and medium intake of red and processed meat (HR= 1.38, 95% CI = 1.06 to 1.80) compared with the group with high intake of fiber and low intake of red and processed meat. The risk reduction associated with high fiber intake was of similar magnitude in all categories of intake of red and processed meat.

Discussion

The results reported here are from one of the largest cohorts of men and women that has been developed specifically to examine the relationship between diet and cancer. We found a consistent positive association between high intake of red and processed meat and colorectal cancer and an inverse association between high intake of fish and colorectal cancer. These findings held in models adjusted for age, sex, and energy and in models adjusted for other covariates.

In this study population, the absolute risk of developing colorectal cancer within 10 years for a study subject aged 50 years was 1.71% for the highest category of red meat intake and 1.28% for the lowest category of intake; risk was 1.86% for subjects in the lowest category of fish intake and 1.28% for subjects in the highest category of fish intake. We found that the associations of red meat and fish intake with cancer risk were stronger for tumors of the rectum and left side of the colon than for right-sided colon tumors, although differences were not statistically significant. The opposing associations of red meat and fish intake were not explained by the displacement of one by the other, because the associations did not disappear when fish and red meat were mutually adjusted for each other. Colorectal cancer risk was not associated with poultry intake.

The mechanisms underlying the association between colorectal cancer risk and high intake of red and processed meat are uncertain. Controlled human intervention studies have raised the possibility that the endogenous nitrosation that arises from ingestion of heme iron but not of inorganic iron or protein may account for the increased risk associated with red and processed meat consumption *(34,35)*. Heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAH) in diet may pose a potential risk of cancer to humans *(36)*, depending on the extent to which the compounds are activated in vivo by metabolic enzymes. HCAs are formed as a byproduct of reactions during the cooking of meat, poultry, and fish at high temperatures, such as pan-frying or grilling with charcoal or on a gas grill; PAHs are formed in grilled and barbecued meat and in cured, processed foods *(36)*. The results of studies of the association of polymorphisms of genes encoding for enzymes associated with the metabolism and disposition of HCAs and PAHs and risk of colorectal cancer are inconsistent *(37–41)*. Information on cooking methods to estimate dietary exposure to HCAs and PAHs produced from pyrolysis of meat and fish was not systematically collected in the baseline EPIC dietary questionnaires. However, this information was systematically collected in the 24-hour diet recall study. Chicken is a major contributor to HCA intake, but we observed no association between poultry intake and colorectal cancer risk in this study. Furthermore, although analyses of

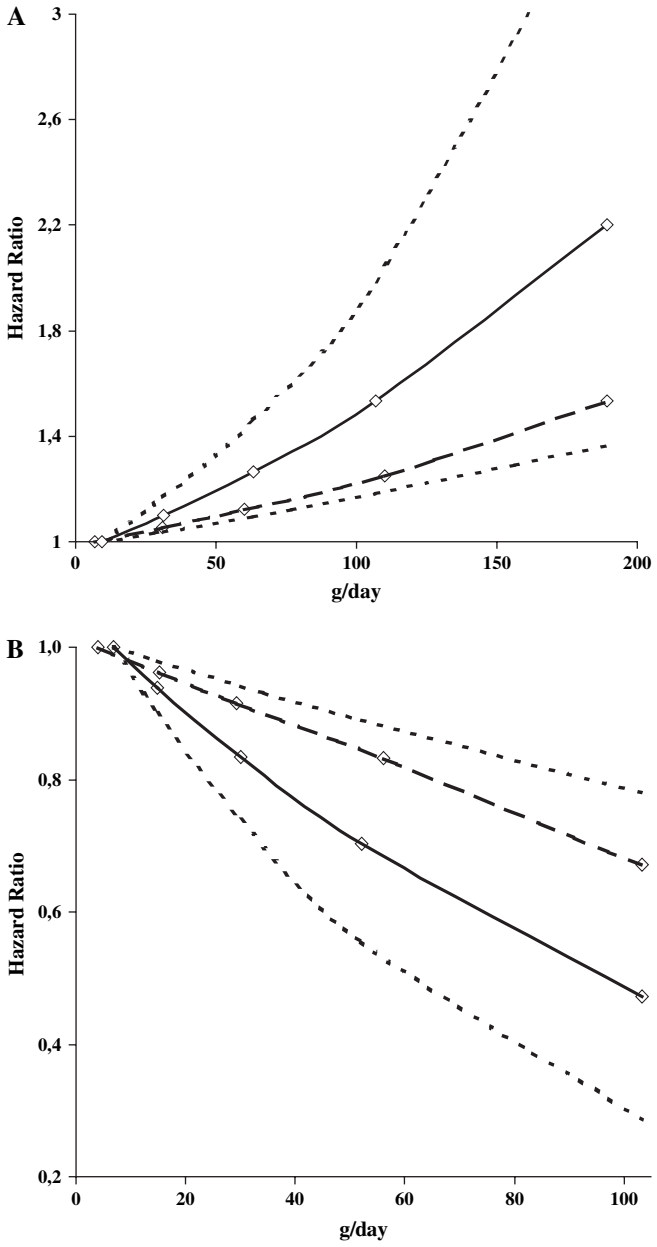


Fig. 1. Hazard ratios of colorectal cancer in the European Prospective Investigation into Cancer and Nutrition Cohort. Hazard ratios by **A**) intake of red and processed meat and **B**) by intake of fish. Hazard ratios were calculated from Cox regression models adjusted for age, sex, energy from nonfat sources (continuous variable), energy from fat sources (continuous variable), height (tertiles defined for each sex and center), weight (tertiles defined for each sex and center), work-related physical activity (no activity, sedentary, standing, manual, or heavy manual) smoking status (never, former, or current smoker), alcohol consumption (grams per day) and stratified for center. **Points** in the figure represent median intakes in each category of consumption. Curves generated from calibrated data (**solid line**) and uncalibrated data (**hatched line**) and upper and lower confidence intervals for calibrated data (**dotted lines**) are shown.

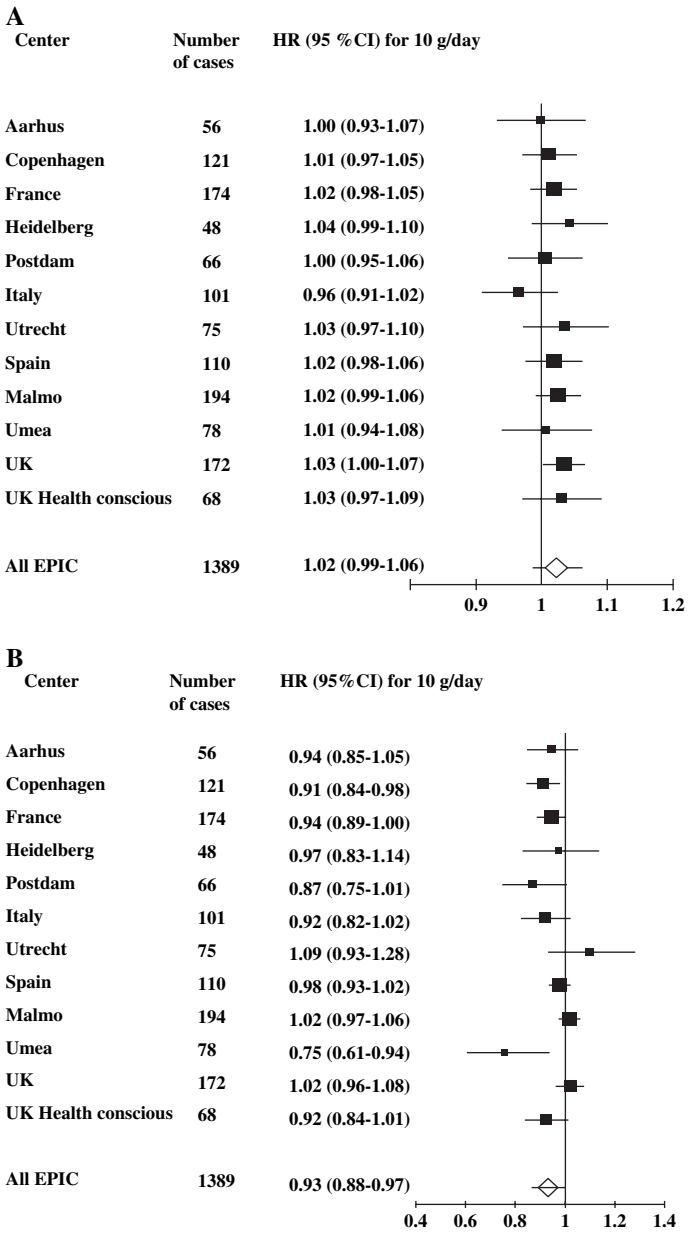


Fig. 2. Multivariable hazard ratios and 95% confidence intervals from calibrated analyses of colorectal cancer for individual center in the European Prospective Investigation into Cancer and Nutrition (EPIC) Cohort. Hazard ratios (HRs) per 100-g increase in intake and 95% confidence intervals (CIs) were calculated for calibrated intakes of **A**) red and processed meat and **B**) fish. Hazard ratios were calculated from β coefficients from Cox regression models adjusted for age, sex, energy from nonfat sources (continuous variable), energy from fat sources (continuous variable), height (tertiles defined for each sex and center), weight (tertiles defined for each sex and center), work-related physical activity (no activity, sedentary, standing, manual, or heavy manual) smoking status (never, former, or current smoker), fiber intake (grams per day) and alcohol consumption (grams of day). Centers with fewer than 50 case patients with colorectal cancer are not included. The **black squares and horizontal lines** correspond to the center-specific hazard ratios (per 100-g increase in intake) and 95% confidence intervals. The area of the square reflects the center-specific statistical weight (inverse of the variance). The **diamond and horizontal lines** represent the hazard ratio and 95% confidence intervals in EPIC.

the 24-hour recall data showed a high variation in meat and fish cooking practices across cohorts participating in EPIC *(33)*, we did not observe heterogeneity of association of colorectal cancer risk with red meat intake across the centers (Fig. 2).

It has been suggested that processed meat intake has a stronger association with colorectal cancer than red meat intake *(3,7)*. Indeed, in this European study, we found that the overall association with colorectal cancer risk was stronger for processed than for unprocessed red meat. However, we could not determine whether one particular type of either red meat or processed meat was more strongly associated with colorectal cancer risk than others. In Europe, processed meat is a mixed category of mainly pork and beef meats that are preserved by mechanical, chemical,

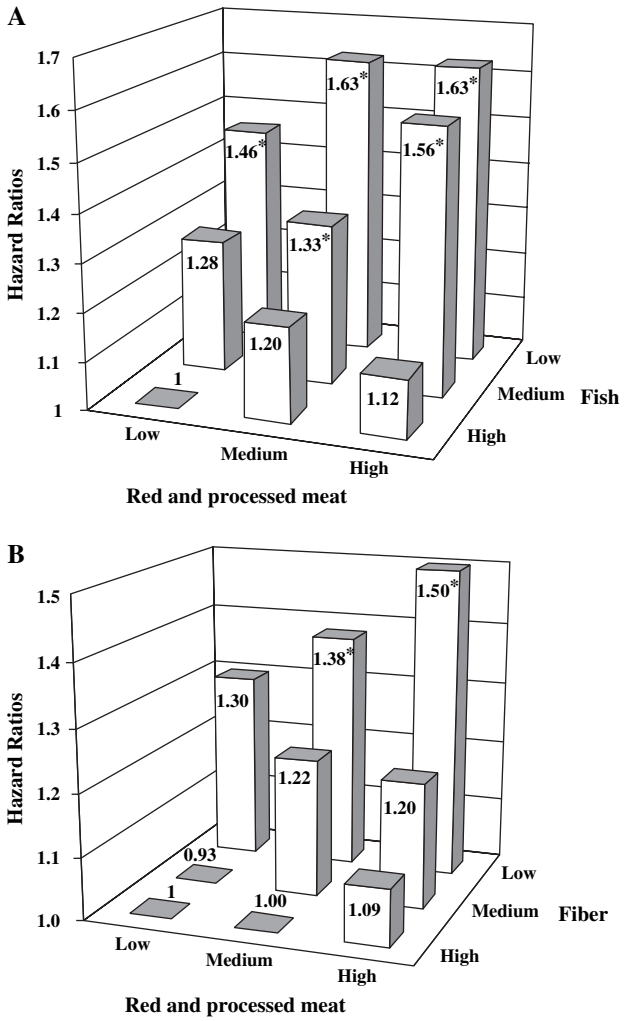


Fig. 3. Multivariable hazard ratios for colorectal cancer in the European Prospective Investigation into Cancer and Nutrition Cohort. Hazard ratios for intakes of **A**) red and processed meat and fish and **B**) red and processed meat and fiber. Multivariable analysis was performed using Cox regression models adjusted for age, sex, energy from nonfat sources (continuous variable), energy from fat sources (continuous variable), height (tertiles defined for each sex and center), weight (tertiles defined for each sex and center), work-related physical activity (no activity, sedentary, standing, manual, or heavy manual) smoking status (never, former, or current smoker), alcohol consumption (grams per day) and stratified by center. Low, medium, and high represent sex-specific tertiles. For red meat intake, low was less than 30 g/day of red and processed meat in men and less than 13 g/day in women, medium was 30–129 g/day in men and 13 to 85 g/day in women, and high was more than 129 g/day in men and 85 g/day in women. Cut points for fish intake were the same for men and women, with low being less than 14 g/day, medium being 14–50 g/day, and high being more than 50 g/day. For fiber intake, low was less than 17 g/day in men and women, medium was 17–28 g/day in men and 17–26 g/day in women, and high was more than 28 g/day in men and 26 g/day in women. *P<.05 relative to the group of subjects with low red and processed meat and high fish intake (**A**) or high fiber intake (**B**).

or enzymatic procedures. The methods of preparing processed meat vary across Europe and have changed over time. Common ingredients used in processed meats are salt, phosphates, nitrite, nitrate, water, sugar, fat, and spices *(26)*. To our knowledge, there are no clearly demonstrated biologic mechanisms that could explain why the observed association of colorectal cancer risk with processed meat might be stronger than that with unprocessed red meat. Nitrites or nitrates added to meat for preservation could increase exogenous exposure to nitrosamines, other *N*-nitrosocompounds, and their precursors, but not all processed meats contain added nitrites—for example, most sausages and air-dried hams do not.

All of the red and virtually all of the processed meat studied here would have contained greater amounts of heme, which is known to stimulate production of endogenous *N*-nitroso compounds in the human gastrointestinal system *(34)*, than poultry, which contains much lower amounts of heme and does not stimulate endogenous *N*-nitroso compound formation *(35)*. Endogenous *N*-nitrosation, arising from ingestion of heme, may account for the increased risk of colorectal cancer associated with high consumption of red meat and the lack of association with intake of poultry.

The trend in the association between increased fish consumption and decreased colorectal cancer risk was highly statistically significant (P_{trend}<.001). Results from animal and in vitro studies indicate that n−3 fatty acids, especially the long-chain polyunsaturated fatty acids eicosapentaenoic and docosahexaenoic acids, which are present in fatty cold-water fish and fish oils, inhibit carcinogenesis *(42)*. However, we were unable to differentiate between intakes of fatty fish, which contains the majority of n–3 fatty acids and other fish. Furthermore, heterogeneity was encountered among the different cohorts, and it is not clear whether this heterogeneity could be explained by unaccounted for differences in the fat content of fish *(27)*, in cooking practices across EPIC cohorts *(33)*, or by the small numbers of case patients in some centers.

Our study has several limitations. Most important, methods used in nutritional epidemiology are known to provide imprecise estimates of food intake. Random measurement errors of food intake lead to the attenuation of the disease risk estimates *(43)*. We attempted to correct for this error by adjusting for total energy intake and body weight, because adjustment for self-reported total energy intake is thought to partially correct for measurement error *(44)*. Body weight was also included because it has been suggested to be a better measure of real, unmeasured energy intake than energy intake derived from dietary questionnaires *(45)*. Furthermore, as a novel procedure to correct the relative risk estimates for de-attenuation, we calibrated the dietary questionnaires using a more detailed reference method, the 24-hour diet recall, under the assumption that a single 24-hour recall provides unbiased estimates of dietary intake at a group level. This choice maximizes the statistical power for adjusting relative risk estimates, but it does not permit the correction of hazard ratios associated with quantiles of intakes *(43)*. The method of calibration that we used assumes that there are no correlations of errors produced by the reference method (24-hour diet recall) and the dietary questionnaire *(46,47)*. In practice, however, there is evidence that the individual errors of dietary measurements obtained with dietary questionnaires and 24-hour diet recalls tend to be positively correlated *(48)*; such correlation would lead to an underestimation of the de-attenuation factor and therefore would bias the hazard ratio estimates toward the null value of 1.

The assumption that the more detailed reference method provides unbiased estimates of dietary intake at a group level was tested using biomarkers of intake in a validation study involving 1103 volunteers of both sexes from 12 centers participating in EPIC *(49)*. Group mean nitrogen intakes obtained with the 24-hour diet recalls, used as the reference for calibration, were compared against mean 24-hour urinary nitrogen,

a quantitative marker of protein intake. The sex-adjusted partial Pearson's correlation coefficient between urinary and dietary nitrogen at the mean group level was .84 (.90 after exclusion of outliers), and the calculated β regression coefficients were not statistically significantly different from 1, suggesting that, overall, systematic bias across centers was modest and of uniform magnitude. Nevertheless, because calibration adjusts only partially for measurement error, the almost two-fold increase in colorectal cancer risk for the highest versus lowest daily intake of red and processed meat, estimated after the calibration (Fig. 1), should still be considered a conservative estimate of the real underlying association.

It has been recently estimated that approximately 70% of colorectal cancer could be avoided by changes in lifestyle in Western countries *(50)*. Risk factors included in this recent estimate were obesity, physical inactivity, high alcohol consumption, early adulthood cigarette smoking, high red meat consumption, and low intake of folic acid. The investigation of the combined association of these factors with colorectal cancer risk is ongoing in EPIC. Our results published here support the hypothesis that colorectal cancer risk is positively associated with high consumption of red and processed meat and inversely associated with the intake of fish and confirm in a larger number of case patients our previous results *(22)* of a statistically significant inverse association between intake of fiber and colorectal cancer risk.

References

(1) Willett WC, Stampfer MJ, Colditz GA, Rosner BA, Speizer FE. Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women. N Engl J Med 1990;323:1664–72.

(2) WCRF-AICR. Food, nutrition and the prevention of cancer: a global perspective. Washington DC: American Institute for Cancer Research, 1997.

(3) Norat T, Lukanova A, Ferrari P, Riboli E. Meat consumption and colorectal cancer risk: dose-response meta-analysis of epidemiological studies. Int J Cancer 2002;98:241–56.

(4) Sandhu MS, White IR, McPherson K. Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analytical approach. Cancer Epidemiol Biomarkers Prev 2001;10: 439–46.

(5) Wei EK, Giovannucci E, Wu K, Rosner B, Fuchs CS, Willett WC, et al. Comparison of risk factors for colon and rectal cancer. Int J Cancer 2004;108:433–42.

(6) Flood A, Velie EM, Sinha R, Chaterjee N, Lacey JV Jr, Schairer C, et al. Meat, fat, and their subtypes as risk factors for colorectal cancer in a prospective cohort of women. Am J Epidemiol 2003;158:59–68.

(7) Chao A, Thun MJ, Connell CJ, McCullough ML, Jacobs EJ, Flanders WD, et al. Meat consumption and risk of colorectal cancer. JAMA 2005;293: 172–82.

(8) Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC. Intake of fat, meat, and fiber in relation to risk of colon cancer in men. Cancer Res 1994;54:2390–7.

(9) Bostick RM, Potter JD, Kushi LH, Sellers TA, Steinmetz KA, McKenzie DR, et al. Sugar, meat, and fat intake, and non-dietary risk factors for colon cancer incidence in Iowa women (United States). Cancer Causes Control 1994;5:38–52.

(10) Goldbohm RA, van den Brandt PA, van't Veer P, Brants HA, Dorant E, Sturmans F, et al. A prospective cohort study on the relation between meat consumption and the risk of colon cancer. Cancer Res 1994;54: 718–23.

(11) Gaard M, Tretli S, Loken EB. Dietary factors and risk of colon cancer: a prospective study of 50,535 young Norwegian men and women. Eur J Cancer Prev 1996;5:445–54.

(12) Kato I, Akhmedkhanov A, Koenig K, Toniolo PG, Shore RE, Riboli E. Prospective study of diet and female colorectal cancer: the New York University Women's Health Study. Nutr Cancer 1997;28:276–81.

(13) Pietinen P, Malila N, Virtanen M, Hartman TJ, Tangrea JA, Albanes D, et al. Diet and risk of colorectal cancer in a cohort of Finnish men. Cancer Causes Control 1999;10:387–96.

(14) Singh PN, Fraser GE. Dietary risk factors for colon cancer in a low-risk population. Am J Epidemiol 1998;148:761–74.

(15) Hsing AW, McLaughlin JK, Chow WH, Schuman LM, Co CH, Gridley G, et al. Risk factors for colorectal cancer in a prospective study among U.S. white men. Int J Cancer 1998;77:549–553.

(16) Tiemersma EW, Kampman E, Bueno de Mesquita HB, Bunschoten A, van Schothorst EM, Kok FJ, et al. Meat consumption, cigarette smoking, and genetic susceptibility in the etiology of colorectal cancer: results from a Dutch prospective study. Cancer Causes Control 2002;13:383–93.

(17) Kobayashi M, Tsubono Y, Otani T, Hanaoka T, Sobue T, Tsugane S; JPHC Study Group. Fish, long-chain n-3 polyunsaturated fatty acids, and risk of colorectal cancer in middle-aged Japanese: the JPHC study. Nutr Cancer 2004;49:32–40.

(18) English DR, MacInnis RJ, Hodge AM, Hopper JL, Haydon AM, Giles GG. Red meat, chicken, and fish consumption and risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2004;13:1509–14.

(19) Larsson SC, Rafter J, Holmberg L, Bergkvist L, Wolk A. Red meat consumption and risk of cancers of the proximal colon, distal colon and rectum: the Swedish Mammography Cohort. Int J Cancer 2005;113: 829–34.

(20) Bingham S, Riboli E. Diet and cancer—the European Prospective Investigation into Cancer and Nutrition. Nat Rev Cancer 2004;4:206–15.

(21) Two studies find high-fiber diet lowers colon cancer risk. CA Cancer J Clin 2003;53:201–2.

(22) Bingham SA, Day NE, Luben R, Ferrari P, Slimani N, Norat T, et al. Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. Lancet 2003;361:1496–501.

(23) Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113–24.

(24) Ferrari P, Slimani N, Ciampi A, Trichopoulou A, Naska A, Lauria C, et al. Evaluation of under- and overreporting of energy intake in the 24-hour diet recalls in the European Prospective Investigation into Cancer and Nutrition (EPIC). Public Health Nutr 2002;5:1329–45.

(25) European Prospective Investigation into Cancer and Nutrition. Validity studies on dietary assessment methods. Int J Epidemiol 1997;26 Suppl. 1: S1–S189.

(26) Linseisen J, Kesse E, Slimani N, Bueno-de-Mesquita HB, Ocke MC, Skeie G, et al. Meat consumption in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts: results from 24-hour dietary recalls. Public Health Nutr 2002;5:1243–58.

(27) Welch AA, Lund E, Amiano P, Dorronsoro M, Brustad M, Kumle M, et al. Variability of fish consumption within the 10 European countries participating in the European Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr 2002;5:1273–85.

(28) Slimani N, Kaaks R, Ferrari P, Casagrande C, Clavel-Chapelon F, Lotze G, et al. European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study: rationale, design and population characteristics. Public Health Nutr 2002;5:1125–45.

(29) Kaaks R, Plummer M, Riboli E, Esteve J, van Staveren W. Adjustment for bias due to errors in exposure assessments in multicenter cohort studies on diet and cancer: a calibration approach. Am J Clin Nutr 1994;59(Suppl 1): 245S–50S.

(30) Ferrari P, Kaaks R, Fahey M, Slimani N, Day NE, Pera G, et al. Within and between cohort variation in measured macro-nutrient intakes in the EPIC study taking account of measurement errors. Am J Epidemiol 2004;160: 814–22.

(31) Rosner B, Gore R. Measurement error correction in nutritional epidemiology based on individual foods, with application to the relation of diet to breast cancer. Am J Epidemiol 2001;154:827–35.

(32) Greenland S, Rothman KJ. Introduction to stratified analysis. In: Rothman KJ, Greenland S, editors. Modern epidemiology. 2nd ed. Philadelphia (PA): Lippincott-Raven; 1998. p. 53–79.

(33) Rohrmann S, Linseisen J, Becker N, Norat T, Sinha R, Skeie G, et al. Cooking of meat and fish in Europe—results from the European Prospective Investigation into Cancer and Nutrition (EPIC). Eur J Clin Nutr 2002;56: 1216–30.
(34) Cross AJ, Pollock JR, Bingham SA. Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. Cancer Res 2003;63:2358–60.
(35) Bingham SA, Hughes R, Cross AJ. Effect of white versus red meat on endogenous N-nitrosation in the human colon and further evidence of a dose response. J Nutr 2002;132(11 Suppl):3522S–5S.
(36) Sugimura T. Nutrition and dietary carcinogens. Carcinogenesis 2000;21: 387–95.
(37) Sachse C, Smith G, Wilkie MJ, Barrett JH, Waxman R, Sullivan F, et al. A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. Carcinogenesis 2002;23:1839–49.
(38) Kampman E, Slattery ML, Bigler J, Leppert M, Samowitz W, Caan BJ, et al. Meat consumption, genetic susceptibility, and colon cancer risk: a United States multicenter case-control study. Cancer Epidemiol Biomarkers Prev 1999;8:15–24.
(39) Lang NP, Butler MA, Massengill J, Lawson M, Stotts RC, Hauer-Jensen M, et al. Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increase the risk for colorectal cancer or polyps. Cancer Epidemiol Biomarkers Prev 1994;3:675–82.
(40) Roberts-Thomson IC, Butler WJ, Ryan P. Meat, metabolic genotypes and risk for colorectal cancer. Eur J Cancer Prev 1999;8:207–11.
(41) Murtaugh MA, Sweeney C, Ma KN, Caan BJ, Slattery ML. The CYP 1A1 genotype may alter the association of meat consumption patterns and preparation with the risk of colorectal cancer in men and women. J Nutr 2005;135:179–86.
(42) Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am J Clin Nutr 2004;79:935–45.
(43) Kaaks R, Riboli E, van Staveren W. Calibration of dietary intake measurements in prospective cohort studies. Am J Epidemiol 1995;142:548–56.
(44) Willett W. Commentary: Dietary diaries versus food frequency questionnaires—a case of undigestible data. Int J Epidemiol 2001;30:317–9.
(45) Jakes RW, Day NE, Luben R, Welch A, Bingham S, Mitchell J, et al. Adjusting for energy intake—what measure to use in nutritional epidemiological studies? Int J Epidemiol 2004;33:1382–6.
(46) Day N, McKeown N, Wong M, Welch A, Bingham S. Epidemiological assessment of diet: a comparison of a 7-day diary with a food frequency questionnaire using urinary markers of nitrogen, potassium and sodium. Int J Epidemiol 2001;30:309–17.
(47) Kipnis V, Carroll RJ, Freedman LS, Li L. Implications of a new dietary measurement error model for estimation of relative risk: application to four calibration studies. Am J Epidemiol 1999;150:642–51.
(48) Kipnis V, Midthune D, Freedman L, Bingham S, Day NE, Riboli E, et al. Bias in dietary-report instruments and its implications for nutritional epidemiology. Public Health Nutr 2002;5:915–23.
(49) Slimani N, Bingham S, Runswick S, Ferrari P, Day NE, Welch AA, et al. Group level validation of protein intakes estimated by 24-hour diet recall and dietary questionnaires against 24-hour urinary nitrogen in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study. Cancer Epidemiol Biomarkers Prev 2003;12:784–95.
(50) Willett WC. Balancing life-style and genomics research for disease prevention. Science 2002;296:695–8.

Notes

The work described in this article was carried out with financial support of the "Europe Against Cancer Program" of the European Commission (SANCO); the Danish Cancer Society; German Cancer Aid; Ligue Nationale contre le Cancer, the 3M Company, INSERM; the German Cancer Research Center; the German Federal Ministry of Education and Research; the Dutch Ministry of Public Health, Welfare and Sports; the National Cancer Registry and the Regional Cancer Registries Amsterdam, East and Maastricht of The Netherlands; the Norwegian Cancer Society; the Norwegian Research Council; the Health Research Fund (FIS) of the Spanish Ministry of Health; the Greek Ministry of Health; the Greek Ministry of Education; the Italian Association for Research on Cancer; Spanish Regional Governments of Andalucia, Asturias, Basque Country, Murcia and Navarra, and ISCIII; Red de Centros RCESP, C03/09; the Swedish Cancer Society; the Swedish Scientific Council; the Regional Government of Skane, Sweden; Cancer Research UK; the Medical Research Council, UK; the Stroke Association, UK; the British Heart Foundation; the Department of Health, UK; the Food Standards Agency, UK; and the Wellcome Trust, UK.

Manuscript received November 16, 2004; revised April 7, 2005; accepted May 3, 2005.

Re: Meat, Fish, and Colorectal Cancer Risk: The European Prospective Investigation into Cancer and Nutrition

In a large cohort comprising 10 populations in the European Prospective Investigation into Cancer and Nutrition, Norat et al. *(1)* reported that processed and red meat intake was associated with elevated rates of colorectal cancer and its subtypes. Although the authors considered several study limitations, they may have omitted one that is key: the possibility that confounding by socioeconomic position may be responsible for the diet–disease gradients.

In populations drawn from some of the countries featured in the article, markers of socioeconomic position have been shown to be associated with self-reported dietary characteristics, including meat consumption *(2)*. Thus, persons who are socioeconomically disadvantaged are more likely to report higher intake than their affluent counterparts *(2)*. A raised risk of colorectal cancer has also been found in persons from deprived social groups, as indexed by lower levels of educational attainment *(3)*.

In exploring the relationship between meat consumption (indeed, most indicators of food intake) and colon cancer (indeed, most chronic disease outcomes), surprisingly few investigators adjust for socioeconomic indices, so judging the impact of this covariate on the diet-disease relationship is problematic. However, a suggestion that socioeconomic deprivation may have a role as a confounder in the meat–colon cancer relationship can be found in a study that appears to comprise a socioeconomically homogenous group of women. As cited by the authors *(1)*, but not discussed in the present context, an early report from the Nurses' Health Study *(4)* found a positive relationship of both unprocessed meat (beef, pork, or lamb) and processed meat intake with incident colon cancer. This association was essentially lost in a later follow-up study of the same population *(5)* containing over four times the number of cases (n = 670) and therefore greater statistical precision than the earlier report.

In a series of articles *(3,6,7)*, the European Union Working Group on Socioeconomic Inequalities in Health has reported that the methodologic issues of comparing the relationship between mortality and socioeconomic indices (i.e., education and occupational social class) across culturally disparate European settings can be surmounted. Assuming that similar data are available in at least some of the cohorts comprising the present report *(1)*, as they should be, presumably the potentially confounding role of socioeconomic position in the meat–colon cancer relationship could be explored in a subgroup of study participants and reported by the authors.

G. David Batty

References

(1) Norat T, Bingham S, Ferrari P, Slimani N, Jenab M, Mazuir M, et al. Meat, fish, and colorectal cancer risk: the European Prospective Investigation into Cancer and Nutrition. J Natl Cancer Inst 2005;97:906–16.

(2) Dowler E. Inequalities in diet and physical activity in Europe. Public Health Nutr 2001;4:701–9.

(3) Huisman M, Kunst AE, Bopp M, Borgan JK, Borrell C, Costa G, et al. Educational inequalities in cause-specific mortality in middle-aged and older men and women in eight western European populations. Lancet 2005; 365:493–500.

(4) Willett WC, Stampfer MJ, Colditz GA, Rosner BA, Speizer FE. Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women. N Engl J Med 1990;323:1664–72.

(5) Wei EK, Giovannucci E, Wu K, Rosner B, Fuchs CS, Willett WC, et al. Comparison of risk factors for colon and rectal cancer. Int J Cancer 2004;108:433–42.

(6) Borrell C, Plasencia A, Huisman M, Costa G, Kunst A, Andersen O, et al. Education level inequalities and transportation injury mortality in the middle aged and elderly in European settings. Inj Prev 2005;11:138–42.

(7) Kunst AE, Groenhof F, Mackenbach JP, Health EW. Occupational class and cause specific mortality in middle aged men in 11 European countries: comparison of population based studies. EU Working Group on Socioeconomic Inequalities in Health. BMJ 1998;316:1636–42.

Notes

Dr. Batty is supported by a Wellcome Advanced Training Fellowship.

Correspondence to: G. David Batty, PhD, MRC Social & Public Health Sciences Unit, University of Glasgow, 4 Lilybank Gardens, Glasgow, U.K. G12 8RZ (e-mail: E.david-b@msoc.mrc.gla.ac.uk).

DOI: 10.1093/jnci/dji407

We read with interest the article by Norat et al. *(1)* about meat, fish, and colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, which found no statistically significant association between the intake of red meat and colorectal cancer. Only when red and processed meats were combined was the observed positive association statistically significant. We would like to ask why the authors did not mention in their abstract the fact that red meat alone does not statistically significantly increase the relative risk and why they did not adjust their data for vegetable intake. Because vegetables and fruit are a good source of fiber and folate and reduce the energy density of a meal, all of which could diminish colorectal cancer risk, they are an important confounding factor. The cross-classification of red and processed meat intake with fiber intake clearly showed no increase in cancer risk if both intakes were in the highest category. The same was true for high fish intake. Many people enjoy eating red meat, fish, and poultry but do not eat processed meat.

Although the authors choose their wording very carefully, stating only that they found a "positive association," the media took the results of this study as a proof for a causal relationship between red meat consumption and colorectal cancer. We want to point out that such an association is far from proven, as none of the prospective European studies published to date found a statistically significant association *(2–5)*. The authors state that they could not explain the association, and there is no known mechanism. One possible explanation could be the higher content of heme iron, but in the same issue of the Journal, Chan et al. *(6)* published data indicating that dietary iron was not associated with colorectal adenoma in women. In addition, it was recently shown that chlorophyll from green vegetables prevents the cytotoxic and hyperproliferative effects of heme in a rat model *(7)*.

The EPIC study offers an important opportunity to learn more about diet and cancer. Its results, however, should be communicated in a very clear way because otherwise it would only increase the already existing confusion among consumers and health care providers. Because lean (red) meat is an important contributor to mineral, vitamin, and protein nutrition and many people like to eat meat, we feel it unjustified, based on the EPIC data, to give the impression that this habit could lead to such a severe illness. Perhaps the message of this EPIC publication should read: Enjoy our meat but have your veggies with it.

Ulrike Gonder
Nicolai Worm

References

(1) Norat T, Bingham S, Ferrari P, Slimani N, Jenab M, Mazuir M, et al. Meat, fish, and colorectal cancer risk: the European Prospective Investigation into Cancer and Nutrition. J Natl Cancer Inst 2005;97:906–16.

(2) Gaard M, Tretli S, Loken EB. Dietary factors and risk of colon cancer: a prospective study of 50,535 young Norwegian men and women. Eur J Cancer Prev 1996;5:445–54.

(3) Knekt P, Steineck G, Jarvinen R, Hakulinen T, Aromaa A. Intake of fried meat and risk of cancer: a follow-up study in Finland. Int J Cancer 1994;59:756–60.

(4) Goldbohm RA, van den Brandt PA, van 't Veer P, Brandts HA, Dorant E, Sturmans F, et al. A prospective cohort study on the relation between meat consumption and the risk of colon cancer. Cancer Res 1994;54:718–23.

(5) Truswell AS. Meat consumption and cancer of the large bowel. Eur J Clin Nutr 2002; 56 Suppl 1:S19–24.

(6) Chan AT, Ma J, Tranah GJ, Giovannucci EL, Rifai N, Hunter DJ, et al. Hemochromatosis gene mutations, body iron stores, dietary iron, and risk of colorectal adenoma in women. J Natl Cancer Inst 2005;97:917–26.

(7) de Vogel J, Jonker-Termont DS, van Lieshout EM, Katan MB, van der Meer R. Green vegetables, red meat and colon cancer: chlorophyll prevents the cytotoxic and hyperproliferative effects of haem in rat colon. Carcinogenesis 2005;26:387–93.

Notes

Ulrike Gonder and Dr. Worm are both independent consultants and authors and have occasionally worked for the German Agriculture Marketing Board (CMA).

Correspondence to: Ulrike Gonder, DIPL OEC TROPH, Taunusblick 21, D-65510 Huenstetten, Germany or Nicolai Worm, Maxhoehe 40, D-82335 Berg, Germany (e-mail: nicolai.worm@t-online.de).

DOI: 10.1093/jnci/dji408

RESPONSE

Batty suggests that our findings of a positive association between high intake of red and processed meat and colorectal cancer risk might be the result of confounding by socioeconomic position. In our study population, meat intake is inversely associated with educational attainment *(1)*, an indicator of socioeconomic position. The relative risks of colorectal cancer associated to secondary school, professional school, and university compared with primary school or less were 1.00, 1.12, and 1.02, respectively, indicating that colorectal cancer risk does not vary by educational attainment in this population. It is therefore not surprising that adjustment for educational attainment did not modify the relationship of red meat, processed meat, and fish with colorectal cancer risk. We have previously shown that the inverse association of colorectal cancer risk with fiber intake persisted after adjustment for this indicator of socioeconomic status *(2)*.

We appreciate the fact that initial results based on a relatively small number of cancer patients may change when precision increases with longer follow-up and when confounding is attenuated by better adjustment for covariates, as may have been the case for the study *(3)* cited by Batty. The number of colorectal cancer case patients in the European Prospective Investigation into Cancer and Nutrition (EPIC) study is comparable to the number included in the recent combined analysis of the Nurses' Health Study and the Health Professionals Follow Up Study *(3)*, and our results are adjusted for main potential confounders. The cohorts in EPIC are heterogeneous in diet, lifestyle, and other potential confounders *(4)*, but there was not a statistically significant heterogeneity in the association of red and processed meat with colorectal cancer risk when the analyses were conducted by country ($P_{\text{heterogeneity}} = .82$).

Gonder and Worm ask why we did not mention in the abstract that red meat alone does not statistically significantly increase the relative risk of colorectal cancer. The association of red meat with colorectal cancer was statistically significant when the variable was modeled as continuous ($P_{\text{trend}} = .03$) and close to statistical significance in categorical models ($P_{\text{trend}} = .08$). These

results do not provide evidence of a lack of association of high consumption of red meat and colorectal cancer risk. As discussed in our article, previous studies have provided possible explanations for the association with red meat, processed and unprocessed, but not with processed meat only. One possibility could be increased exposure to N-nitroso compounds and their precursors due to nitrites or nitrates added to meat for preservation. However, not all processed meats contain added nitrites, and we could not identify one particular type of processed meat that was more strongly associated with colorectal cancer risk than others.

Gonder and Worm ask why our analyses were not adjusted for vegetables, good sources of fiber and folate. Adjustment for vegetables did not modify our results, and this variable was not kept in the model. Our results were adjusted for fiber intake, and we showed that they were unchanged after adjustment for dietary folate in a subset of the cohort for whom dietary folate was available. As noted in the correspondence, there was some evidence that the association of red and processed meat with colorectal cancer risk might be weaker in the group of subjects with higher intake of fiber. Because, to the best of our knowledge, this possible modification by fiber of the association between meat intake and colorectal cancer risk had not been previously reported by large prospective studies, further research is needed before drawing any firm conclusions.

Finally, we see as a strong point supporting our results the fact that there was no heterogeneity of the association for red and processed meat across countries in EPIC, despite the high variability of vegetable and fiber consumption across EPIC cohorts *(5)*. Our results support our conclusion that red and processed meat are positively associated with risk of colorectal cancer, but they do not demonstrate that high intake of red meat accompanied by high intake of vegetables is not associated with colorectal cancer risk.

Teresa Norat
Sheila Bingham
Elio Riboli

References

(1) Linseisen J, Kesse E, Slimani N, Bueno-De-Mesquita HB, Ocke MC, Skeie G, et al. Meat consumption in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts: results from 24-hour dietary recalls. Public Health Nutr 2002;5:1243–58.

(2) Bingham SA, Norat T, Moskal A, Ferrari P, Slimani N, Clavel-Chapelon F, et al. Is the association with fiber from foods in colorectal cancer confounded by folate intake? Cancer Epidemiol Biomarkers Prev 2005;14:1552–6.

(3) Wei EK, Giovannucci E, Wu K, Rosner B, Fuchs CS, Willett WC, et al. Comparison of risk factors for colon and rectal cancer. Int J Cancer 2004;108:433–42.

(4) Bingham S, Riboli E. Diet and cancer. The European Prospective Investigation into Cancer and Nutrition. Nat Rev Cancer 2004;4: 206–15.

(5) Agudo A, Slimani N, Ocke MC, Naska A, Miller AB, Kroke A, et al. Consumption of vegetables, fruit and other plant foods in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts from 10 European countries. Public Health Nutr 2002;5:1179–96.

Notes

Affiliations of authors: Infections and Cancer Epidemiology Group, International Agency for Research on Cancer, Lyon, France (TN); Medical Research Council Dunn Human Nutrition Unit, Cambridge, UK (SB); Nutrition and Hormones Group, International Agency for Research on Cancer, Lyon, France (ER).

Correspondence to: Elio Riboli, MD, MPH, ScM, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372, Lyon Cedex 08, France (e-mail: riboli@iarc.fr).

DOI: 10.1093/jnci/dji409

DISCUSSION

1. Difficultés méthodologiques de l'étude de la relation causale entre l'alimentation et les cancers dans les études épidémiologiques.

2. Synthèse méta-analytiques des données apportées par les études de cohorte sur la relation entre la viande rouge, le poisson et le risque de cancer colorectal.

3. Hypothèses sur les mécanismes expliquant un effet promoteur des viandes rouges.

    3.1 Les lipides de la viande

    3.2 Le fer

    3.3 Les protéines

    3.4 Les composés N-nitrosés

    3.5 Carcinogènes potentiels formés pendant la cuisson et préservation de la viande.

4. Hypothèses sur les mécanismes expliquant un effet protecteur du poisson.

Nous discuterons d'abord les difficultés d'ordre méthodologique des études épidémiologiques en nutrition et cancer, et en particulier celles liées aux études prospectives de cohorte. Nous ferons ensuite une synthèse quantitative des données épidémiologiques sur la relation entre la viande rouge et le poisson avec le cancer colorectal à l'aide de la méta-analyse. Nous conclurons par une synthèse des principales hypothèses de mécanismes pouvant expliquet cette relation.

1. Difficultés méthodologiques de l'étude de la relation causale entre l'alimentation et les cancers dans les études épidémiologiques.

Il existe deux problèmes principaux pour évaluer les relations entre alimentation et cancer. Le premier est qu'on ne sait pas à quels moments du processus de la cancérogenèse l'alimentation intervient, et que cela varie probablement en fonction de la localisation du cancer. La seconde raison, d'ordre plutôt méthodologique, est la difficulté à déterminer quels composants l'on doit mesurer dans l'alimentation et comment. Une approche réductionniste – un nutriment - présente l'intérêt d'examiner la possibilité d'association causale, mais cette approche ne tient pas compte des possibles interactions entre composants de l'alimentation. Il est donc absolument nécessaire de développer des études comprenant plusieurs niveaux de mesure de l'exposition et des nutriments qui sont eux spécifiques aux aliments, aux groupes d'aliments, au type d'alimentation ou au profil alimentaire.

Les méthodes les plus fréquemment utilisées en épidémiologie du cancer visent à mesurer avec quelle fréquence et en quelles quantités sont habituellement consommés les aliments qui font partie de l'alimentation d'une population donnée. Ces méthodes, connues sous le nom générique de « questionnaires de fréquence », incluent une liste d'aliments pour lesquels la fréquence de consommation, et parfois le poids moyen de la portion, sont systématiquement recensés. On demande généralement aux sujets interrogés de faire référence à leur

consommation habituelle de l'année précédente. Une liste détaillée d'aliments est normalement nécessaire pour couvrir l'ensemble des groupes alimentaires. Une méthode modifiée (« histoire alimentaire ») permet en général au sujet de décrire son alimentation sans être restreint par une liste préétablie. Il existe d'autres méthodes d'enquête alimentaire pouvant donner une estimation plus précise de la consommation alimentaire pendant une courte période de temps (plusieurs rappels de 24 heures, journaux des consommations sur plusieurs jours). Toutefois, l'implémentation de ces méthodes est difficile et très coûteuse dans des populations de grande taille.

Les méthodes de questionnaires posent des problèmes de validité. Ces problèmes sont particulièrement importants lorsqu'on applique un questionnaire à une population qui n'est pas celle où le questionnaire a été développé et validé (146). La mesure des erreurs systématiques se fait pendant des études de calibration ou de validation des questionnaires, et la mesure des erreurs aléatoires se fait avec des études de reproductibilité (154;155). Dans les études épidémiologiques, les erreurs de mesure des apports alimentaires (non-différentiels entre les cas et les témoins) ont comme conséquence une sous-estimation de la valeur des risques relatifs observés (156).

Un avantage de l'étude EPIC est d'avoir développé de procédures pour corriger la sous-estimation des risques relatifs due aux erreurs de mesure. Ces procédures incluent la réalisation d'une deuxième enquête alimentaire plus detaillée (rappel de 24 heures) dans un échantillon de la population (156), ainsi que le développement de stratégies d'analyse statistique des données (154;157).

Plusieurs approches sont employées par les épidémiologistes pour étudier la relation entre l'alimentation et le cancer (ou les maladies chroniques en général). Ces études diffèrent largement en ce qui concerne leur possibilité de (a) identifier, éliminer ou ajuster par des facteurs de confusion, (b) établir une relation temporelle de précédence de l'effet causal sur

l'effet, (c) minimiser les biais, (d) déterminer des événements d'intérêt, y compris des événements fatals (e) évaluer le risque attribuable aux niveaux de la population (f) obtenir des résultats généralisables.

Les premières indications sur le rôle potentiel de l'alimentation dans l'étiologie du cancer chez l'homme proviennent des études de corrélation. Ces études ont pour objectif de comparer, au niveau de populations, des taux de cancer et des niveaux de consommation d'aliments, nutriments et d'autres composants de l'alimentation. Une limite évidente de ces études est leur vulnérabilité aux effets des facteurs de confusion. Bien qu'elles permettent de mettre en évidence une corrélation entre un composé donné de l'alimentation et la fréquence du cancer au niveau d'une population, cette relation peut être due à d'autres facteurs méconnus qui se trouvent être associés à certaines habitudes alimentaires.

Les études cas-témoins sont des études incluant des patients atteints d'un cancer donné (cas) et des sujets issus de la même population que les cas (témoins) mais sans cancer. Des informations sur le(s) facteur(s) d'exposition d'intérêt pour l'étude sont donc recueillies à la fois auprès des cas et des témoins, ainsi que sur un ensemble de variables considérées comme des facteurs de confusion potentiels (ex. âge, sexe, autres expositions) qui doivent être contrôlés, soit lors de l'inclusion dans l'étude, avec un appariement des cas et des témoins, soit lors de l'analyse statistique. L'association entre le facteur d'exposition (ex. consommation de viande) et la maladie (ex. cancer colorectal) peut donc ainsi être analysée. Ces études présentent cependant certaines limitations. En particulier, les individus interrogés peuvent rapporter de façon plus ou moins exacte leur alimentation passée selon qu'ils sont atteints d'un cancer ou pas, et cela peut induire des différences systématiques dans la qualité et la fiabilité des données qui compromettent la comparaison qui s'ensuit. Les études cas-

témoins ne sont pas appropriées pour étudier la séquence temporelle et très souvent sont soumise au biais de « survie », rendant impossible l'étude des événements fatals.

Les études de cohorte constituent en général la meilleure méthode d'observation en épidémiologie. Des grands groupes d'individus, ne présentant aucune des maladies d'intérêt pour l'étude sont suivis sur une longue période, durant laquelle certains sujets de la cohorte vont développer un cancer et d'autres, non. L'exposition étudiée (l'alimentation) est mesurée chez l'ensemble des sujets lors de leur recrutement dans la cohorte. Comme l'alimentation est mesurée longtemps avant l'apparition du cancer, des biais de sélection ou les biais différentiels de mesure de l'alimentation entre les futurs cas et les témoins peuvent être évités. Les études de cohorte permettent également de recueillir et conserver des échantillons biologiques (sang, tissus, urine) pour des études ultérieures en relation avec l'apparition du cancer.

Les principaux obstacles que rencontrent les études de cohorte sont leur coût et leur durée. Même pour des cancers à forte incidence comme le cancer du côlon, il est nécessaire de recruter plusieurs dizaines de milliers de participants afin de disposer d'un nombre suffisant de cas de cancer et par conséquence de bénéficier d'une puissance statistique suffisante pour étudier les relations entre alimentation et cancer. Un nombre encore plus important est nécessaire pour étudier des effets d'interaction.

Grâce aux registres de mortalité et de cancers il est possible de suivre actuellement de manière efficace les sujets participants à des larges études pour l'identification de l'événement d'intérêt. Sinon, un suivi actif, plus coûteux et laborieux doit être mis en place. Un problème particulier est posé par l'implémentation de techniques de dépistage précoce, qui conduit à l'identification sélective des types particuliers de cancers ou des groupes de personnes plus attentives à leur santé- et diminuent la comparabilité des études dans de régions avec des

pratiques de dépistage diverses. Ces divergences doivent être prises en compte dans l'analyse et l'interprétation des données.

La sélection des populations des études de cohorte et de certains études d'intervention avec des volontaires, induit fréquemment « l'effet de santé ou *healthy effect* », c'est-à-dire qu'il s'agit de populations avec des habitudes de vie plus saines que la population générale. Les risques attribuables qui peuvent être dérivés de ces études, ou les mesures d'effets pour les études d'intervention, ne sont pas extrapolables à la population générale. C'est pourquoi des mesures relatives (risques relatifs, ratio de mortalité) doivent être utilisées et correctement interprétées.

De manière générale, les résultats des études de type cas-témoins en nutrition et cancer n'ont pas été confirmés par les études de cohorte. Soit l'association est confirmée, mais la force de l'association est plus faible dans les études de cohorte que dans les études cas-témoins, soit les résultats son négatifs dans les études de cohorte (98;149). Des exceptions se retrouvent dans la relation entre le cancer colorectal avec l'alcool et avec la viande rouge (49;149), pour lesquelles les résultats sont assez convergents dans leur ensemble.

Il est reconnu que l'approche méthodologique la moins susceptible de biais est l'étude d'intervention randomisée chez l'homme. Il existe différents types d'études assimilables au concept d'intervention. En pratique, la plupart des études d'intervention réalisées en nutrition et cancer sont des études randomisées où chaque sujet est assigné, de façon aléatoire au(x) groupe(s) d'intervention à qui l'on donne le composé à tester ou au groupe témoin qui recevra un placebo ou un suivi équivalent. En dépit des avantages scientifiques, l'application de cette méthode à l'évaluation de la relation entre l'alimentation et le cancer s'avère difficile, et parfois impossible.

Une première difficulté fondamentale est que pour des raisons éthiques évidentes on ne peut pas tester chez l'homme des composants soupçonnés d'augmenter le risque de cancer, mais seulement des substances supposées réduire ce risque. Les interventions ne sont justifiées qu'en possession d'indications scientifiques très fortes sur les effets bénéfiques du composé à tester sur la prévention de cancer, et de l'assurance qu'un effet nuisible est très improbable.

C'est ainsi que l'étude de la relation viande rouge-cancer colorectal ne serait pas justifiée dans le contexte d'un essai randomisé.

Pour des raisons culturelles, il peut être difficile de s'assurer qu'une grande population adhère à un régime strict, tel qu'un régime riche en poisson, sur une longue période de temps. D'autre part, les interventions menant aux modifications de la consommation d'aliments ne peuvent pas être effectué en aveugle. Les individus du groupe « témoin » (sans l'intervention) peuvent modifier leurs habitudes alimentaires vers un régime présumé sain, selon l'adaptation sociale aux informations divulguées et de ce fait limiter la puissance de l'étude.

La réalisation des essais randomisés semble davantage applicable à l'évaluation des inhibiteurs potentiels de cancer, tels que certaines vitamines, des minéraux, et d'autres agents chimiopréventifs, qui peuvent être incorporés aux comprimés ou capsules et administrés aux participants en double-aveugle (158). Dans ce contexte spécifique il existe une difficulté supplémentaire liée à la nature de l'alimentation, qui est un ensemble extrêmement vaste et varié de composés chimiques dont il est difficile, dans les conditions normales, d'isoler et d'étudier l'action spécifique ou combinée. Il est toujours possible que d'autres micronutriments actifs présents dans les aliments ne soient pas présents dans la supplémentation utilisée dans l'étude expérimentale. De plus, les composants utilisés, bien qu'apparemment protecteurs lorsqu'ils sont contenus dans les aliments, peuvent avoir un effet inattendu lorsqu'ils sont considérés individuellement, et tout particulièrement lorsqu'ils sont

utilisés à des doses très éloignées de celles que l'on observe dans une alimentation normale (159).

En ce qui concerne le cancer, en raison de la durée de la période d'induction pour la plupart des cancers, le temps nécessaire entre l'intervention diététique et l'apparition d'un effet mesurable peut être des plusieurs années ou décennies. L'inclusion des individus à risque élevé de cancer en raison des facteurs génétiques ou de la présence d'une maladie pré-maligne (polypes du côlon, par exemple) comme sujets d'expérimentation peut augmenter la fréquence avec laquelle des résultats d'intérêt se produisent avec le temps. Un critère de réponse intermédiaire peut être employé comme indicateur d'efficacité, tel qu'appliqué, par exemple, dans les études du rôle de la supplémentation au son de blé sur la prolifération cellulaire de la muqueuse colique et la formation de polypes chez des patients à risque élevé de cancer du côlon (160). Cependant, les critères intermédiaires peuvent ne pas prévoir entièrement le risque de cancer.

L'interprétation des études d'intervention peut être problématique. La modification des composants simples du régime alimentaire dans des essais d'intervention modifie le régime au-delà de ces changements. Par exemple, l'augmentation de la consommation de fruits et de légumes ou la réduction des apports caloriques des lipides alimentaires modifient les apports d'un certain nombre d'aliments qui sont soit substitués par les fruits et les légumes, soit des substituts des apports caloriques des lipides alimentaires, ainsi que de plusieurs autres nutriments. Outre l'absence réelle d'effet, les résultats négatifs pourraient presque toujours être expliqués par des niveaux inadéquats d'apport des nutriments ou des aliments testés, par une faible conformité des participants à l'intervention, une durée insuffisante de l'intervention ou du suivi des participants. Finalement, les études d'intervention dans la population constituent un laboratoire pour évaluer l'effet des interventions en étude, mais ils sont

susceptibles de contamination, biais de confusion et en général, de manque de puissance statistique.

Malgré les avantages des études d'intervention, les difficultés méthodologiques et pratiques de leur réalisation et interprétation font que les études d'observation analytiques restent encore la méthode d'étude chez l'homme la plus utilisée en nutrition et cancer. C'est ainsi que de nombreuses études de cohorte prospectives sur la nutrition et le cancer (et d'autres maladies) sont en cours actuellement, principalement aux Etats Unis, en Europe, parmi lesquelles l'étude EPIC, en Australie et au Japon.

Une méthode d'analyse statistique, la « méta-analyse » a été élaborée afin de synthétiser les résultats de plusieurs études, augmenter la puissance statistique et donc la probabilité de détecter des effets modestes, difficilement identifiables lorsque les études sont considérées séparément. A défaut de pouvoir rassembler au moyen des critères de sélection rigoureux l'ensemble des études traitant du sujet d'intérêt, ces études peuvent répéter et parfois même amplifier les biais des études individuelles.

2. Synthèse méta-analytique des données des études de cohorte sur la relation entre la viande rouge, le poisson et le risque de cancer colorectal.

Deux grands rapports de consensus sur le cancer et l'alimentation, le comité du Word Cancer Research Fund/American Institute for Cancer Research (50) et le comité (69) ont conclu que les données scientifiques indiquent que la consommation élevée de viande rouge augmente probablement le risque de cancer colorectal. Le rapport WCRF/AIRC a recommandé de limiter la prise quotidienne de viande rouge et de viande traitée à 80 grammes par jour. Les experts du COMA ont recommandé d'éviter l'augmentation du niveau moyen de consommation par personne, et de diminuer la consommation chez les sujets avec des apports moyens supérieurs à 140 grammes par jour. Cependant, les deux comités ont convenu que les données épidémiologiques n'étaient pas concordantes.

Dans une méta-analyse des études de type cas-témoins et de cohorte publiées entre 1973 et 1999, nous avons estimé à 22% l'augmentation du risque de cancer colorectal pour chaque 120 grammes d'augmentation de la consommation de viande rouge, et en 54% pour 30 grammes d'apports de viande traitée (149).

Après la publication de notre méta-analyse, d'autres données épidémiologiques ont été publiées (105;151;161). La plupart de ces études trouvent une relation positive entre la viande rouge et le risque de cancer colorectal, relation qui en général,.à l'échelle individuelle, n'atteint pas la significativité statistique Deux études de cohorte américaines (82;151) ne trouvent pas d'association significative entre la viande rouge et le risque de cancer colorectal. Une étude australienne (162), une étude suédoise (163) et l'étude EPIC, ont montré des relations statistiquement significatives. Plus récemment, une étude japonaise a identifié un risque relatif de 1.14 (95% CI=0.85-1.53) pour le niveau de consommation de viande le plus bas par rapport au niveau le plus haut, mais les volailles ont été inclues dans le même groupe

que les viandes rouges (164). Une autre étude japonaise a trouvé une augmentation de risque associé a la consommation de viande traitée chez les hommes, mais pas chez les femmes (165).

Nous ici avons synthetisé de manière quantitative les résultats des études de cohorte publiées entre 1990 et juin 2006, à l'aide de la méta-analyse. La mesure de l'effet utilisée est le risque relatif moyen associé au niveau de consommation le plus élevé de viande rouge et du poisson par rapport au niveau le plus faible rapporté dans chaque étude. Les méthodes ont été décrites en détail dans le chapitre II (1) de ce rapport (166).

Le sommaire de 14 études de cohorte incluant 6634 hommes et femmes chez lesquels un cancer colorectal ou de côlon a été diagnostiqué durant le suivi indique que dans ces populations, les gros consommateurs de viande rouge ont un risque significativement plus élevé par rapport aux sujets avec les niveaux de consommation plus faibles dans les populations (RR= 1,21 ; 95% CI= 1,09-1,34 ; Figure 2). Les risques relatifs moyens sont plus élevés pour le cancer du rectum (RR=1.56 ; 95% CI=1.21-2.01) que pour le côlon (RR=1.17 ; 95% CI=1.02-1.34) mais la différence n'est pas statistiquement significative. Toutes les études n'ont pas publié des résultats pour le côlon et le rectum séparément et la différence pourrait être due aux facteurs autres que le site de cancer. La relation du cancer colorectal avec la consommation de viande traitée est en moyenne de la même magnitude que celle observée pour la viande rouge (RR= 1.19 ; 95% CI=1.09-1.30). Il s'avère difficile d'identifier avec précision les études qui ont étudié séparément la viande non-traitée, en raison du manque de détail des classifications utilisées dans les publications.

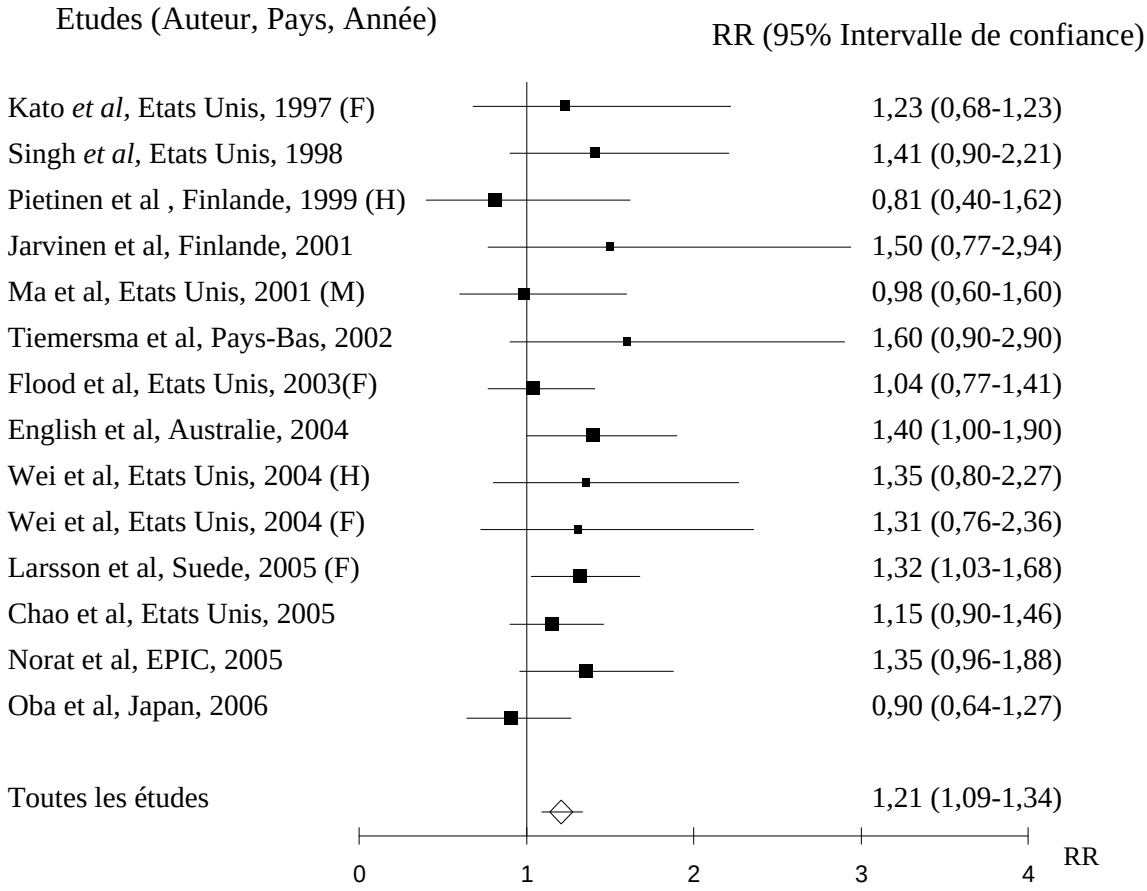
Les études de cohorte disponibles en 2006, prises dans leur ensemble, confirment la magnitude de l'association estimée dans notre méta-analyse précédente (Chapitre II. Article 1, Tableau I) (149). Les études plus récentes apportent des données qui suggèrent que l'association pourrait être plus forte avec le cancer du rectum que celle avec le cancer du

côlon, mais la différence n'est pas significative. Il est intéressant de constater que les données de l'étude EPIC suggèrent un gradient de la force de l'association du côlon droit vers le côlon gauche (Chapitre IV. Article 1. Tableau 3) (166). Ce résultat doit être confirmé par des études statistiquement plus puissantes.

En ce qui concerne le poisson, nous avons identifié treize études de cohorte avec 4506 cas au total. Le risque relatif moyen de cancer colorectal pour le niveau de consommation de poisson le plus haut par rapport au niveau le plus bas dans les populations étudiées est de 0.87 (95%CI=0.78-0.97) (Figure 3). Très peu d'études ont investigué la relation entre la consommation de poisson et le cancer du côlon et du rectum séparément (162) (166) (167). Pour cette raison nous ne présentons pas d'estimations méta-analytiques selon localisation anatomique.

Le possible effet protecteur de la consommation du poisson sur le cancer colorectal requiert une confirmation ultérieure. Tout d'abord, l'estimation méta-analytique est très influencée par l'étude EPIC, avec 1 329 cas, ce qui représente un tiers des cas dans l'analyse. D'autre part, nous avons détecté que la relation entre le poisson et le risque de cancer colorectal diffère parmi les cohortes de l'étude EPIC, selon le test d'hétérogénéité effectué.

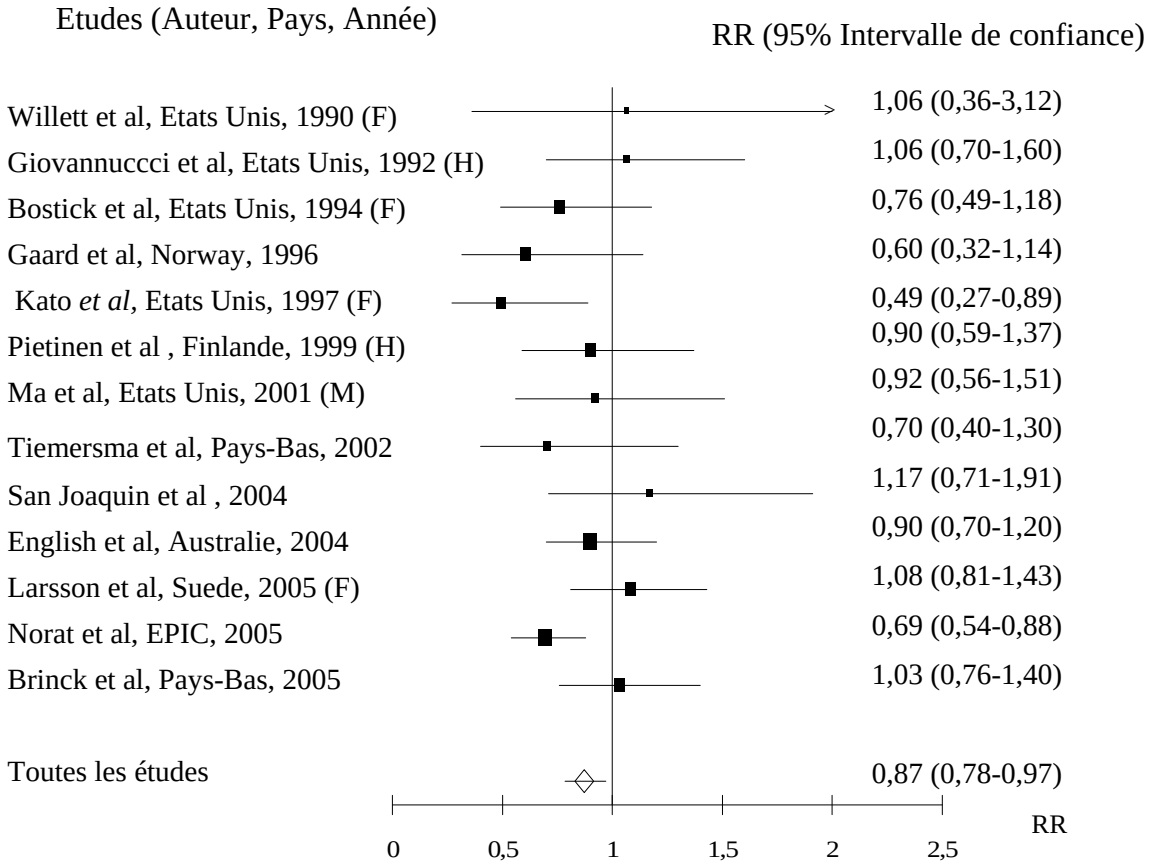
Figure 2: Risques relatifs de cancer colorectal comparant la catégorie de consommation de viandes rouges la plus haute versus la plus basse dans les études de cohortes publiées (1997-2005)



Les carrés noirs et les lignes horizontales représentent les risques relatifs estimés de chaque étude ainsi que leurs IC à 95%. La taille du carré noir reflète le poids de l'étude dans l'estimation du risque moyen. Les losanges représentent les estimations des risques relatifs moyens obtenus par la méta-analyse et les estimations des IC à 95% respectifs.

(H) : Cohorte des hommes, (F) : Cohorte de femmes.

Figure 3 : Risques relatifs de cancer colorectal comparant la catégorie de consommation de poisson la plus haute versus la plus basse dans les études de cohortes publiées (1997-2005)



Les carrés noirs et les lignes horizontales représentent les risques relatifs estimés de chaque étude ainsi que leurs IC à 95%. La taille du carré noir reflète le poids de l'étude dans l'estimation du risque moyen. Les losanges représentent les estimations des risques relatifs moyens obtenus par la méta-analyse et les estimations des IC à 95% respectifs.

(H) : Cohorte des hommes, (F) : Cohorte de femmes.

3. Hypothèses sur les mécanismes expliquant un effet promoteur des viandes rouges.

Plusieurs mécanismes ont été proposés pour expliquer l'association entre la consommation de viande rouge et le cancer colorectal. Ces mécanismes impliquent des composés de la viande comme agents étiologiques - les lipides ou leurs acides gras, les protéines, le fer, des composées nitrosés- ou des produits de leur métabolisme, ainsi que des carcinogènes potentiels formés pendant la cuisson ou la préservation de la viande.

3.1. Les lipides de la viande

L'hypothèse selon laquelle les lipides alimentaires augmentent le risque de cancer colorectal a été suggéré par les études écologiques qui ont rapporté des coefficients de corrélation entre les taux de cancer du côlon et les apports *per capita* de lipides situés entre 0.8 et 0.9 (66;76).

La teneur en graisse de la viande rouge est très variable et elle dépend entre autres facteurs, de facteurs génétiques et environnementaux, de l'âge de l'animal et du type de viande. En général, la viande rouge contient des quantités similaires d'acides gras saturés (approximativement 3.2 g/100 g de bœuf cuit et dégraissé) et mono-insaturés (3.41 g/100 g de bœuf). Les niveaux d'acides gras polyinsaturés sont très faibles (0.38 g/100 grammes de bœuf). Une partie de la graisse peut être éliminée mécaniquement pour la commercialisation sur la forme de « viande maigre », dont le contenu en lipides n'est pas précisément défini.

Nous avons déjà exposé (Chapitre I) l'hypothèse selon laquelle les lipides alimentaires peuvent favoriser l'apparition d'un cancer colorectal en augmentant la sécrétion d'acides biliaires nécessaires à leur digestion. Ces acides biliaires peuvent être convertis en acides biliaires secondaires et tertiaires par la population bactérienne colique. Selon cette hypothèse, l'effet irritant non spécifique de produits des acides biliaires sur la muqueuse du côlon peut induire une prolifération compensatrice des cellules épithéliales et de ce fait augmenter le risque de mutation endogène (168;169).

Parmi les mécanismes suggérés par des études de laboratoires pour expliquer le fait que les lipides alimentaires peuvent influencer de manière indirecte le risque de cancer colorectal nous trouvons la modification de la composition en acides gras des membranes cellulaires (Meterission et al, 1995 Cancer Letter 89 :145-52), le rôle des acides gras saturés sur les mécanismes de résistance à l'insuline (170) et l'influence de lipides alimentaires sur la réponse anti-inflammatoire et immunologique [une révision détaillée de ces hypothèses a été publiée par Giovannucci et Goldin (171)].

D'après les expériences réalisées sur les rongeurs, l'effet carcinogénique attribué aux lipides alimentaires semble dépendre du type d'acide gras, saturé ou non. Pour les acides gras polyinsaturés, l'effet dépendrait de la place de la double liaison. Or, les acides gras polyinsaturés de la série n-6 seraient promoteurs, ceux de la série n-3 inhiberaient au contraire la croissance des tumeurs et les acides gras mono-insaturés seraient sans effet sur la cancérogenèse (77;172;173).

En général, les données scientifiques actuellement disponibles montrent que la relation entre le cancer colorectal et la consommation de viande rouge est indépendante de la teneur en lipides de la viande (79;80). Dans la plus grande étude prospective publiée, l'étude EPIC (166), la relation viande rouge-cancer colorectal persiste après l'ajustement des risques relatifs par les apports caloriques provenant de lipides alimentaires. Dans des études cas-témoins montrant une relation positive entre les lipides et le risque de cancer du côlon, une association positive entre les apports énergétiques élevés et le risque de cancer du côlon a été aussi observée (174). Ceci, couplé aux résultats de plusieurs études qui montrent que l'activité physique réduit le risque de cancer du côlon (34) laisse penser que les apports caloriques et l'inactivité physique pourraient jouer le rôle de facteurs de confusion sur la relation graisse alimentaire-cancer. En présence de confusion résiduelle, le résultat serait une fausse association positive.

3.2. Le fer.

L'hypothèse impliquant le fer dans la cancérogenèse colorectale a été suggérée par l'absence de relation entre le cancer colorectal et la viande blanche (en générale, viande de volailles, et plus spécifiquement, du poulet) moins riche en fer que la viande rouge.

Le fer est un métal essentiel chez les mammifères pour le transport de l'oxygène par l'hémoglobine et pour l'activité de plusieurs enzymes, y compris les cytochromes. Le fer libre intervient dans la réaction de Fenton, qui provoque la formation de radicaux hydroxylés, notamment le radical OH. Ce radical, très instable et très réactif, provoque, entre autres, la peroxydation des lipides, des dommages à l'ADN et la réduction des mécanismes de protection contre le stress oxydatif (175). Tous les éléments invoqués peuvent être liés à l'effet mutagène et carcinogène du fer libre observé chez les rats (176).

La viande rouge est l'aliment qui apporte le plus de fer à l'organisme, sous une forme très assimilable (fer héminique). Il n'existe pas de bases de données utilisables dans des études épidémiologiques pour estimer avec précision le contenu de fer héminique des viandes (177), ce qui fait que très peu d'études épidémiologiques ont abordé cette problématique. Une base de données est actuellement en préparation à l'Institut national du cancer (*National Cancer Institute*, *NCI*) des Etats-Unis. Cette base des données tiendra en compte la modification de la biodisponibilité du fer selon le mode de cuisson (Sinha, communication personnelle).

Une relation significative entre les apports en fer héminique et le risque de cancer du côlon a été observée dans deux études prospectives(178;179) où des mesures approximées ont été utilisées, mais l'association disparaît dans une des études (178) quand la viande rouge et le fer héminique sont inclus simultanément dans le modèle.

3.3. Les protéines.

La viande est une source importante de protéines qui peuvent être impliquées dans la cancérogenèse colique au travers de la formation d'ammonium. Des niveaux d'ammonium aussi bas que 5-10 mMol peuvent modifier la morphologie, le métabolisme intermédiaire des cellules intestinales, la synthèse de l'ADN et le renouvellement des cellules du côlon (180). Tous ces dommages cellulaires prédisposent à la croissance néoplasique.

Les molécules azotées (principalement les acides aminés) résiduels de la digestion des protéines arrivent dans le côlon et sont métabolisées en ammonium par la flore colique. Dans une étude sur quatre volontaires sains, l'augmentation des protéines du régime alimentaire (de 63 à 136 g/jour) a élevé de façon significative la concentration d'ammonium fécale (de 15 à 30 mM) (181) et dans une autre étude, la concentration est augmentée de 2,7 à 6,5 mM chez les sujets suivant un régime riche en viande rouge (182).

Le mécanisme de l'ammonium est concordant avec l'observation que les patients ayant subi des urétérosigmoidostomies et qui ont des concentrations très élevées en ammonium dans le lumen du côlon, présentent un risque élevé de développer des tumeurs distales à l'emplacement de l'implantation de l'urètre (183). En dépit de la plausibilité biologique de ce mécanisme et des pistes expérimentales, l'hypothèse de l'ammonium n'a pas fait l'objet d'évaluation dans des études épidémiologiques, en raison des difficultés à déterminer les niveaux d'exposition dans des populations de grande taille.

3.4. Les composés N-nitrosés.

Les composés N-nitrosés (NOCs) sont parmi les carcinogènes chimiques les plus puissants. La cancérogenèse de NOCs a été examinée chez 39 espèces animales différentes, y compris six espèces de primates. Des tumeurs ont été induites par des NOCs dans plusieurs sites

anatomiques, y compris le côlon et le rectum de toutes les espèces animales examinées (184;185).

Les NOCs sont des agents alkylants qui peuvent agir sur l'ADN des tissus cibles, donnant lieu à la formation de bases altérées. Certaines mutations accumulées pendant la progression du cancer colorectal sont caractéristiques des réactions d'alkylations; par exemple, les transitions de la guanine vers l'adénosine (G->A) dans le codon 12 ou 13 du gène K-ras (186). Ils peuvent par cette voie jouer un rôle dans l'initiation de la cancérogenèse (187).

Les sources exogènes principales des NOCs sont le tabac et les aliments, y compris les viandes qui ont été en contact direct avec des flammes (188). Une autre source des NOCs sont les sels de nitrite ajoutés à la viande comme antibactérien et agent cosmétique pour donner, par réaction avec la myoglobine, la couleur rose caractéristique des produits carnés traités.

La formation endogène est la source plus importante de NOCs dans l'organisme humain. Une flore microbienne normale est nécessaire pour que la N-nitrosation endogène se produise (189). La population microbienne intestinale fournit des substrats nitrosables tels que des amines et des amides, par activité catalytique et décarboxylation des acides aminés. Chez l'homme, un certain nombre de bactéries facultatives et anaérobiques peuvent catalyser la formation de NOCs au pH neutre par l'intermédiaire de la réductase de nitrate (190-192). La formation endogène des NOCs peut se produire aussi par l'intermédiaire de catalyse acide, qui a lieu principalement dans l'estomac, où le pH est bas et l'agent nitrosant, l'acide nitrique, peut exister sous sa forme libre (193).

La contribution de la viande rouge sur la formation endogène de NOCS a été examinée dans une série d'études sur des volontaires masculins sains avec des régimes alimentaires soigneusement contrôlés (182) (194;195), utilisant une mesure indirecte de N-nitrosation

endogène dérivée de l'excrétion fécale des composés N-nitrosés (“*apparent total nitroso-compounds* » ou ATNC).

Les études ont montré l’existence d’une relation dose-réponse entre les apports en viande rouge et les niveaux d’ATNC. Les niveaux observés d’ATNC ne peuvent être expliqués que par formation endogène de composés nitrosés, puisque les niveaux estimés d’ATCN préformés fournis par des régimes de viande rouge étaient inférieurs aux niveaux mesurés dans les selles. Remarquablement, les concentrations d'ATNC pour les niveaux plus élevées de viande rouge (600 mg par jour) sont du même ordre que la concentration des NOCs qui se retrouve dans la fumée de cigarette. Le régime riche en viande rouge a augmenté aussi le niveau de l’ammonium fécale (195). Les régimes alimentaires basés en viande blanche (volailles) ou en protéines d’origine végétale n’ont pas été accompagnés d’une augmentation des niveaux d’ATNC.

Nous avions déjà exposé l’hypothèse selon laquelle le fer héminique pourrait jouer un rôle dans la carcinogénèse chimique par la formation des radicaux hydroxylés. Le fer héminique semble jouer aussi un rôle spécifique sur la nitrosation endogène, tel qu’il a été observé dans une étude portant sur 21 hommes volontaires sains (196). Les niveaux d’ATNC ont été significativement plus élevés chez les sujets qui ont suivi un régime enrichi avec 8 mg par jour de fer héminique, par rapport aux niveaux d’ATNC chez des sujets suivants un régime faible en viande rouge (60 grammes par jour). Les niveaux d’ATNC n’ont pas varié chez les sujets qui ont suivi un régime enrichie avec 35 mg de fer inorganique par jour (196). La constatation que le fer héminique a un effet sur la formation de NOCs suggère que la catalyse chimique, en plus de la N-nitrosation bactérienne, est responsable de l'effet dose-réponse de la viande rouge sur la N-nitrosation intestinale endogène. La N-nitrosohæmoglobine et la N-nitrosomyoglobine peuvent être formés par réaction du nitrite avec l'hémoglobine et la

myoglobine, ainsi que d'autres composés nitrosés par l'action directe de l'oxyde nitrique sur l'hémoglobine et la myoglobine (197).

Il n'y a pas de preuves directes de l'effet écotoxique des NOCs sur la muqueuse intestinale. L'effet génotoxique des NOCs a été étudié de manière indirecte en utilisant l'ADN des cellules exfoliés de la surface colique récupérées dans les selles des volontaires sains (198). Une relation directe entre le pourcentage de cellules positives à l'O^6-carboxyméthyl guanine (O^6CMG), un adduit de l'ADN spécifique des NOCs, et les niveaux d'ATNC dans les selles a été observé chez des sujets suivant un régime riche en viande rouge. Le pourcentage de cellules positives à l' O^6CMG a diminué, mais de manière non-significative, quand les sujets ont suivi des régimes riches simultanément en fibre et en viande rouge et il était plus bas quand les sujets ont suivi un régime végétarien. Ce résultat est concordant avec l'effet de modification de la relation viande rouge-cancer colorectal par intermédiaire de la fibre alimentaire observé dans l'étude EPIC (166).

3.5. Carcinogènes potentiels formés pendant la cuisson et préservation de la viande.

Les amines hétérocycliques (HCAs) sont des mutagènes et des cancérogènes qui peuvent être formés dans la viande pendant la cuisson prolongée à haute température. Plus de 20 HCA différents ont été identifiés, dont la plupart avec activité mutagène selon le test d'Ames. L'effet cancérogène sur le foie, le poumon, le côlon et les glandes mammaires de quatre HCA (IQ, 2-amino-3-méthylimidazo [4,5-f]quinoline; MeIQ, 2-amino-3,4-diméthylimidazo[4,5-f]quinoline; MeIQx, 2-amino-3,8-diméthylimidazo[4,5-f]quinoxaline; and PhIP, 2-amino-1-méthyl-6-phenylimidazo[4,5-b]pyridine) à doses très élevées (10-400 mg par kilogramme de poids corporel) a été démontré dans des études chez des rongeurs et des primates non humains (199). L'effet cancérogène des HCAs chez l'homme n'a pas être démontré, mais les tumeurs du côlon induites par HCAs ont une fréquence élevée d'instabilité microsatellitaire semblable

à celle constatée sous les formes héritées et sporadiques de cancer colorectal chez l'homme (200).

Les HCAs les plus abondants dans la viande cuite sont 2-amino-1-méthyl-6-phenylimidazo (b) pyridine 4.5- (PhIP) et 2-amino-3,8-diméthylimidazo [f]quinoxaline 4.5- (MeIQx). Ce sont également les deux HCAs majoritairement absorbés.

D'autres carcinogènes potentiels formés dans la viande pendant sa cuisson sont les hydrocarbures polycycliques aromatisés (PAHs). Les jus de la viande grillée sur une flamme directe provoquent une fumée contenant des PAHs, tel que le benzoapyrène (BaP), qui adhèrent à la surface de la nourriture. A la différence des HCA, dont la voie d'exposition pour l'homme est surtout la viande cuite à haute température, les PAH se trouvent dans le tabac, les aliments grillés et fumés et dans l'environnement. Les PAHs se forment lorsqu'il y a combustion incomplète (pyrolyse) des matériaux organiques.

L'activation métabolique est indispensable pour que les HCAs puissent agir comme agents mutagènes/cancérogènes; l'activation a lieu principalement par N-oxydation par l'enzyme hépatique du cytochrome P-450 1A2 (CYP1A2), suivi par l'O-acétylation par l'N-acétyltransférase hépatique (NAT1) et/ou l'N-acétyltransférase 2 (NAT2), ou bien par des sulfotransférases. Les métabolites N-hydroxylaminés des HCAs peuvent subir des réactions de conjugaison par l'intermédiaire de l'enzyme UDPglucuronosyltransférase hépatique ou du côlon (UGTs) pour former les conjugués correspondants de N-glucuronide, ce qui est considéré une voie significative de désintoxication des HCAs (97). L'expression de l'enzyme CYP1A2 varie jusqu'à 40 fois chez l'homme. L'enzyme peut être induite par le tabagisme, l'alimentation et l'hépatite chronique, ainsi que par un régime riche en HCAs. L'expression d'UGTs est également connue pour être induisible, mais l'expression de NAT2 ne l'est pas.

La contribution des HCAs et des PAHs alimentaires au risque de cancer colorectal demeure incertaine en raison des difficultés pour mesurer l'exposition individuelle et l'absence de biomarqueurs appropriés de l'exposition aux HCAs et PAHs alimentaires (200).

Les résultats de certaines études cas-témoins concordent avec l'hypothèse que l'exposition aux produits de la pyrolyse dans la viande très cuite augmente le risque de cancer colorectal (201). La viande rouge est associée au cancer colorectal chez les individus avec génotypes susceptibles NAT1 et NAT2 (202;203), UGT1A7 (204) ou avec des phénotypes d'acétylation rapide NAT2 et CYP1A (205;206). Néanmoins, les résultats d'une grande étude pharmacogénétique de type cas-témoins ne confirment pas l'hypothèse que les amines hétérocycliques sont liées au risque du cancer colorectal. Aucun des polymorphismes de gènes des enzymes métaboliques de la phase I et la phase II examinées, tels que le cytochrome P450, la glutathione S-transférase, la sulfotransférases, la N-acetyl transférase 2 (NAT2) n'était associé au risque de cancer colorectal (207).

4. Hypothèses sur les mécanismes expliquant un effet protecteur du poisson.

L'effet potentiel du poisson sur le cancer a été attribué fondamentalement à sa teneur en acides gras insaturés (n-3) à longue chaîne. L'acide éicosopentanoïque (EPA) a particulièrement retenu l'attention. Cet acide gras a 20 carbones et 5 insaturations. Il peut être metabolisé en plusieurs substances comme les prostaglandines, les leucotriènes et les thromboxanes. Le poisson de type gras -originaire des eaux froides-, tel que le saumon et les sardines ont des concentrations des acides gras plus élevées. Les poissons non-gras, typiques des eaux non-froides, présentent des concentrations plus faibles en EPA et il possède parfois des niveaux plus élevés d'acide arachidonique. Or, différents types de poisson peuvent avoir des effets différents sur le développement du cancer.

Plusieurs mécanismes ont été proposés pour expliquer l'effet potentiel des acides gras marins sur la carcinogénèse. Parmi eux, l'inhibition de la biosynthèse d'eicosanoïdes à partir de

l'acide arachidonique (AA; 20:4n-6). Les eicosanoïdes sont une famille de composés dérivés des acides polyinsaturés, parmi lesquels se trouvent les prostaglandines, les acides hydroxy-éicosatétraénoïques, et les leukotriènes. Les prostaglandines sont des acides gras insaturés cycliques qui exercent une action de type hormonal. Les acides gras marins inhibent la cyclooxygénase-2 et le métabolisme oxydatif des AA vers PGE_2 et par cette voie, peuvent réduire la prolifération cellulaire et induire d'appose dans la muqueuse colique (208;209). Des études chez les animaux montrent que les acides gras n-3 marins réduisent la prolifération cellulaire et augmentent l'appoptose des cellules de la muqueuse des cryptes (210;211).

Chez l'homme, la diminution de l'expression de la cyclooxygénase 2 est liée à la réduction du risque de cancer colorectal et des adénomes (212-214) et à l'augmentation de la survie après cancer colorectal (215). Des essais thérapeutiques ont montré que les apports des acides gras n-3 à doses élevées (2.5 et 7.7 grammes par jour) réduisent la prolifération cellulaire de la muqueuse rectale des patients avec adénome rectal sporadique (216;217) et des patients polypéctomisés pour adénome ou cancer (218).

Malgré les données scientifiques qui suggèrent un effet protecteur des acides gras du poisson contre le cancer colorectal, très peu d'études épidémiologiques ont évalué cette association et les résultats sont peu cohérents(85;209;219). Une méta-analyse récente des études de cohorte et des essais cliniques randomisés a conclu que les données accumulées sont insuffisantes pour affirmer que les acides gras ω3 ont un effet sur la réduction du risque de cancer (220).

Considérations finales

Depuis le début du XXème siècle, l'espérance de vie a connu une amélioration spectaculaire, témoignant de l'ampleur des évolutions tant sociales que médicales qui ont marqué cette période, surtout dans les sociétés économiquement les plus riches. Cette évolution s'explique principalement par un recul considérable de la mortalité par maladies infectieuses et respiratoires pendant cette période, alors que les maladies cardio-vasculaires et les cancers sont devenus les deux principales causes de mortalité. Outre leur impact sur la mortalité globale, ces évolutions sont accompagnées d'une proportion croissante d'individus avec un poids excessif et un style de vie sédentaire (221).

Au cours de l'évolution humaine, les approvisionnements alimentaires, souvent précaires, se caractérisaient en général par une composition faible en lipides et élevée en hydrates de carbone et fibres. Au cours des deux derniers siècles, les améliorations des techniques de production, la préservation, le stockage, et la distribution de la nourriture ont conduit à des modifications importantes de la composition du régime alimentaire dans les nations économiquement développées et à moindre échelle, dans les pays en voie de développement.

Le régime alimentaire est une source de nutriments essentiels pour le bon fonctionnement de l'organisme, mais il sert aussi de véhicule aux nutriments et autres substances qui ont le potentiel d'inhiber ou de participer à la cancérogenèse. Des méthodes de préservation ou de préparation des aliments (e.g., salaison, cuisson à haute température des viandes) ont été impliquées en tant que sources de carcinogènes ou de promoteurs de la croissance tumorale (142;201). Certains substances phytochimiques possèdent des propriétés anti-cancérogènes (50). Des colorants, des édulcorants, des aromatisants et d'autres additifs semblent très peu contribuer à l'incidence de cancer dans le monde (50). Les risques potentiels des pesticides,

des herbicides, des hormones, des déchets industriels et d'autres contaminants synthétiques qui entrent dans la chaîne alimentaire n'ont pas encore été clairement définis.

Le régime humain est une combinaison complexe d'aliments qui montre des variations journalières et saisonnières significatives. L'étude du rôle de l'alimentation dans le développement du cancer chez l'homme est caractérisée par l'imprécision de la quantification des apports alimentaires et de sa variation intra-individuelle dans le temps (222;223).

Des progrès considérables ont été accomplis récemment dans le domaine de l'épidémiologie nutritionnelle permettant de mieux cerner la relation entre l'alimentation et le risque de cancer. Il s'agit des résultats d'études de cohorte initiées 10 à 20 ans auparavant, d'études avec utilisation de biomarqueurs et d'études d'intervention nutritionnelle ; de l'incorporation des méthodes pour la correction partielle des erreurs de mesure de l'alimentation dans des études épidémiologiques et de l'amélioration du contrôle de facteurs de confusion ; de la réalisation de méta-analyses et d'analyses combinées des études épidémiologiques ; et enfin d'une meilleure connaissance des mécanismes impliqués, en particulier de l'étude des interactions entre prédisposition génétique et facteurs environnementaux .

Les études épidémiologiques peuvent apporter des données scientifiques sur le bénéfice ou le risque posé par des constituants de l'alimentation ou d'autres facteurs. L'épidémiologie est nécessaire mais néanmoins non suffisante pour traduire ces résultats en actions dans le domaine des politiques de santé. L'analyse des synthèses exhaustives du rôle de l'alimentation dans l'étiologie des cancers montre que la littérature existante est complexe, incomplète et parfois contradictoire (50;65;69). Les recommandations alimentaires pour la prévention des maladies sont, jusqu'à présent, basées sur l'intégration d'informations dérivées d'une variété d'approches, d'investigations épidémiologiques et en laboratoire. Les progrès à venir dépendront de la réplication des résultats dans des populations différentes et des

stratégies épidémiologiques innovantes utilisant des indicateurs biochimiques et moléculaires, ainsi que les progrès de la génétique.

L'étude EPIC a contribué à la connaissance de la relation entre la nutrition et le risque de cancer colorectal en montrant que l'obésité (42), l'inactivité physique (36), la consommation élevée d'alcool (51) et de viande rouge sont des facteurs de risque de cancer colorectal. La fibre alimentaire et le poisson semblent diminuer le risque. Des études au sein de la population EPIC sont actuellement en cours afin d'examiner l'interaction entre facteurs nutritionnels, métaboliques et génétiques. Certains de nos résultats sont cohérents avec l'ensemble des données disponibles, en particulier nos résultats sur la relation entre viande rouge et cancer colorectal. D'autres résultats, tels que la relation entre fibres alimentaires, poisson et le cancer colorectal, requièrent d'ultérieures confirmations.

Nos résultats démontrent que la consommation quotidienne de viande rouge peut augmenter le risque de cancer colorectal et que la diminution de la consommation de viande rouge chez les gros consommateurs doit aboutir à la diminution du risque de cancer colorectal au niveau de la population.

BIBLIOGRAPHIE

(1) International Agency for Research on Cancer. *World Cancer Report*. Lyon: *IARC Press*, 2000.

(2) Parkin DM PP. Estimates of the worldwide incidence of 25 major cancers in 1990. Int J Cancer 1999; 80(6):827-841.

(3) Remontet L, Esteve J, Bouvier AM et al. Cancer incidence and mortality in France over the period 1978-2000. Rev Epidemiol Sante Publique 2003; 51(1 Pt 1):3-30.

(4) Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55(2):74-108.

(5) Ferlay J, Pisani P., Parkin D.M. GLOBOCAN 2002: Cancer Incidence,Mortality and Prevalence Worldwide. IARC CancerBase .version 2.0 No.5. 2004. IARC Press,Lyon. 31-12-2004.

(6) McMichael AJ, Potter JD. Reproduction, endogenous and exogenous sex hormones, and colon cancer: a review and hypothesis. J Natl Cancer Inst 1980; 65(6):1201-1207.

(7) Parkin DM, Bray FI, Devesa SS. Cancer burden in the year 2000. The global picture. Eur J Cancer 2001; 37 Suppl 8:S4-66.

(8) Parkin DM, , Ferlay J, , Thomas DB (eds). Cancer Incidence in Five Continents Vol. VIII. . Lyon, France: International Agency for Research on Cancer, 2002. IARC Scientific Publications No.155.

(9) Le Marchand L. Combined influence of genetic and dietary factors on colorectal cancer incidence in Japanese Americans. J Natl Cancer Inst Monogr 1999;(26):101-105.

(10) Flood DM, Weiss NS, Cook LS, Emerson JC, Schwartz SM, Potter JD. Colorectal cancer incidence in Asian migrants to the United States and their descendants. Cancer Causes Control 2000; 11(5):403-411.

(11) Czene K, Lichtenstein P, Hemminki K. Environmental and heritable causes of cancer among 9.6 million individuals in the Swedish Family-Cancer Database. Int J Cancer 2002; 99(2):260-266.

(12) Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science 2005; 307(5717):1915-1920.

(13) Vonk RJ, Kalivianakis M, Minich DM, Bijleveld CM, Verkade HJ. The metabolic importance of unabsorbed dietary lipids in the colon. Scand J Gastroenterol Suppl 1997; 222:65-7.:65-67.

(14) Lipkin M, Reddy B, Newmark H, Lamprecht SA. Dietary factors in human colorectal cancer. Annu Rev Nutr 1999; 19:545-586.

(15) Silk DB. Digestion and absorption of dietary protein in man. Proc Nutr Soc 1980; 39(1):61-70.

(16) Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990; 61(5):759-767.

(17) Morson BC. Precancerous conditions of the large bowel. Proc R Soc Med 1971; 64(9):959-962.

(18) Radtke F, Clevers H, Riccio O. From gut homeostasis to cancer. Curr Mol Med 2006; 6(3):275-289.

(19) Grambsch P, Louis TA, Bostick RM et al. Statistical analysis of proliferative index data in clinical trials. Stat Med 1994; 13(16):1619-1634.

(20) Shih IM, Wang TL, Traverso G et al. Top-down morphogenesis of colorectal tumors. Proc Natl Acad Sci U S A 2001; 98(5):2640-2645.

(21) Groden J, Thliveris A, Samowitz W et al. Identification and characterization of the familial adenomatous polyposis coli gene. Cell 1991; 66(3):589-600.

(22) de la CA, Peltomaki P. The genetics of hereditary common cancers. Curr Opin Genet Dev 1998; 8(3):298-303.

(23) Powell SM, Zilz N, Beazer-Barclay Y et al. APC mutations occur early during colorectal tumorigenesis. Nature 1992; 359(6392):235-237.

(24) Korinek V, Barker N, Morin PJ et al. Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-/- colon carcinoma. Science 1997; 275(5307):1784-1787.

(25) Vogelstein B, Fearon ER, Kern SE et al. Allelotype of colorectal carcinomas. Science 1989; 244(4901):207-211.

(26) Lothe RA, Peltomaki P, Meling GI et al. Genomic instability in colorectal cancer: relationship to clinicopathological variables and family history. Cancer Res 1993; 53(24):5849-5852.

(27) Itzkowitz SH, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. Am J Physiol Gastrointest Liver Physiol 2004; 287(1):G7-17.

(28) Brentnall TA, Crispin DA, Rabinovitch PS et al. Mutations in the p53 gene: an early marker of neoplastic progression in ulcerative colitis. Gastroenterology 1994; 107(2):369-378.

(29) Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. Nat Genet 1994; 7(4):536-540.

(30) Iacopetta B. Are there two sides to colorectal cancer? Int J Cancer 2002; 101(5):403-408.

(31) Thomas G, Olschwang S. Genetic predispositions to colorectal cancer. Pathol Biol (Paris) 1995; 43(3):159-164.

(32) Bodmer WF. Cancer genetics: colorectal cancer as a model. J Hum Genet 2006; 51(5):391-396.

(33) Fuchs CS, Giovannucci EL, Colditz GA, Hunter DJ, Speizer FE, Willett WC. A prospective study of family history and the risk of colorectal cancer. N Engl J Med 1994; 331(25):1669-1674.

(34) Vainio H, Bianchini F (eds). IARC Handbooks of Cancer Prevention. Weight Control and Physical Activity. 6. 2002. IARC Press, Lyon.
Ref Type: Generic

(35) Giovannucci E, Colditz GA, Stampfer MJ, Willett WC. Physical activity, obesity, and risk of colorectal adenoma in women (United States). Cancer Causes Control 1996; 7(2):253-263.

(36) Friedenreich C., Norat T, Steindorf K et al. Physical Activity and Risk of Colon and Rectal Cancers: the European Prospective Investigation into Cancer and Nutrition. In press 2006.

(37) Samad AK, Taylor RS, Marshall T, Chapman MA. A meta-analysis of the association of physical activity with reduced risk of colorectal cancer. Colorectal Dis 2005; 7(3):204-213.

(38) Slattery ML, Edwards SL, Ma KN, Friedman GD, Potter JD. Physical activity and colon cancer: a public health perspective. Ann Epidemiol 1997; 7(2):137-145.

(39) Slattery ML. Physical activity and colorectal cancer. Sports Med 2004; 34(4):239-252.

(40) Friedenreich CM, Orenstein MR. Physical activity and cancer prevention: etiologic evidence and biological mechanisms. J Nutr 2002; 132(11 Suppl):3456S-3464S.

(41) Slattery ML, Potter JD. Physical activity and colon cancer: confounding or interaction? Med Sci Sports Exerc 2002; 34(6):913-919.

(42) Pischon T, Lahmann PH, Boeing H et al. Body Size and Risk of Colon and Rectal Cancer in the European Prospective Investigation Into Cancer and Nutrition (EPIC). J Natl Cancer Inst 2006; 98(13):920-931.

(43) Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 2004; 4(8):579-591.

(44) Watkins LF, Lewis LR, Levine AE. Characterization of the synergistic effect of insulin and transferrin and the regulation of their receptors on a human colon carcinoma cell line. Int J Cancer 1990; 45(2):372-375.

(45) Bjork J, Nilsson J, Hultcrantz R, Johansson C. Growth-regulatory effects of sensory neuropeptides, epidermal growth factor, insulin, and somatostatin on the non-transformed intestinal epithelial cell line IEC-6 and the colon cancer cell line HT 29. Scand J Gastroenterol 1993; 28(10):879-884.

(46) Corpet DE, Jacquinet C, Peiffer G, Tache S. Insulin injections promote the growth of aberrant crypt foci in the colon of rats. Nutr Cancer 1997; 27(3):316-320.

(47) Tran TT, Medline A, Bruce WR. Insulin promotion of colon tumors in rats. Cancer Epidemiol Biomarkers Prev 1996; 5(12):1013-1015.

(48) Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 2004; 363(9418):1346-1353.

(49) Moskal A., Norat T, Ferrari P, Riboli E. Alcohol intake and colorectal cancer risk: a dose-response meta-analysis of published cohort studies. International Journal of Cancer.(In press)

(50) World cancer research Fund. Food, nutrition and the prevention of cancer: a global perspective. American Institute for Cancer Research, editor. 1997. Washington,DC.

(51) Ferrari P, Jenab M., Norat T et al. Lifetime and current alcohol consumption and the risk of colorectal cancer in the European Prospective Investigation into Cancer and Nutrition. Submitted for publication (Jounal of the National Cancer Institute) 2006.

(52) Cho E, Smith-Warner SA, Ritz J et al. Alcohol intake and colorectal cancer: a pooled analysis of 8 cohort studies. Ann Intern Med 2004; 140(8):603-613.

(53) Freudenheim JL, Graham S, Marshall JR, Haughey BP, Cholewinski S, Wilkinson G. Folate intake and carcinogenesis of the colon and rectum. Int J Epidemiol 1991; 20(2):368-374.

(54) Giovannucci E, Stampfer MJ, Colditz GA et al. Folate, methionine, and alcohol intake and risk of colorectal adenoma. J Natl Cancer Inst 1993; 85(11):875-884.

(55) Kuper H, Boffetta P, Adami HO. Tobacco use and cancer causation: association by tumour type. J Intern Med 2002; 252(3):206-224.

(56) Giovannucci E. Should smokers be considered a high-risk group for colorectal cancer? Dig Liver Dis 2004; 36(10):643-645.

(57) Giovannucci E, Colditz GA, Stampfer MJ et al. A prospective study of cigarette smoking and risk of colorectal adenoma and colorectal cancer in U.S. women. J Natl Cancer Inst 1994; 86(3):192-199.

(58) Hilmi I, I. Chemoprevention of colorectal cancer with nonsteroidal anti-inflammatory drugs. Chinese journal of digestive diseases 2006; 7(1):1-6.

(59) Chan AT, Giovannucci EL, Meyerhardt JA, Schernhammer ES, Curhan GC, Fuchs CS. Long-term use of aspirin and nonsteroidal anti-inflammatory drugs and risk of colorectal cancer. JAMA 2005; 294(8):914-923.

(60) Benamouzig R, Uzzan B, Little J, Chaussade S. Low dose aspirin, COX-inhibition and chemoprevention of colorectal cancer. Curr Top Med Chem 2005; 5(5):493-503.

(61) Grodstein F, Newcomb PA, Stampfer MJ. Postmenopausal hormone therapy and the risk of colorectal cancer: a review and meta-analysis. Am J Med 1999; 106(5):574-582.

(62) Rossouw JE, Anderson GL, Prentice RL et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 2002; 288(3):321-333.

(63) Hulley S, Furberg C, Barrett-Connor E et al. Noncardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/progestin Replacement Study follow-up (HERS II). JAMA 2002; 288(1):58-66.

(64) Hormone therapy for the prevention of chronic conditions in postmenopausal women: recommendations from the U.S. Preventive Services Task Force. Ann Intern Med 2005; 142(10):855-860.

(65) CNERNA-CNRS. Alimentation et Cancer. Evaluation des données scientifiques. Riboli E, Decloitre F, Collet-Ribbing CC, editors. 1996. Paris, Technique & Documentation.

(66) Armstrong B, Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. Int J Cancer 1975; 15(4):617-631.

(67) Prentice RL, Sheppard L. Dietary fat and cancer: consistency of the epidemiologic data, and disease prevention that may follow from a practical reduction in fat consumption. Cancer Causes Control 1990; 1(1):81-97.

(68) McKeown-Eyssen G. Epidemiology of colorectal cancer revisited: are serum triglycerides and/or plasma glucose associated with risk? Cancer Epidemiol Biomarkers Prev 1994; 3(8):687-695.

(69) COMA. Report of the Working Group on Diet and cancer. Nutritional aspects of the development of cancer. The Stationery Office, editor. 1998. London.
Ref Type: Serial (Book,Monograph)

(70) Bostick RM, Potter JD, Kushi LH et al. Sugar, meat, and fat intake, and non-dietary risk factors for colon cancer incidence in Iowa women (United States). Cancer Causes Control 1994; 5(1):38-52.

(71) Slattery ML, Benson J, Berry TD et al. Dietary sugar and colon cancer. Cancer Epidemiol Biomarkers Prev 1997; 6(9):677-685.

(72) Michaud DS, Fuchs CS, Liu S, Willett WC, Colditz GA, Giovannucci E. Dietary glycemic load, carbohydrate, sugar, and colorectal cancer risk in men and women. Cancer Epidemiol Biomarkers Prev 2005; 14(1):138-147.

(73) Larsson SC, Giovannucci E, Wolk A. Dietary Carbohydrate, Glycemic Index, and Glycemic Load in Relation to Risk of Colorectal Cancer in Women. Am J Epidemiol 2006; .

(74) McCarl M, Harnack L, Limburg PJ, Anderson KE, Folsom AR. Incidence of colorectal cancer in relation to glycemic index and load in a cohort of women. Cancer Epidemiol Biomarkers Prev 2006; 15(5):892-896.

(75) Angres G., Beth M. Human nutrition: a comprehensive treatise. Alfin-Slater R.B., Kritchevsky D., editors. Cancer and nutrition. Effects of dietary constituents on carcinogenesis in different tumor models: an overview from 1975 to 1988. [7], 51. 1991. New York. Plenum.

(76) Rose DP, Boyar AP, Wynder EL. International comparisons of mortality rates for cancer of the breast, ovary, prostate, and colon, and per capita food consumption. Cancer 1986; 58(11):2363-2371.

(77) Bougnoux P, Menanteau J. [Dietary fatty acids and experimental carcinogenesis]. Bull Cancer 2005; 92(7):685-696.

(78) Howe GR, Hirohata T, Hislop TG et al. Dietary factors and risk of breast cancer: combined analysis of 12 case-control studies. J Natl Cancer Inst 1990; 82(7):561-569.

(79) Willett WC, Stampfer MJ, Colditz GA, Rosner BA, Speizer FE. Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women [see comments]. N Engl J Med 1990; 323(24):1664-1672.

(80) Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC. Intake of fat, meat, and fiber in relation to risk of colon cancer in men. Cancer Res 1994; 54(9):2390-2397.

(81) Goldbohm RA, van den Brandt PA, van ', V, Dorant E, Sturmans F, Hermus RJ. Cholecystectomy and colorectal cancer: evidence from a cohort study on diet and cancer. Int J Cancer 1993; 53(5):735-739.

(82) Flood A, Velie EM, Sinha R et al. Meat, fat, and their subtypes as risk factors for colorectal cancer in a prospective cohort of women. Am J Epidemiol 2003; 158(1):59-68.

(83) Kune S, Kune GA, Watson LF. Case-control study of dietary etiological factors: the Melbourne Colorectal Cancer Study. Nutr Cancer 1987; 9(1):21-42.

(84) Jain M, Cook GM, Davis FG, Grace MG, Howe GR, Miller AB. A case-control study of diet and colo-rectal cancer. Int J Cancer 1980; 26(6):757-768.

(85) Pietinen P, Malila N, Virtanen M et al. Diet and risk of colorectal cancer in a cohort of Finnish men. Cancer Causes Control 1999; 10(5):387-396.

(86) Slattery ML, Schumacher MC, Smith KR, West DW, Abd-Elghany N. Physical activity, diet, and risk of colon cancer in Utah. Am J Epidemiol 1988; 128(5):989-999.

(87) Terry P, Bergkvist L, Holmberg L, Wolk A. No association between fat and fatty acids intake and risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2001; 10(8):913-914.

(88) Thun MJ, Calle EE, Namboodiri MM et al. Risk factors for fatal colon cancer in a large prospective study. J Natl Cancer Inst 1992; 84(19):1491-1500.

(89) Larsson SC, Bergkvist L, Wolk A. High-fat dairy food and conjugated linoleic acid intakes in relation to colorectal cancer incidence in the Swedish Mammography Cohort. Am J Clin Nutr 2005; 82(4):894-900.

(90) Schatzkin A, Lanza E, Corle D et al. Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. Polyp Prevention Trial Study Group. N Engl J Med 2000; 342(16):1149-1155.

(91) Beresford SA, Johnson KC, Ritenbaugh C et al. Low-fat dietary pattern and risk of colorectal cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. JAMA 2006; 295(6):643-654.

(92) Burkitt DP, Walker AR, Painter NS. Effect of dietary fibre on stools and the transit-times, and its role in the causation of disease. Lancet 1972; 2(7792):1408-1412.

(93) Bingham SA, Day NE, Luben R et al. Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. Lancet 2003; 361(9368):1496-1501.

(94) Bingham SA, Norat T, Moskal A et al. Is the association with fiber from foods in colorectal cancer confounded by folate intake? Cancer Epidemiol Biomarkers Prev 2005; 14(6):1552-1556.

(95) Park Y, Hunter DJ, Spiegelman D et al. Dietary fiber intake and risk of colorectal cancer: a pooled analysis of prospective cohort studies. JAMA 2005; 294(22):2849-2857.

(96) Lampe JW. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. Am J Clin Nutr 1999; 70(3 Suppl):475S-490S.

(97) Potter JD. Colorectal cancer: molecules and populations. J Natl Cancer Inst 1999; 91(11):916-932.

(98) Riboli E, Norat T. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. Am J Clin Nutr 2003; 78(3 Suppl):559S-569S.

(99) Willett WC. Diet and cancer: one view at the start of the millennium. Cancer Epidemiol Biomarkers Prev 2001; 10(1):3-8.

(100) Terry P, Giovannucci E, Michels KB et al. Fruit, vegetables, dietary fiber, and risk of colorectal cancer. J Natl Cancer Inst 2001; 93(7):525-533.

(101) Schatzkin A, Kipnis V. Could exposure assessment problems give us wrong answers to nutrition and cancer questions? J Natl Cancer Inst 2004; 96(21):1564-1565.

(102) Willett W. Nutritional Epidemiology. Second Edition. Oxford University Press, editor. [30]. 1998. Monographs in Epidemiology and Biostatistic Volume 30.
Ref Type: Serial (Book,Monograph)

(103) Khosraviani K, Weir HP, Hamilton P, Moorehead J, Williamson K. Effect of folate supplementation on mucosal cell proliferation in high risk patients for colon cancer. Gut 2002; 51(2):195-199.

(104) Terry P, Jain M, Miller AB, Howe GR, Rohan TE. Dietary intake of folic acid and colorectal cancer risk in a cohort of women. Int J Cancer 2002; %20;97(6):864-867.

(105) Wei EK, Giovannucci E, Wu K et al. Comparison of risk factors for colon and rectal cancer. Int J Cancer 2004; 108(3):433-442.

(106) Konings EJ, Goldbohm RA, Brants HA, Saris WH, van den Brandt PA. Intake of dietary folate vitamers and risk of colorectal carcinoma: results from The Netherlands Cohort Study. Cancer 2002; 95(7):1421-1433.

(107) Sohn KJ, Stempak JM, Reid S, Shirwadkar S, Mason JB, Kim YI. The effect of dietary folate on genomic and p53-specific DNA methylation in rat colon. Carcinogenesis 2003; 24(1):81-90.

(108) Flood A, Caprario L, Chaterjee N, Lacey JV, Jr., Schairer C, Schatzkin A. Folate, methionine, alcohol, and colorectal cancer in a prospective study of women in the United States. Cancer Causes Control 2002; 13(6):551-561.

(109) Baylin SB, Herman JG, Graff JR, Vertino PM, Issa JP. Alterations in DNA methylation: a fundamental aspect of neoplasia. Adv Cancer Res 1998; 72:141-96.:141-196.

(110) Bollheimer LC, Buettner R, Kullmann A, Kullmann F. Folate and its preventive potential in colorectal carcinogenesis. How strong is the biological and epidemiological evidence? Crit Rev Oncol Hematol 2005; 55(1):13-36.

(111) Norat T, Riboli E. Dairy products and colorectal cancer. A review of possible mechanisms and epidemiological evidence. Eur J Clin Nutr 2003; 57(1):1-17.

(112) Benamouzig R, Chaussade S. Calcium supplementation for preventing colorectal cancer: where do we stand? Lancet 2004; 364(9441):1197-1199.

(113) Kesse E, Boutron-Ruault MC, Norat T, Riboli E, Clavel-Chapelon F. Dietary calcium, phosphorus, vitamin D, dairy products and the risk of colorectal adenoma and cancer among French women of the E3N-EPIC prospective study. Int J Cancer 2005; 117(1):137-144.

(114) Genkinger JM, Hunter DJ, Spiegelman D et al. Dairy products and ovarian cancer: a pooled analysis of 12 cohort studies. Cancer Epidemiol Biomarkers Prev 2006; 15(2):364-372.

(115) Cho E, Smith-Warner SA, Spiegelman D et al. Dairy foods, calcium, and colorectal cancer: a pooled analysis of 10 cohort studies. J Natl Cancer Inst 2004; 96(13):1015-1022.

(116) Lipkin M, Newmark H. Effect of added dietary calcium on colonic epithelial-cell proliferation in subjects at high risk for familial colonic cancer. N Engl J Med 1985; 313(22):1381-1384.

(117) Llor X, Jacoby RF, Teng BB, Davidson NO, Sitrin MD, Brasitus TA. K-ras mutations in 1,2-dimethylhydrazine-induced colonic tumors: effects of supplemental dietary calcium and vitamin D deficiency. Cancer Res 1991; 51(16):4305-4309.

(118) Van Der MR, Kleibeuker JH, Lapre JA. Calcium phosphate, bile acids and colorectal cancer. Eur J Cancer Prev 1991; 1 Suppl 2:55-62.:55-62.

(119) Wargovich MJ, Lynch PM, Levin B. Modulating effects of calcium in animal models of colon carcinogenesis and short-term studies in subjects at increased risk for colon cancer. Am J Clin Nutr 1991; 54(1 Suppl):202S-205S.

(120) Welberg JW, Kleibeuker JH, Van Der MR et al. Effects of oral calcium supplementation on intestinal bile acids and cytolytic activity of fecal water in patients with adenomatous polyps of the colon. Eur J Clin Invest 1993; 23(1):63-68.

(121) Alder RJ, Keown-Eyssen G, Bright-See E. Randomized trial of the effect of calcium supplementation on fecal risk factors for colorectal cancer. Am J Epidemiol 1993; 138(10):804-814.

(122) Govers MJ, Termont DS, Lapre JA, Kleibeuker JH, Vonk RJ, Van Der MR. Calcium in milk products precipitates intestinal fatty acids and secondary bile acids and thus inhibits colonic cytotoxicity in humans. Cancer Res 1996; 56(14):3270-3275.

(123) Lipkin M. Application of intermediate biomarkers to studies of cancer prevention in the gastrointestinal tract: introduction and perspective. Am J Clin Nutr 1991; 54(1 Suppl):188S-192S.

(124) Slattery ML, Berry TD, Potter J, Caan B. Diet diversity, diet composition, and risk of colon cancer (United States). Cancer Causes Control 1997; 8(6):872-882.

(125) DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986; 7(3):177-188.

(126) Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. Am J Epidemiol 1992; 135(11):1301-1309.

(127) Sugimura T, Wakabayashi K, Ohgaki H, Takayama S, Nagao M, Esumi H. Heterocyclic amines produced in cooked food: unavoidable xenobiotics. Princess Takamatsu Symp 1990; 21:279-288.

(128) Nagao M, Sugimura T. Carcinogenic factors in food with relevance to colon cancer development. Mutat Res 1993; 290(1):43-51.

(129) Knize MG, Salmon CP, Pais P, Felton JS. Food heating and the formation of heterocyclic aromatic amine and polycyclic aromatic hydrocarbon mutagens/carcinogens. Adv Exp Med Biol 1999; 459:179-193.

(130) Sinha R, Knize MG, Salmon CP et al. Heterocyclic amine content of pork products cooked by different methods and to varying degrees of doneness. Food Chem Toxicol 1998; 36(4):289-297.

(131) Sinha R, Rothman N. Exposure assessment of heterocyclic amines (HCAs) in epidemiologic studies. Mutat Res 1997; 376(1-2):195-202.

(132) Sinha R, Rothman N, Salmon CP et al. Heterocyclic amine content in beef cooked by different methods to varying degrees of doneness and gravy made from meat drippings. Food Chem Toxicol 1998; 36(4):279-287.

(133) Sinha R, Chow WH, Kulldorff M et al. Well-done, grilled red meat increases the risk of colorectal adenomas. Cancer Res 1999; 59(17):4320-4324.

(134) Sinha R, Kulldorff M, Curtin J, Brown CC, Alavanja MC, Swanson CA. Fried, well-done red meat and risk of lung cancer in women (United States). Cancer Causes Control 1998; 9(6):621-630.

(135) Ward MH, Sinha R, Heineman EF et al. Risk of adenocarcinoma of the stomach and esophagus with meat cooking method and doneness preference. Int J Cancer 1997; 71(1):14-19.

(136) Zheng W, Gustafson DR, Sinha R et al. Well-done meat intake and the risk of breast cancer. J Natl Cancer Inst 1998; 90(22):1724-1729.

(137) Delfino RJ, Sinha R, Smith C et al. Breast cancer, heterocyclic aromatic amines from meat and N-acetyltransferase 2 genotype. Carcinogenesis 2000; 21(4):607-615.

(138) Roberts-Thomson IC, Butler WJ, Ryan P. Meat, metabolic genotypes and risk for colorectal cancer. Eur J Cancer Prev 1999; 8(3):207-211.

(139) Mann N. Dietary lean red meat and human evolution. Eur J Nutr 2000; 39(2):71-79.

(140) Sugimura T. Nutrition and dietary carcinogens. Carcinogenesis 2000; 21(3):387-395.

(141) Krauss RM, Eckel RH, Howard B et al. AHA Dietary Guidelines: revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation 2000; 102(18):2284-2299.

(142) Bingham SA. High-meat diets and cancer risk. Proc Nutr Soc 1999; 58(2):243-248.

(143) Trichopoulou A, Lagiou P. The DAFNE food data bank as a tool for monitoring food availability in Europe. DAta Food NEtworking. Public Health Rev 1998; 26(1):65-71.

(144) Skog KI, Johansson MA, Jagerstad MI. Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. Food Chem Toxicol 1998; 36(9-10):879-896.

(145) Shirai T, Tamano S, Sano M, Masui T, Hasegawa R, Ito N. Carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in rats: dose-response studies. Princess Takamatsu Symp 1995; 23:232-239.

(146) Slimani N, Ferrari P, Ocke M et al. Standardization of the 24-hour diet recall calibration method used in the european prospective investigation into cancer and nutrition (EPIC): general concepts and preliminary results. Eur J Clin Nutr 2000; 54(12):900-917.

(147) Linseisen J, Kesse E, Slimani N et al. Meat consumption in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts: results from 24-hour dietary recalls. Public Health Nutr 2002; 5(6B):1243-1258.

(148) Ferrari P, Slimani N, Ciampi A et al. Evaluation of under- and overreporting of energy intake in the 24-hour diet recalls in the European Prospective Investigation into Cancer and Nutrition (EPIC). Public Health Nutr 2002; 5(6B):1329-1345.

(149) Norat T, Lukanova A, Ferrari P, Riboli E. Meat consumption and colorectal cancer risk: dose-response meta-analysis of epidemiological studies. Int J Cancer 2002; 98(2):241-256.

(150) Sandhu MS, White IR, McPherson K. Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analytical approach. Cancer Epidemiol Biomarkers Prev 2001; 10(5):439-446.

(151) Chao A, Thun MJ, Connell CJ et al. Meat consumption and risk of colorectal cancer. JAMA 2005; 293(2):172-182.

(152) Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997; 26 Suppl 1:S6-14.:S6-14.

(153) Slimani N, Kaaks R, Ferrari P et al. European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study: rationale, design and population characteristics. Public Health Nutr 2002; 5(6B):1125-1145.

(154) Kaaks R, Plummer M, Riboli E, Esteve J, van Staveren W. Adjustment for bias due to errors in exposure assessments in multicenter cohort studies on diet and cancer: a calibration approach. Am J Clin Nutr 1994; 59(1 Suppl):245S-250S.

(155) Kipnis V, Midthune D, Freedman L et al. Bias in dietary-report instruments and its implications for nutritional epidemiology. Public Health Nutr 2002; 5(6A):915-923.

(156) Kaaks R, Riboli E. Validation and calibration of dietary intake measurements in the EPIC project: methodological considerations. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997; 26 Suppl 1:S15-S25.

(157) Ferrari P, Day NE, Boshuizen HC et al. The evaluation of the diet/disease relations in the EPIC study: practical considerations for the calibration and the disease model. Int J Epidemiol 2006;(In press).

(158) Wattenberg L.W.. Inhibitors of chemical carcinogens. Burchenal J.H., Oettgen H.F., editors. [1], 517. 1981. New York, NY, Grune and Stratton. Cancer: achievements, challenges, and prospects for the 1980s.

(159) Albanes D, Heinonen OP, Huttunen JK et al. Effects of alpha-tocopherol and beta-carotene supplements on cancer incidence in the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study. Am J Clin Nutr 1995; 62(6 Suppl):1427S-1430S.

(160) DeCosse JJ, Miller HH, Lesser ML. Effect of wheat fiber and vitamins C and E on rectal polyps in patients with familial adenomatous polyposis. J Natl Cancer Inst 1989; 81(17):1290-1297.

(161) Ma J, Giovannucci E, Pollak M et al. Milk intake, circulating levels of insulin-like growth factor-I, and risk of colorectal cancer in men. J Natl Cancer Inst 2001; 93(17):1330-1336.

(162) English DR, MacInnis RJ, Hodge AM, Hopper JL, Haydon AM, Giles GG. Red meat, chicken, and fish consumption and risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2004; 13(9):1509-1514.

(163) Larsson SC, Rafter J, Holmberg L, Bergkvist L, Wolk A. Red meat consumption and risk of cancers of the proximal colon, distal colon and rectum: the Swedish Mammography Cohort. Int J Cancer 2005; 113(5):829-834.

(164) Sato Y, Nakaya N, Kuriyama S, Nishino Y, Tsubono Y, Tsuji I. Meat consumption and risk of colorectal cancer in Japan: the Miyagi Cohort Study. Eur J Cancer Prev 2006; 15(3):211-218.

(165) Oba S, Shimizu N, Nagata C et al. The relationship between the consumption of meat, fat, and coffee and the risk of colon cancer: A prospective study in Japan. Cancer Lett 2006.

(166) Norat T, Bingham S, Ferrari P et al. Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. J Natl Cancer Inst 2005; 97(12):906-916.

(167) Tiemersma EW, Kampman E, Bueno de Mesquita HB et al. Meat consumption, cigarette smoking, and genetic susceptibility in the etiology of colorectal cancer: results from a Dutch prospective study. Cancer Causes Control 2002; 13(4):383-393.

(168) Chomchai C, Bhadrachari N, Nigro ND. The effect of bile on the induction of experimental intestinal tumors in rats. Dis Colon Rectum 1974; 17(3):310-312.

(169) Preston-Martin S, Pike MC, Ross RK, Jones PA, Henderson BE. Increased cell division as a cause of human cancer. Cancer Res 1990; 50(23):7415-7421.

(170) Kaaks R, Lukanova A. Energy balance and cancer: the role of insulin and insulin-like growth factor-I. Proc Nutr Soc 2001; 60(1):91-106.

(171) Giovannucci E, Goldin B. The role of fat, fatty acids, and total energy intake in the etiology of human colon cancer. Am J Clin Nutr 1997; 66(6 Suppl):1564S-1571S.

(172) Rao CV, Hirose Y, Indranie C, Reddy BS. Modulation of experimental colon tumorigenesis by types and amounts of dietary fatty acids. Cancer Res 2001; 61(5):1927-1933.

(173) Zhou S, Wang G, Chen B, Wang P. Effect of dietary fatty acids on tumorigenesis of colon cancer induced by methyl nitrosourea in rats. J Environ Pathol Toxicol Oncol 2000; 19(1-2):81-86.

(174) Willett WC. Dietary fat intake and cancer risk: a controversial and instructive story. Semin Cancer Biol 1998; 8(4):245-253.

(175) Toyokuni S. Iron-induced carcinogenesis: the role of redox regulation. Free Radic Biol Med 1996; 20(4):553-566.

(176) Okada S. Iron-induced tissue damage and cancer: the role of reactive oxygen species-free radicals. Pathol Int 1996; 46(5):311-332.

(177) Boontaveeyuwat N, Klunklin S. The heme iron content of urban and rural Thai diets. J Med Assoc Thai 2001; 84(8):1131-1136.

(178) Lee DH, Anderson KE, Harnack LJ, Folsom AR, Jacobs DR, Jr. Heme iron, zinc, alcohol consumption, and colon cancer: Iowa Women's Health Study. J Natl Cancer Inst 2004; 96(5):403-407.

(179) Larsson SC, Adami HO, Giovannucci E, Wolk A. Re: Heme iron, zinc, alcohol consumption, and risk of colon cancer. J Natl Cancer Inst 2005; 97(3):232-233.

(180) Lin HC, Visek WJ. Colon mucosal cell damage by ammonia in rats. J Nutr 1991; 121(6):887-893.

(181) Cummings JH, Hill MJ, Bone ES, Branch WJ, Jenkins DJ. The effect of meat protein and dietary fiber on colonic function and metabolism. II. Bacterial metabolites in feces and urine. Am J Clin Nutr 1979; 32(10):2094-2101.

(182) Bingham SA, Pignatelli B, Pollock JR et al. Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? Carcinogenesis 1996; 17(3):515-523.

(183) Stewart M, Hill MJ, Pugh RC, Williams JP. The role of N-nitrosamine in carcinogenesis at the ureterocolic anastomosis. Br J Urol 1981; 53(2):115-118.

(184) Bogovski P, Bogovski S. Animal Species in which N-nitroso compounds induce cancer. Int J Cancer 1981; 27(4):471-474.

(185) Mirvish SS. Role of N-nitroso compounds (NOC) and N-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC [published erratum appears in Cancer Lett 1995 Nov 6;97(2):271]. Cancer Lett 1995; 93(1):17-48.

(186) Bos JL. ras oncogenes in human cancer: a review. Cancer Res 1989; 49(17):4682-4689.

(187) Bartsch H, Ohshima H, Munoz N et al. Assessment of endogenous nitrosation in humans in relation to the risk of cancer of the digestive tract. Dev Toxicol Environ Sci 1983; 11:299-309.

(188) Mirvish SS. Role of N-nitroso compounds (NOC) and N-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. Cancer Lett 1995; 93(1):17-48.

(189) Massey RC, Key PE, Mallett AK, Rowland IR. An investigation of the endogenous formation of apparent total N-nitroso compounds in conventional microflora and germ-free rats. Food Chem Toxicol 1988; 26(7):595-600.

(190) Calmels S, Ohshima H, Vincent P, Gounot AM, Bartsch H. Screening of microorganisms for nitrosation catalysis at pH 7 and kinetic studies on nitrosamine formation from secondary amines by E. coli strains. Carcinogenesis 1985; 6(6):911-915.

(191) Calmels S, Bereziat JC, Ohshima H, Bartsch H. Bacterial formation of N-nitroso compounds from administered precursors in the rat stomach after omeprazole-induced achlorhydria. Carcinogenesis 1991; 12(3):435-439.

(192) Pignatelli B, Malaveille C, Rogatko A et al. Mutagens, N-nitroso compounds and their precursors in gastric juice from patients with and without precancerous lesions of the stomach. Eur J Cancer 1993; 29A(14):2031-2039.

(193) Ohshima H, Bartsch H. Quantitative estimation of endogenous nitrosation in humans by monitoring N-nitrosoproline excreted in the urine. Cancer Res 1981; 41(9 Pt 1):3658-3662.

(194) Hughes R, Cross AJ, Pollock JR, Bingham S. Dose-dependent effect of dietary meat on endogenous colonic N-nitrosation. Carcinogenesis 2001; 22(1):199-202.

(195) Bingham SA, Hughes R, Cross AJ. Effect of White Versus Red Meat on Endogenous N-Nitrosation in the Human Colon and Further Evidence of a Dose Response. J Nutr 2002; 132(11):3522S-3525.

(196) Cross AJ, Pollock JR, Bingham SA. Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. Cancer Res 2003; 63(10):2358-2360.

(197) Wade RS, Castro CE. Redox reactivity of iron(III) porphyrins and heme proteins with nitric oxide. Nitrosyl transfer to carbon, oxygen, nitrogen, and sulfur. Chem Res Toxicol 1990; 3(4):289-291.

(198) Lewin MH, Bailey N, Bandaletova T et al. Red meat enhances the colonic formation of the DNA adduct O6-carboxymethyl guanine: implications for colorectal cancer risk. Cancer Res 2006; 66(3):1859-1865.

(199) Wakabayashi K, Totsuka Y, Fukutome K, Oguri A, Ushiyama H, Sugimura T. Human exposure to mutagenic/carcinogenic heterocyclic amines and comutagenic beta-carbolines. Mutat Res 1997; 376(1-2):253-259.

(200) Sinha R, Cross A, Curtin J et al. Development of a food frequency questionnaire module and databases for compounds in cooked and processed meats. Mol Nutr Food Res 2005; 49(7):648-655.

(201) Sinha R, Norat T. Meat and colon cancer. In: Colon Cancer: The Causes and Prevention of Cancer. 2003. Cancer causes and control.

(202) Lilla C, Verla-Tebit E, Risch A et al. Effect of NAT1 and NAT2 genetic polymorphisms on colorectal cancer risk associated with exposure to tobacco smoke and meat consumption. Cancer Epidemiol Biomarkers Prev 2006; 15(1):99-107.

(203) Tamer L, Ercan B, Ates NA et al. N-acetyltransferase 2 gene polymorphism in patients with colorectal carcinoma. Cell Biochem Funct 2006; 24(2):131-135.

(204) Butler LM, Duguay Y, Millikan RC et al. Joint effects between UDP-glucuronosyltransferase 1A7 genotype and dietary carcinogen exposure on risk of colon cancer. Cancer Epidemiol Biomarkers Prev 2005; 14(7):1626-1632.

(205) Le Marchand L, Hankin JH, Pierce LM et al. Well-done red meat, metabolic phenotypes and colorectal cancer in Hawaii. Mutat Res 2002; 506-507:205-214.

(206) Chen J, Stampfer MJ, Hough HL et al. A prospective study of N-acetyltransferase genotype, red meat intake, and risk of colorectal cancer. Cancer Res 1998; 58(15):3307-3311.

(207) Sachse C, Smith G, Wilkie MJ et al. A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. Carcinogenesis 2002; 23(11):1839-1849.

(208) Roynette CE, Calder PC, Dupertuis YM, Pichard C. n-3 polyunsaturated fatty acids and colon cancer prevention. Clin Nutr 2004; 23(2):139-151.

(209) Nkondjock A, Shatenstein B, Maisonneuve P, Ghadirian P. Specific fatty acids and human colorectal cancer: an overview. Cancer Detect Prev 2003; 27(1):55-66.

(210) Hendrickse CW, Keighley MR, Neoptolemos JP. Dietary omega-3 fats reduce proliferation and tumor yields at colorectal anastomosis in rats. Gastroenterology 1995; 109(2):431-439.

(211) Latham P, Lund EK, Johnson IT. Dietary n-3 PUFA increases the apoptotic response to 1,2-dimethylhydrazine, reduces mitosis and suppresses the induction of carcinogenesis in the rat colon. Carcinogenesis 1999; 20(4):645-650.

(212) Sano H, Kawahito Y, Wilder RL et al. Expression of cyclooxygenase-1 and -2 in human colorectal cancer. Cancer Res 1995; 55(17):3785-3789.

(213) Eberhart CE, DuBois RN. Eicosanoids and the gastrointestinal tract. Gastroenterology 1995; 109(1):285-301.

(214) Sheng H, Shao J, Morrow JD, Beauchamp RD, DuBois RN. Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. Cancer Res 1998; 58(2):362-366.

(215) Sheehan KM, Sheahan K, O'Donoghue DP et al. The relationship between cyclooxygenase-2 expression and colorectal cancer. JAMA 1999; 282(13):1254-1257.

(216) Anti M, Marra G, Armelao F et al. Effect of omega-3 fatty acids on rectal mucosal cell proliferation in subjects at risk for colon cancer. Gastroenterology 1992; 103(3):883-891.

(217) Anti M, Armelao F, Marra G et al. Effects of different doses of fish oil on rectal cell proliferation in patients with sporadic colonic adenomas. Gastroenterology 1994; 107(6):1709-1718.

(218) Cheng J, Ogawa K, Kuriki K et al. Increased intake of n-3 polyunsaturated fatty acids elevates the level of apoptosis in the normal sigmoid colon of patients polypectomized for adenomas/tumors. Cancer Lett 2003; 193(1):17-24.

(219) Slattery ML, Potter JD, Duncan DM, Berry TD. Dietary fats and colon cancer: assessment of risk associated with specific fatty acids. Int J Cancer 1997; 73(5):670-677.

(220) Hooper L, Thompson RL, Harrison RA et al. Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. BMJ 2006; 332(7544):752-760.

(221) World Health Organization (WHO). The World Health Report. Geneva (Switzerland):WHO 1997.

(222) Block G. A review of validations of dietary assessment methods. Am J Epidemiol 1982; 115(4):492-505.

(223) Medlin C, Skinner JD. Individual dietary intake methodology: a 50-year review of progress. J Am Diet Assoc 1988; 88(10):1250-1257.

LISTE DES ANNEXES

Les fibres alimentaires comme facteur de protection contre le cancer colorectal dans l'Etude Prospective Européenne sur la Nutrition et le Cancer (EPIC). *Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. Lancet 2003; 361: 1496-501*

La consommation de noix et le risque de cancer colorectal dans l'Etude Prospective Européenne sur la Nutrition et le Cancer (EPIC). *Association of nut and seed intake with colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). Cancer Epidemiol Biomarkers Prev 2004; 13(10);1595-603*

Le folate, agit-il comme facteur de confusion de la relation entre les fibres alimentaires et le cancer colorectal ? *Is the association of fibre from foods in colorectal cancer confounded by folate intake? Cancer Epidemiol Biomarkers Prev 2005; 14(6);1552-6*

Corpulence physique et risque de cancer du côlon et du rectum dans l'Etude Prospective Européenne sur la Nutrition et le Cancer (EPIC). *Body size and risk of côlon and rectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). J Natl Cancer Inst 2006; 98:920-31*

Activité physique et risque de cancer du côlon et du rectum : l'Etude Prospective Européenne sur la Nutrition et le Cancer (EPIC). *Physical activity and risk of colon and rectal cancers: the European prospective investigation into cancer and nutrition. Cancer Epidemiol Biomarkers Prev. 2006 ;15(12):2398-407*

Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study

Sheila A Bingham, Nicholas E Day, Robert Luben, Pietro Ferrari, Nadia Slimani, Teresa Norat, Françoise Clavel-Chapelon, Emmanuelle Kesse, Alexandra Nieters, Heiner Boeing, Anne Tjønneland, Kim Overvad, Carmen Martinez, Miren Dorronsoro, Carlos A Gonzalez, Timothy J Key, Antonia Trichopoulou, Androniki Naska, Paolo Vineis, Rosario Tumino, Vittorio Krogh, H Bas Bueno-de-Mesquita, Petra HM Peeters, Göran Berglund, Göran Hallmans, Eiliv Lund, Guri Skeie, Rudolf Kaaks, Elio Riboli

Summary

Background Dietary fibre is thought to protect against colorectal cancer but this view has been challenged by recent prospective and intervention studies that showed no protective effect.

Methods We prospectively examined the association between dietary fibre intake and incidence of colorectal cancer in 519 978 individuals aged 25–70 years taking part in the EPIC study, recruited from ten European countries. Participants completed a dietary questionnaire in 1992–98 and were followed up for cancer incidence. Relative risk estimates were obtained from fibre intake, categorised by sex-specific, cohort-wide quintiles, and from linear models relating the hazard ratio to fibre intake expressed as a continuous variable.

Findings Follow-up consisted of 1 939 011 person-years, and data for 1065 reported cases of colorectal cancer were included in the analysis. Dietary fibre in foods was inversely related to incidence of large bowel cancer (adjusted relative risk 0·75 [95% CI 0·59–0·95] for the highest versus lowest quintile of intake), the protective effect being greatest for the left side of the colon, and least for the rectum. After calibration with more detailed dietary data, the adjusted relative risk for the highest versus lowest quintile of fibre from food intake was 0·58 (0·41–0·85). No food source of fibre was significantly more protective than others, and non-food supplement sources of fibre were not investigated.

Interpretation In populations with low average intake of dietary fibre, an approximate doubling of total fibre intake from foods could reduce the risk of colorectal cancer by 40%.

Lancet 2003; **361:** 1496–501
See Commentary page 1487

MRC Dunn Human Nutrition Unit, Cambridge, UK (S A Bingham PhD); **Strangeways Research Laboratory, University of Cambridge, Cambridge** (Prof N E Day PhD, R Luben BSc); **International Agency for Research on Cancer (IARC-WHO), Lyon, France** (P Ferrari PhD, N Slimani PhD, T Norat PhD, R Kaaks PhD, E Riboli MD); **INSERM, U 521, Institut Gustave Roussy, Villejuif** (F Clavel-Chapelon PhD, E Kesse PhD) **German Cancer Research Centre, Heidelberg, Germany** (A Nieters PhD); **German Institute of Human Nutrition, Potsdam-Rehbücke** (H Boeing PhD); **Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark** (A Tjønneland MD); **Department of Clinical Epidemiology, Aalborg Hospital and Aarhus University Hospital, and Department of Epidemiology and Social Medicine, University of Aarhus, Denmark** (K Overvad MD); **Andalusian School of Public Health, Granada, Spain** (C Martinez MD); **Department of Public Health of Guipuzkoa, San Sebastian, Spain** (M Dorronsoro MD); **Catalan Institute of Oncology, Barcelona** (C A Gonzalez PhD); **Cancer Research UK, Radcliffe Infirmary, Oxford, UK** (T J Key PhD) **University of Athens Medical School, Greece** (Prof A Trichopoulou MD, A Naska PhD); **University of Torino and CPO-Piemonte, Torino, Italy** (P Vineis MD); **Ragusa Cancer Registry, Sicily** (R Tumino MD); **Department of Epidemiology, National Cancer Institute, Milan** (V Krogh MD); **National Institute of Public Health and the Environment, Bilthoven, Netherlands** (H B Bueno-de-Mesquita MD); **Julius Centre for Health Sciences and Primary Care, University Medical Center, Netherlands** (P H M Peeters MD); **Malmö Diet and Cancer Study, Lund University, Malmö, Sweden** (Prof G Berglund MD); **Department of Nutritional Research, University of Umeå, Sweden** (Prof G Hallmans MD); **and Institute of Community Medicine, University of Tromsø, Norway** (Prof E Lund MD, G Skeie MSc)

Correspondence to: Dr Elio Riboli, Unit of Nutrition and Cancer, International Agency for Research on Cancer (IARC-WHO), 150 Cours Albert-Thomas, 69372 Lyon cedex 08, France
(e-mail: ntr@iarc.fr)

Introduction

Whether dietary fibre (non-starch polysaccharides) lowers the risk of colorectal cancer is debatable. In reports from large prospective studies in the USA, Finland, and Sweden, no protective effects of fibre were seen.[1–3] In addition, results of large intervention trials have shown that supplements of bran, soluble fibre, or vegetables have not reduced recurrence rates of adenomatous colorectal polyps.[4–6] Death rates for colorectal cancer in vegetarians are no different from those in non-vegetarians.[7]

These findings of no effect have challenged consensus recommendations, drawn from a large body of epidemiological and experimental findings, that population intakes of fibre should be increased to reduce the risk of colorectal cancer.[8,9] However, all prospective studies of diet and cancer have been done in single populations in whom dietary habits are more or less homogeneous, so that the extent of measurement error would have obscured all but very large underlying associations with diet.[10,11] Measurement error can be reduced by studying populations with diverse dietary practices, thus increasing the between-person variance in diet, and enabling measurement error to be kept to a minimum.[10] Such was the approach behind the large prospective collaborative project done in ten different European countries, the European Prospective Investigation of Cancer and Nutrition (EPIC).[12] Other reports[13,14] have shown the heterogeneity of dietary intakes of foods supplying dietary fibre in this collaborative cohort. For example, there is over a three-fold range in total average population consumption of fruit and vegetables (excluding potatoes) between centres in Sweden and in southern Spain.[13,14]

Methods

Participants

The EPIC cohort consists of subcohorts recruited from 22 centres from Denmark, France, Germany, Greece,

Italy, the Netherlands, Norway, Spain, Sweden, and the UK, allowing comparisons between regions with very different rates of cancer occurrence and distribution of lifestyle and food habits. We administered food-related questionnaires and lifestyle and personal questionnaires and obtained anthropometric measurements from all participants at the time of enrolment. The methods have been reported in full by Riboli and colleagues.[15]

The 519 978 eligible participants were mostly aged 25–70 years and recruited from the general population residing in a specific geographical region—a town or a province. Exceptions were the French cohort (based on female members of the health insurance for state school employees), the Utrecht cohort, Netherlands (based on women attending breast cancer screening), the Ragusa cohort, Italy (based on blood donors and their spouses), and most of the Oxford cohort, UK (based on vegetarian and health-conscious volunteers).

Procedures

As a result of several studies done in the early 1990s,[12] we measured diet by country-specific questionnaires designed to capture local dietary habits and to provide high compliance. For calibration, we obtained a second dietary measurement from an 8% random sample (36 000 individuals) of the cohort using a computerised 24-h diet recall method developed ad hoc.[16] The extensive lifestyle questionnaires included questions on education and socioeconomic status, occupation, history of previous illness and disorders or surgical operations, lifetime history of consumption of tobacco and alcoholic beverages, and physical activity.

Follow-up was based on population cancer registries in seven of the participating countries: Denmark, Italy, the Netherlands, Spain, Sweden, the UK, and Norway. In France, Germany, and Greece, we used a combination of methods including health insurance records, cancer and pathology registries, and active follow-up through participants and their next-of-kin. Mortality data were also obtained from either the cancer registry or mortality registries at the regional or national level. By June, 2002, for all centres using cancer registry data, reports to the IARC represented complete follow-up until December, 1998, or December, 1999. For France and Germany—countries using individually based follow-up—the end of the follow-up was 2002, when the last known contact, or date of diagnosis, or death was available. We included the results from all centres except for Greece and Norway (because of the small numbers of cases accruing there from a short follow-up time).

We used the 10th revision of the International Statistical Classification of Diseases, Injuries and Causes of Death (ICD). Mortality data were coded following the rules of ICD-10, and cancer incidence data following ICD-0-2. Cancer of the rectum included tumours in the rectosigmoid junction (C19) and rectum (C20). We excluded anal canal tumours. Right colon tumours included the caecum, appendix, ascending colon, hepatic flexure, transverse colon, and splenic flexure (C18.0–18.5). Left colon tumours included the descending and sigmoid colon (C18.6–18.7). We included all incident cases of colorectal cancer ICD-0-2 C18, C19, C20 with dietary data for the period of complete follow-up, but excluded prevalent cases.

Statistical analysis

To convert the weight of foods derived from the food questionnaires to daily fibre intakes, participating countries used country-specific food tables, incorporating analyses for dietary fibre or plant polysaccharides, which are based on different definitions and analytical techniques and give different values for common foods.[17] To assess the possible effects of sex and different analytical procedures for fibre analysis, we used sex-specific, and sex-and-country-specific quintiles of dietary fibre in the analysis. Daily fibre intake—derived from the Englyst and the Southgate methods[18]—was calculated in the UK cohorts. There was no significant difference between the estimates of relative risk for colorectal cancer using these methods, and we used sex-specific quintiles and the Englyst non-starch polysaccharide values for the UK cohorts. Data for individuals in the top and bottom 1% of the ratio of energy intake to estimated energy requirement (calculated from age, sex, and bodyweight)[19] and from the top 1% of sex-specific fibre intakes, were excluded from the analysis to reduce the effect on the analysis of implausible extreme values.

Analyses were done with Cox's regression and were stratified by centre to control for centre effects related to different methods of fibre analysis,[17] follow-up procedures, and questionnaire design. The 20 French administrative regions were condensed into four geographical regions (northeast, northwest, south, and south coast). The five Italian centres were combined into one, as were the four Spanish centres, because of small numbers of cases in each centre. The UK Oxford centre was divided into two, for general population and health conscious participants. Age was used as the primary time variable in all Cox's regression models, with year of follow-up and sex included as covariates. The analysis focused on dietary fibre—with other dietary variables included as covariates—and anthropometric variables. These quantitative variables were categorised according to sex-specific quintiles defined over the entire cohort. We analysed the data using variables as categorical and as continuous, scored from 1 to 5 according to the interquintile interval in which an observation lay. Trend tests were calculated on the basis of these quintile based scores. Categorical relative risks were calculated from the hazard ratio.[20]

For the purpose of making isoenergetic comparisons, we included weight in all models. Estimated total energy intake was included to control partly for the error in the estimated intake of fibre, since there is high correlation between the errors of estimation of different dietary components. To improve this error correction, estimated energy intake was divided into energy from fat and energy from non-fat sources, since it is mostly the non-fat components of the diet that contribute to fibre intake. Models were run including non-fat energy and with and without energy from fat, and the results did not differ; models including both non-fat and fat energy were included in the results. Partial correlations (adjusting for age and centre) for fibre with energy excluding fat were 0·473 in men and 0·529 in women, and with energy from fat were 0·037 in men and –0·030 in women. Smoking and physical exercise had no significant effect, and these were therefore not included in the models. To control for body size and obesity, we included weight and height in the models.

	Large bowel cancer cases			Cohort numbers			Fibre intake (g per day)		
	Men	Women	All	Men	Women	All	Men	Women	All
Country									
France	0	166	166	0	71 874	71 874	.. (..)	22·92 (6·94)	22·92 (6·94)
Italy	35	49	84	13 508	30 489	43 997	24·93 (9·12)	22·02 (7·29)	22·92 (8·01)
Spain	41	33	74	15 025	24 910	39 935	29·12 (8·34)	23·35 (7·08)	25·52 (8·08)
UK	94	112	206	23 905	55 957	79 862	20·00 (7·50)	20·39 (7·06)	20·27 (7·20)
Netherlands	14	86	100	9 998	28 913	38 911	26·77 (7·61)	22·55 (5·49)	23·63 (6·38)
Germany	59	34	93	22 137	29 354	51 491	23·83 (7·51)	21·55 (6·41)	22·53 (7·00)
Sweden	110	122	232	22 840	29 519	52 359	21·33 (7·78)	19·08 (6·47)	20·06 (7·16)
Denmark	58	52	110	26 599	29 181	55 780	20·48 (6·70)	19·51 (6·51)	19·97 (6·62)
Age (years)									
<35	1	0	1	7 637	18 404	18 404	23·32 (8·73)	20·53 (7·13)	21·35 (7·74)
35–44	13	21	34	22 172	53 336	53 336	24·67 (8·72)	21·85 (7·02)	22·68 (7·67)
45–54	91	188	279	48 118	118 198	118 198	23·08 (8·35)	21·59 (6·90)	22·02 (7·38)
55–64	225	300	525	46 853	90 319	90 319	22·48 (7·86)	21·51 (6·80)	21·84 (7·20)
⩾65	81	145	226	9 232	19 940	19 940	20·64 (7·29)	21·09 (6·74)	20·95 (6·92)
All	411	654	1065	134 012	300 197	300 197	22·98 (8·26)	21·52 (6·90)	21·97 (7·38)

Values are number of participants or mean (SD).

Table 1: **Description of the EPIC cohort**

To correct for centre-specific bias and for regression dilution within each centre-sex stratum, the 24-h recall nutrient values were regressed on the main study dietary questionnaire values, within each centre, for men and women separately, thus providing sex-specific and centre-specific regression coefficients.[21,22] This analysis was a multivariate procedure, including fibre, energy from fat, and energy from non-fat sources simultaneously. For every individual in the main study, we obtained adjusted values of these three variables by applying both the intercept and slope variables of the appropriate sex-specifc and centre-specific regression to the main study questionnaire values: adjusted value=intercept+slope (observed questionnaire value). Use of both the intercept and slope variables, age-specific and centre-specific, ensured that centre-specific bias was addressed, as was that of regression dilution. We then ran Cox's regression models with the adjusted values for each individual. The SE of the de-attenuated coefficient was calculated with bootstrap sampling in the calibration and disease models consecutively.[23] 300 repetitions gave a sufficiently stable estimate of the SE of the corrected regression coefficient. The p value for trend for the de-attenuated β was obtained by dividing the de-attenuated β by the bootstrap-derived SE approximating the standard normal distribution.

Role of the funding source

EPIC was funded jointly by the European Commission funding authorities listed in the acknowledgments. These funding authorities agreed to fund our proposed design and running of the study, but had no role in the subsequent management of the study or in the writing of the report. The report was not submitted to them for approval before publication.

Results

Since 1992, we have obtained 1 939 011 person-years (average 4·5 years) of follow-up. Our study is based on 1065 incident colorectal cancer cases with complete and satisfactory data as described above. Of these, 706 tumours were located in the colon (287 right side, 286 left side, 133 overlapping or unspecified) and 359 in the rectum. Histological confirmation was available for 1035 cases. Table 1 shows numbers of colorectal cancer tumours according to country, age and sex, and country-specific, sex-specific, and age-specific intakes of fibre.

Table 2 shows baseline characteristics by sex-specific quintiles of fibre intake. Height, weight, and body-mass index did not differ by much across quintiles despite some significant trends due to the very large sample size. Across the quintiles of fibre there was a significant

	Quintile					p
	1	2	3	4	5	
Women						
Fibre (g per day)	12·64 (2·27)	17·45 (1·03)	20·89 (0·99)	24·69 (1·28)	31·91 (3·97)	..
Number in each quintile	60 043	60 074	60 003	60 038	60 039	..
Age (years)	50·98 (10·66)	51·37 (10·09)	51·51 (9·93)	51·32 (9·85)	51·04 (9·77)	0·588
Weight (kg)	65·35 (11·75)	65·74 (11·70)	65·70 (11·56)	65·71 (11·52)	65·69 (11·71)	<0·0001
Height (m)	1·62 (0·07)	1·62 (0·07)	1·62 (0·07)	1·62 (0·07)	1·62 (0·07)	<0·0001*
Body-mass index (kg/m^2)	24·98 (4·38)	25·06 (4·35)	25·00 (4·32)	24·97 (4·34)	25·01 (4·48)	0·570
Energy from fat (MJ)	2·40 (0·79)	2·73 (0·85)	2·94 (0·91)	3·17 (0·98)	3·52 (1·12)	<0·0001
Non-fat energy (MJ)	4·16 (1·04)	4·85 (1·07)	5·33 (1·14)	5·83 (1·22)	6·72 (1·45)	<0·0001
Men						
Fibre (g per day)	12·77 (2·41)	18·03 (1·17)	21·97 (1·15)	26·51 (1·55)	35·61 (5·30)	..
Number in each quintile	26 810	26 796	26 806	26 798	26 802	..
Age (years)	53·23 (10·22)	53·38 (9·75)	52·79 (9·80)	51·94 (9·89)	50·35 (9·88)	<0·0001
Weight (kg)	81·09 (12·10)	81·45 (11·99)	81·29 (11·81)	81·17 (11·77)	80·86 (11·87)	0·237
Height (m)	1·75 (0·07)	1·75 (0·07)	1·75 (0·07)	1·75 (0·07)	1·75 (0·08)	<0·0001*
Body-mass index (kg/m^2)	26·42 (3·59)	26·50 (3·56)	26·45 (3·57)	26·44 (3·62)	26·56 (3·72)	0·0002
Energy from fat (MJ)	2·88 (0·96)	3·30 (1·02)	3·59 (1·12)	3·88 (1·20)	4·29 (1·38)	<0·0001
Non-fat energy (MJ)	5·26 (1·44)	6·11 (1·40)	6·69 (1·46)	7·33 (1·56)	8·38 (1·82)	<0·0001

Values are mean (SD). *Differences were highly significant but in the order of 3 mm across quintile 1–5.

Table 2: **Baseline characteristics by quintile of fibre intake**

	Quintile					Hazard ratio (95% CI) for each quintile increase	p
	1	2	3	4	5		
Colorectal cancers							
Number	237	234	200	200	194	..	
Hazard ratio (95% CI)	1·00	0·94 (0·78–1·13)	0·77 (0·63–0·95)	0·76 (0·61–0·95)	0·75 (0·59–0·95)	0·923 (0·873–0·976)	0·005
Colon cancers							
Number	156	158	131	130	131	..	
Hazard ratio (95% CI)	1·00	0·95 (0·75–1·19)	0·75 (0·58–0·96)	0·71 (0·55–0·94)	0·72 (0·54–0·97)	0·908 (0·848–0·972)	0·006
Left colon cancer (n=286)	1·00	0·66 (0·46–0·93)	0·55 (0·37–0·80)	0·51 (0·34–0·77)	0·65 (0·43–0·99)	0·891 (0·804–0·989)	0·030
Right colon cancer (n=287)	1·00	1·21 (0·84–1·71)	0·93 (0·63–1·37)	0·89 (0·59–1·35)	0·73 (0·46–1·19)	0·911 (0·819–1·013)	0·087
Rectal cancers							
Number	81	76	69	70	63	..	
Hazard ratio (95% CI)	1·00	0·92 (0·66–1·27)	0·83 (0·59–1·18)	0·85 (0·59–1·24)	0·80 (0·53–1·22)	0·952 (0·864–1·048)	0·319

Cox's regression using age, weight, height, sex, non-fat energy, energy from fat, and stratified by centre.

Table 3: **Numbers of cancers and hazard ratios by quintile of sex-specific total fibre intake**

trend with energy derived from non-fat sources. This trend reflected the contribution of the non-fat components of the diet that contribute to fibre intake, such as cereals and fruit. The trend with fat energy was also significant but less pronounced, reflecting lower correlations between fat energy and fibre.

Table 3 shows the number of cancers by quintile and the hazard ratios. The hazard ratio for colorectal cancer for the highest quintile was 0·75 (95% CI 0·59–0·95)—a significant reduction in risk. The trend in hazard ratio across quintiles was also significant (p=0·005), the regression coefficient (β=0·080) predicting an 8% reduction in risk for each quintile increase in fibre. These associations did not significantly differ when analysed according to less than 2, 2–4, and more than 4 years of follow-up. Trend effects were significant for colon cancer (p=0·006) and the reduction in hazard ratio in the top quintile was also significant, 0·72 (0·54–0·97). Neither the trend across quintiles nor the hazard ratio in the highest quintile showed significant differences for rectal cancer (0·80; 0·53–1·22). The protective effect of fibre was no greater for the left-sided colon (0·65; 0·43–0·99) than the right-sided colon (0·73; 0·46–1·19). Further analyses showed that fibre was equally protective in women and men and that adjustment for red and processed meat did not affect these results (data not shown).

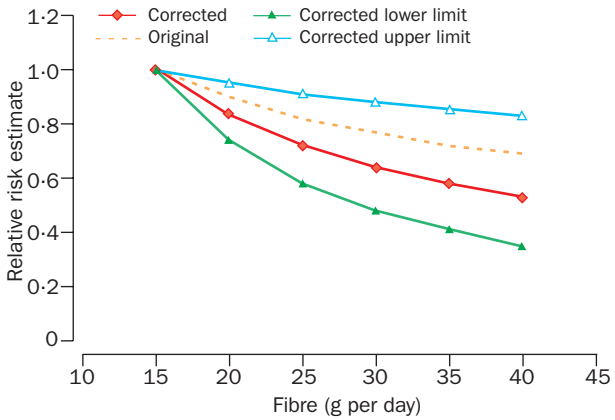


Figure 1: **Relative risk for colorectal cancer according to dietary fibre intake**
Calculated from Cox's regression using age, weight, height, sex, non-fat energy, energy from fat. Original estimates are calculated from the hazard ratio[20] for each quintile increase in energy adjusted fibre (table 3).

Figure 1 shows the continuous and de-attenuated risks for colorectal cancer according to the energy adjusted values of total fibre intake. The β coefficient estimate for log fibre intake based on the observed values was –0·38 (SE 0·13, p=0·002), whereas that for the calibrated values was –0·64 (SE 0·23, p=0·005).

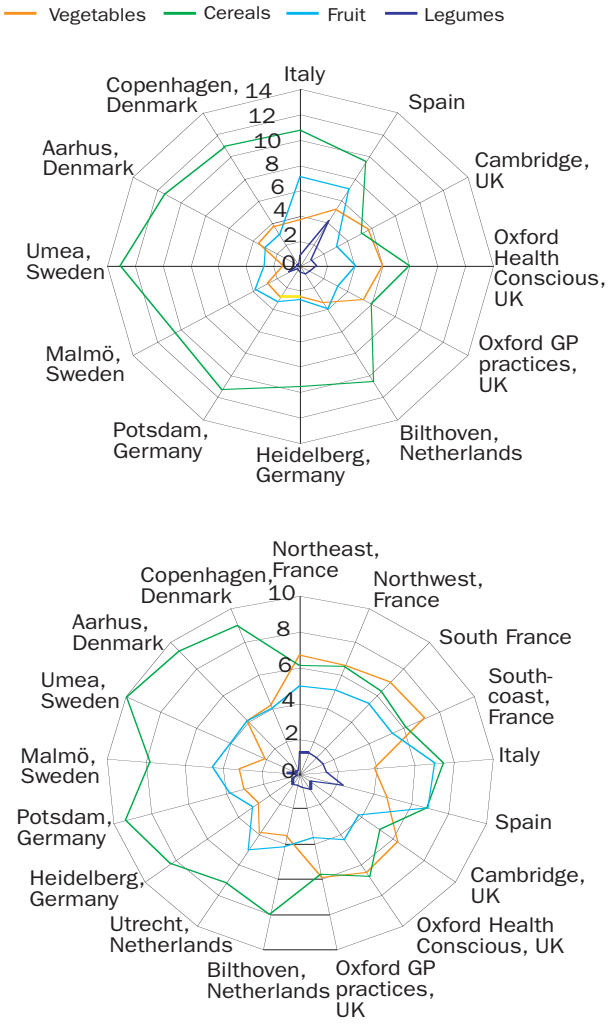


Figure 2: **Average dietary fibre intake in g per day from different food sources for men (upper) and women (lower)**
Calculated from the dietary questionnaires for each of the centres used in this analysis. The vegetables category excludes potatoes, legumes, soy products, and tomato products. The cereals category excludes cakes and biscuits.

	Quintile					Hazard ratio for each quintile increase	p
	1	2	3	4	5		
Cereal fibre (g)	4·72 (2·28)	6·61 (2·82)	7·93 (3·31)	9·35 (3·91)	12·05 (5·71)	..	
Hazard ratio (95% CI)	1·00	0·89 (0·74–1·08)	0·85 (0·69–1·03)	0·88 (0·71–1·08)	0·78 (0·62–0·98)	0·950 (0·901–1·002)	0·060
Vegetable fibre (g)	2·83 (1·72)	3·77 (2·10)	4·42 (2·42)	5·11 (2·81)	6·48 (3·85)	..	
Hazard ratio (95% CI)	1·00	0·94 (0·77–1·15)	0·95 (0·77–1·16)	1·00 (0·81–1·24)	0·88 (0·70–1·11)	0·983 (0·932–1·035)	0·517
Legumes fibre (g)	0·45 (0·68)	0·65 (0·92)	0·85 (1·14)	1·14 (1·47)	1·73 (2·17)	..	
Hazard ratio (95% CI)	1·00	1·02 (0·83–1·26)	1·10 (0·91–1·34)	1·18 (0·97–1·43)	1·04 (0·84–1·30)	1·025 (0·976–1·077)	0·311
Fruit fibre (g)	2·21 (1·56)	3·41 (2·00)	4·29 (2·38)	5·36 (2·87)	7·76 (4·40)	..	
Hazard ratio (g)	1·00	0·69 (0·57–0·85)	0·76 (0·63–0·92)	0·82 (0·66–0·99)	0·78 (0·64–0·97)	0·967 (0·922–1·015)	0·174

Analyses are done with Cox's regression using age, weight, height, sex, non-fat energy, energy from fat, and stratified by centre.

Table 4: **Intakes of fibre and colorectal cancer by quintile of sex-specific source of fibre intake**

This linear decrease in risk corresponds to a relative risk of 0·58 (0·41–0·85) for colorectal cancer incidence at a mean of 35 g dietary fibre (the highest quintile) compared with the baseline mean fibre intake of 15 g in the lowest quintile. Further analysis showed that adjustment for alcohol did not affect these results (data not shown).

Figure 2 shows sources of fibre intake in men and women by the centres used in this analysis. Cereals were the main sources of fibre in the Netherlands, Germany, Sweden, and Denmark, whereas vegetables were most important in France and the UK. Fruit was an important source of fibre in Italy and Spain. Legumes and potatoes contributed small amounts and were more important in Spain, the Netherlands, and Denmark than in other regions, but consumption in other regions averaged 2 g or less. Mean fibre from cakes and biscuits, tomato sauces, and soya products averaged less than 1 g per day.

Table 4 shows that effects for food sources of fibre were not as consistent as those for total fibre. The trends in risks associated with cereal fibre, and fruit, legume, and vegetable sources of fibre were not significant, nor did they differ significantly from each other. Analyses for fibre from cakes and biscuits, potatoes, tomato pastes, and soya products were also not significant.

Discussion

Fibre is one of the most important, if controversial, factors thought to prevent colorectal cancer, with well established biological mechanisms underlying the hypothesis. When entering the large bowel, fibre increases stool weight, reduces transit time, dilutes colonic contents, and stimulates bacterial anaerobic fermentation. This process reduces contact between the intestinal contents and mucosa, and leads to production of short-chain fatty acids, acetate, propionate, and butyrate, which reduce pH and the conversion of primary to secondary bile acids.[24] Butyrate is a major source of energy for the distal colon and in cell lines it reduces cell proliferation and induces apoptosis, factors that are associated with inhibition of the transformation of the colonic epithelium to carcinoma.[24–27]

Our results showed that total dietary fibre consumption was inversely associated with colorectal cancer risk. However, we only studied fibre in foods. Foods supplying fibre also contribute many other nutrients and phytochemicals that have been linked to cancer protection and which could account for the protective effects seen.[8,9] Thus, our results cannot be extrapolated to any potential benefit of dietary supplements or additives containing fibre alone. The association was stronger for colon cancer, especially left-sided colon cancer, than for rectal cancer. The fact that the association was mainly with colon cancer rather than rectal cancer might be expected, since the rectum is empty most of the time,[28] reducing the diluting, antiproliferative, and nutritive effects attributed to fibre.

When fibre intake from the dietary questionnaire was calibrated with intake from the 24-h recall, the Cox's proportional hazard model showed an even greater reduction in risk, with a 40% reduction of risk when average intakes increased from the lowest to highest quintile of fibre intake (figure 1). CIs around the estimates are large, so that the risk may have been reduced by as little as 15 or as great as 60%. Even a moderate increase in fibre intake by those consuming the least in most populations would have significant effects on cancer prevention, and supports recommendations to increase population intakes of fibre in those populations consuming low amounts to reduce high rates of colorectal cancer.[8,9] Although minor differences in hazard ratios were seen between different sources of fibre, they were not significant, so that, on the basis of current data, no firm statement can be made as to whether any one source of dietary fibre is more protective than others.

Three other epidemiological studies have investigated prospectively the role of fibre in colon cancer protection.[1–3] However, only the US Nurses Health Study,[1] was based on a number of colorectal cancer cases as large as in the EPIC study, allowing separate analyses of colon and rectal cancers, and for multivariate analysis including several potential confounding factors. However, in the US nurses cohort, no associations between fibre intake from any source and colon and rectal cancers or adenomas were identified. This difference might reflect lower and less varied fibre intakes in the US Nurses cohort than in our cohort. In EPIC, the mean values in the lowest and highest fifth of the distribution of daily fibre intake are 12·6 g (SD 2·3) and 33·1 g (14·7) respectively. The corresponding figures for the US Nurses study are 9·8 g (1·7) to 24·9 g (5·5) so that about 30% of the EPIC cohort were consuming substantially more fibre than the mean of the highest quintile of the US Nurses study. Cereal fibre is very low in the US Nurses study, the median in the highest fifth of the distribution (4·8 g per day) approximating the mean in the lowest fifth in EPIC (4·7 g per day; 2·28).

Our findings for the potential for reducing colorectal cancer incidence by increasing fibre intake as foods have been adjusted for measurement error by calibrating intake obtained from the food questionnaire against a method that measures specific intake over a single day. We have used this information to calibrate both the

centre means and to deattenuate the individual values on a sex-specific and centre-specific basis by standard methods;[21,22] the crude risks underestimated the protective effects of fibre on colorectal cancer risks when compared with the corrected risks (figure 1). However even the deattenuated risks are probably underestimated since these standard methods assume absence of correlations of errors between the reference and the food intake questionnaire.[10,11] Furthermore, our results are for a limited period of follow-up and the association could strengthen when longer exposure times are assessed. The potential for protection by fibre from foods in populations with current low intakes might therefore be even greater than our findings, which predict a 40% reduction in colorectal cancer risk when intakes of fibre are approximately doubled.

Contributors

E Riboli is overall coordinator of the EPIC study, which he designed and implemented in collaboration with R Kaaks, N Slimani, and the main investigators in the collaborating centres: N E Day, S A Bingham, F Clavel-Chapelon, H Boeing, A Tjønneland, K Overvad, C Martinez, M Dorronsoro, C A Gonzalez, T J Key, A Trichopoulou, P Vineis, R Tumino, H B Bueno de Mesquita, P H M Peeters, G Berglund, G Hallmans, and E Lund. N Slimani, E Kesse, A Nieters, A Naska, V Krogh, and G Skeie supervised the collection and analysis of dietary data. N E Day, S A Bingham, R Luben, P Ferrari, and T Norat analysed the data. S A Bingham wrote the report, taking into account the comments and suggestions of the co-authors.

Conflict of interest statement

None declared.

Acknowledgments

This study was funded by "Europe Against Cancer" Programme of the European Commission (SANCO); Ligue contre le Cancer (France); Société 3M (France); Mutuelle Générale de l'Education Nationale; Institut National de la Santé et de la Recherche Médicale (INSERM); German Cancer Aid; German Cancer Research Center; German Federal Ministry of Education and Research; Danish Cancer Society; Health Research Fund (FIS) of the Spanish Ministry of Health; the participating regional governments and institutions of Spain; Cancer Research UK; Medical Research Council, UK; the Stroke Association, UK; British Heart Foundation; Department of Health, UK; Food Standards Agency, UK; the Wellcome Trust, UK; Greek Ministry of Health; Greek Ministry of Education; Italian Association for Research on Cancer; Italian National Research Council; Dutch Ministry of Public Health, Welfare and Sports; Dutch Ministry of Health; Dutch Prevention Funds; LK Research Funds; Dutch ZON (Zorg Onderzoek Nederland); World Cancer Research Fund (WCRF); Swedish Cancer Society; Swedish Scientific Council; Regional Government of Skane, Sweden; Norwegian Cancer Society.

References

1 Fuchs CS, Giovannucci E, Colditz, GA, et al. Dietary fiber and the risk of colorectal cancer and adenoma in women. *N Engl J Med* 1999; **340:** 169–76.

2 Terry P, Giovannucci E, Michels KB, et al. Fruit, vegetables, dietary fiber, and risk of colorectal cancer. *J Natl Cancer Inst* 2001 **93:** 525–33.

3 Pietinen P, Malila N, Virtanen M, et al. Diet and the risk of colorectal cancer in a cohort of Finnish men. *Cancer Causes Control* 1999; **10:** 387–96.

4 Schatzkin A, Lanza E, Corle D, et al. Lack of effect of low fat high fiber diet on the recurrence of colorectal adenomas. *N Engl J Med* 2000; **342:** 1149–55.

5 Alberts DS, Martinez ME, Roe DJ, et al. Lack of effect of a high fiber cereal supplement on the recurrence of colorectal adenomas. *N Engl J Med* 2000; **342:** 1156–62.

6 Bonithon-Kopp C, Kronborg O, Giacosa A, et al. Calcium and fibre supplementation in prevention of colorectal adenoma recurrence: a randomised intervention trial. *Lancet* 2000; **356:** 1300–06.

7 Key TJ, Fraser GE, Thorogood M, et al. Mortality in vegetarians and non-vegetarians: a collaborative analysis of 8300 deaths among 76 000 men and women in five prospective studies. *Publ Health Nutr* 1998; **1:** 33–41.

8 WCRF-AICR. Food, nutrition and the prevention of cancer: a global perspective. Washington DC: American Institute for Cancer Research, 1997.

9 COMA Working Group on Diet and Cancer. Nutritional aspects of the development of cancer. UK Department of Health Report on Health and Social Subjects No 48. London: HMSO, 1998.

10 Day NE, McKeown N, Wong MY, Welch A, Bingham S. Epidemiological assessment of diet: a comparison of a 7-day diary with a food frequency questionnaire using urinary markers of nitrogen, potassium and sodium *Int J Epidemiol* 2001; **30:** 309–17.

11 Kipnis V, Midthune D, Freedman LS, et al. Empirical evidence of correlated biases in dietary assessment instruments and its implications. *Am J Epidemiol* 2001; **153:** 394–403.

12 Riboli E, Kaaks R. The EPIC project: rationale and study design. *Int J Epidemiol* 1997; **26** (suppl)**:** S6–14.

13 Agudo A, Slimani N, Ocke MC, et al. Consumption of vegetable, fruit and other plant foods in the EPIC cohorts from ten European countries. *Publ Health Nutr* 2002; **5:** 1179–96.

14 Wirfalt E, McTaggart A, Pala V, et al. Food sources of carbohydrates in a European cohort of adults. *Publ Health Nutr* 2002; **5:** 1197–216.

15 Riboli, E, Hunt, KJ, Slimani N, et al. EPIC: study populations and data collection. *Publ Health Nutr* 2002; **5:** 1113–24.

16 Slimani N, Kaaks R, Ferrari P, et al. EPIC Calibration Study: rationale, design and population characteristics. *Publ Health Nutr* 2002; **5:** 1125–46.

17 Deharveng G, Charrondiere UR, Slimani N, Southgate DAT, Riboli E. Comparison of nutrients in the food composition tables in the nine European countries participating in EPIC. *Eur J Clin Nutr* 1999; **53:** 60–79.

18 Holland B, Welch AA, Unwin D, Buss DH, Paul AA, Southgate DAT. McCance and Widdowson's the composition of foods. London: Royal Society of Chemistry and MAFF, 1991.

19 Department of Health. Dietary reference values for food energy and nutrients for the United Kingdom. UK Department of Health Report on Health and Social Subjects No 41. London: Stationery Office, 1991.

20 Breslow NE, Day NE. Statistical methods in cancer research. Volume II: the design and analysis of cohort studies (IARC Sci Publ No 82). Lyon: International Agency for Research on Cancer, 1987.

21 Kaaks R, Riboli E, Van Staveren W. Calibration of dietary-intake measurements in prospective cohort studies *Am J Epidemiol* 1995; **142:** 548–56.

22 Rosner B, Willett WC, Spiegelman D. Correction of logistic-regression relative risk estimates and confidence-intervals for systematic within-person measurement error. *Stat Med* 1989; **8:** 1051–69.

23 Rosner B, Gore R. Measurement error correction in nutritional epidemiology based on individual foods, with application to the relation of diet to breast cancer. *Am J Epidemiol* 2001; **154:** 827–35.

24 Bingham S. Mechanisms and experimental evidence relating dietary fibre and starch to protection against large bowel cancer. *Proc Nutr Soc* 1990; **49:** 153–71.

25 Boffa LC, Luption JR, Mariani MR, et al. Modulation of colonic cell proliferation, histone acetylation and luminal short chain fatty acids by variation of dietary fibre (wheat bran) in rats. *Cancer Res* 1992; **52:** 5906–12.

26 Chai F, Evdoukiou A, Young GP, Zalewski PD. Involvement of p21 and its cleavage by DEVD-caspase during apoptosis of colorectal cancer cells induced by butyrate. *Carcinogenesis* 2000; **21:** 7–14.

27 Domon-Dell C, Wang Q, Kim S, Kedinger M, Evers BM, Freund JN. Stimulation of the intestinal Cdx2 homeobox gene by butyrate in colon cancer cells. *Gut* 2002; **50:** 525–29.

28 McNeil NI, Rampton DS. Is the rectum usually empty? *Dis Colon Rectum* 1981; **24:** 596–99.

Association of Nut and Seed Intake with Colorectal Cancer Risk in the European Prospective Investigation into Cancer and Nutrition

Mazda Jenab,[1] Pietro Ferrari,[1] Nadia Slimani,[1] Teresa Norat,[1] Corinne Casagrande,[1] Kim Overad,[2] Anja Olsen,[3] Connie Stripp,[3] Anne Tjønneland,[3] Marie-Christine Boutron-Ruault,[4,5] Françoise Clavel-Chapelon,[4] Emmanuelle Kesse,[4] Alexandra Nieters,[6] Manuela Bergmann,[7] Heiner Boeing,[7] Androniki Naska,[8] Antonia Trichopoulou,[8] Domenico Palli,[9] Vittorio Krogh,[10] Egidio Celentano,[11] Rosario Tumino,[12] Carlotta Sacerdote,[13] Hendrik B. Bueno-de-Mesquita,[14] Marga C. Ocké,[14] Petra H.M. Peeters,[15] Dagrun Engeset,[16] José R. Quirós,[17] Carlos A. González,[18] Carmen Martínez,[19] Maria D. Chirlaque,[20] Eva Ardanaz,[21] Miren Dorronsoro,[22] Peter Wallström,[23] Richard Palmqvist,[24] Bethany Van Guelpen,[24] Sheila Bingham,[25] Miguel A. San Joaquin,[26] Rodolfo Saracci,[1] Rudolf Kaaks,[1] and Elio Riboli[1]

[1]Nutrition and Hormones Group, IARC-WHO, Lyon, France; [2]Department of Clinical Epidemiology, Aalborg Hospital and Aarhus University Hospital, and Department of Epidemiology and Social Medicine, University of Aarhus, Aarhus, Denmark; [3]Danish Cancer Society, Institute of Cancer Epidemiology, Copenhagen, Denmark; [4]Institut National de la Santé et de la Recherche Médicale, U521, Institut Gustave Roussy, Villejuif, France; [5]Institut National de la Santé et de la Recherche Médicale, U557, Institut Scientifique et Technique de la Nutrition et de l'Alimentation, Conservatoire National des Arts et Métiers, Paris, France; [6]Division of Clinical Epidemiology, German Cancer Research Center, Heidelberg, Germany; [7]Deutsches Institut für Ernährungsforschung, Potsdam, Germany; [8]University of Athens Medical School, Athens, Greece; [9]Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Center (CSPO), Scientific Institute of Tuscany, Florence, Italy; [10]National Cancer Institute, Milan, Italy; [11]Istituto Nazionale Tumori di Napoli, Naples, Italy; [12]Cancer Registry, Azienda Ospedaliera ''Civile M.P. Arezzo,'' Ragusa, Italy; [13]University of Torino and CPO-Piemonte, Turin, Italy; [14]Center for Nutrition and Health, National Institute for Public Health and the Environment, Bilthoven, Netherlands; [15]Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, Netherlands; [16]Institute of Community Medicine, University of Tromsø, Tromsø, Norway; [17]Sección Información Sanitaria, Consejería de Salud y Servicios Sanitarios de Asturias, Asturias, Spain; [18]Department of Epidemiology, Catalan Institute of Oncology, Barcelona, Spain; [19]Andalusian School of Public Health, Granada, Spain; [20]Servicio de Epidemiología, Consejería de Sanidad y Consumo, Murcia, Spain; [21]Public Health Institute, Navarra, Spain; [22]Department of Public Health of Guipuzkoa, San Sebastian, Spain; [23]Medical Research Center, Malmö University Hospital, Malmö, Sweden; [24]Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden; [25]Medical Research Council Dunn Human Nutrition Unit, Cambridge, United Kingdom; and [26]Epidemiology Unit, Cancer Research UK, University of Oxford, Oxford, United Kingdom

Abstract

A link between unsaturated fatty acids or phytonutrients and reduced risk of colorectal cancer has been suggested. However, the effects of higher intake of dietary sources of these nutrients, such as the nuts and seeds food group, are less clear. The objective of this study was to determine the effects of nut and seed intake on colorectal cancer risk within the European Prospective Investigation into Cancer and Nutrition study, a large prospective cohort study involving 10 European countries. Total nut and seed intake was determined from country-specific dietary questionnaires. The data set included 478,040 subjects (141,988 men, 336,052 women) with a total of 855 (327 men, 528 women) colon and 474 (215 men, 259 women) rectal cancer cases. A multivariate Cox proportional hazards model, stratified by center and controlled for fruit intake, dietary fiber, energy, height, weight, sex, age, physical activity, and smoking, was used. The data show no association between higher intake of nuts and seeds and risk of colorectal, colon, and rectal cancers in men and women combined, but a significant inverse association was observed in subgroup analyses for colon cancer in women at the highest (>6.2 g/d) versus the lowest (nonconsumers; hazard ratio, 0.69; 95% confidence interval, 0.50-0.95) category of intake and for the linear effect of log-transformed intake (hazard ratio, 0.89; 95% confidence interval, 0.80-0.98), with no associations in men. It is not evident from this data why there may be a stronger association in women or why it may be limited to the colon, suggesting that much further research is necessary. (Cancer Epidemiol Biomarkers Prev 2004;13(10):1595–603)

Received 1/7/04; revised 5/3/04; accepted 5/6/04.

Grant support: ''Europe Against Cancer'' Programme of the European Commission (SANCO); Ligue contre le Cancer (France); Société 3M (France); Mutuelle Générale de l'Education Nationale; Institut National de la Santé et de la Recherche Médicale; German Cancer Aid; German Cancer Research Center; German Federal Ministry of Education and Research; Danish Cancer Society; Health Research Fund (FIS) of the Spanish Ministry of Health; the participating regional governments and institutions of Spain; Cancer Research UK; Medical Research Council (United Kingdom); Stroke Association (United Kingdom); British Heart Foundation; Department of Health (United Kingdom); Food Standards Agency (United Kingdom); Wellcome Trust (United Kingdom); Greek Ministry of Health; Greek Ministry of Education; Italian Association for Research on Cancer; Italian National Research Council; Dutch Ministry of Public Health, Welfare and Sports; Dutch Ministry of Health; Dutch Prevention Funds; LK Research Funds; Dutch ZON (Zorg Onderzoek Nederland); World Cancer Research Fund; Swedish Cancer Society; Swedish Scientific Council; Regional Government of Skane (Sweden); and Norwegian Cancer Society. The work reported in this article was undertaken during the tenure of a postdoctoral fellowship from the IARC.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Mazda Jenab, Nutrition and Hormones Group, IARC-WHO, 150 cours Albert-Thomas, 69372 Lyon Cedex 08, France. Phone: 33-472-73-80-82; Fax: 33-472-73-83-61. E-mail: jenab@iarc.fr

Introduction

Nuts and seeds may be considered as an important component of a healthy diet. In general, they are nutrient

dense and provide protein, fat (mostly unsaturated fatty acids), dietary fiber, and many bioactive constituents, such as vitamins (e.g., folic acid, niacin, vitamin E, and vitamin B6), minerals (e.g., copper, magnesium, potassium, and zinc), antioxidants, phytoestrogens, and other phytochemicals (1). Given such density and variety of bioactive compounds, extensive research has focused on potential healthy effects of higher nut and seed consumption on the development of heart disease (2-4) and prostate cancer (5-7).

With respect to colorectal cancer (CRC), only limited data are available from one animal (8) and three human case-control studies, which show either no protective effects of combined higher intake of nuts and legumes (9, 10) or a significant linear dose-response protective effect with higher combined intake of pulses, nuts, and seeds in women but not in men (11). However, all of these studies combined intakes of nuts and seeds with legumes and did not measure the direct effects of intake of nuts or seeds as a unique food group, which may be one of the sources of variation observed in their results.

Data from the Adventist Health Study, a prospective observational cohort study, show a protective effect of nuts on colon cancer risk for intakes of one to four times per week compared with less than once per week [relative risk, 0.67; 95% confidence interval (95% CI), 0.45-0.98], but not for higher intakes, likely due to the small number of cases in the higher intake category (12). However, this study did not present data by gender and did not consider rectal cancer.

Due to these mixed results and the limited number of studies, the impact of nut and seed intake on CRC risk is far from being well defined. Thus, the objective of this study was to determine the effects of nut and seed intake on CRC risk within the European Prospective Investigation into Cancer and Nutrition (EPIC) study. EPIC is an ongoing multicenter prospective cohort study, designed specifically to investigate a relationship between cancer and nutrition (13). Currently, the study has enrolled over 520,000 participants from 23 centers in 10 European countries. The strengths of the study lie in its very large size, in allowing comparisons between areas with varying cancer rates, and in its high degree of heterogeneity in dietary intake and variety of foods consumed. As such, it presents an appropriate setting for a study to contribute to the clarification of any potential influence of nuts and seeds on CRC risk.

Methods

Study Population. The rationale and methods of the EPIC study have been described previously in detail (13, 14). The EPIC cohort consists of 23 subcohorts in 10 European countries (Denmark, France, Greece, Germany, Italy, Netherlands, Norway, Spain, Sweden, and United Kingdom), providing a wide range of cancer occurrence rates, lifestyle, and dietary habits. The French and Norwegian cohorts as well as the Naples center of Italy and the Utrecht center in Netherlands are composed of women only. Country-specific dietary questionnaires as well as standardized lifestyle and personal questionnaires, various anthropometric measures, and blood samples have been collected from most participants. Country-specific dietary questionnaires were used to ensure high compliance and to obtain better measures of local dietary habits. In total, 521,468 subjects have been enrolled. For the present study, to reduce the effects of extreme values of intake, subjects in the top and bottom 1% of the ratio of energy intake to estimated energy requirement (calculated from age, sex, height, and body weight) as well as subjects with previous cancer diagnoses or with missing dietary data were excluded. Thus, for the present study, the database consisted of a total of 478,040 (141,988 men, 336,052 women) subjects, including a total of 855 colon cancer cases (327 men, 528 women; proximal: 127 men, 224 women; distal: 154 men, 237 women; unspecified or overlapping colon location: total of 113) and 474 rectal cancer cases (215 men, 259 women). Cases included only those with primary CRC, of which the majority (91%) are adenocarcinoma and the rest are other tumor types.

Follow-up is based on population cancer registries (Denmark, Italy, Netherlands, Norway, Spain, Sweden, and United Kingdom) and other methods such as health insurance records, pathology registries, and active contact of study subjects or next of kin (France, Germany, and Greece). The follow-up period for the present study was for data reports received at IARC to the end of October 2002, representing complete follow-ups until either December 2000 or December 2001 for all centers using cancer registry data and until 2002 for France, Germany, and Greece.

Definitions of colon and rectal cancers were as described previously (15). Tumors in the cecum, appendix, ascending colon, hepatic flexure, transverse colon, and splenic flexure (C18.0-C18.5) were defined as proximal (or right) colon tumors, whereas tumors in the descending and sigmoid colon (C18.6 and C18.7) were defined as distal (or left) colon tumors. Tumors that were overlapping or unspecified (C18.8 and C18.9) were not included in the assignment of proximal and distal colon tumors. Tumors for the proximal and distal colon as well as overlapping or unspecified tumors were combined to define the whole colon. Cancers of the rectum were defined as tumors occurring at the rectosigmoid junction (C19) or rectum (C20). Anal canal tumors were excluded from the analysis.

Determination of Total Nut and Seed Intake. For determination of the total amount of nut and seed intake, this study used the value of total intake of pure nut and seed products (composed of at least 90% nuts and/or seeds) combined as determined from dietary questionnaires. The EPIC questionnaires have been validated by comparing questionnaire data to 12 monthly 24-hour recalls in a subsample of EPIC subjects. For total fruit intake, including nuts and seeds, the correlations range from 0.33 (German men) to 0.72 (Swedish men; ref. 16). Using the same procedure, the assessment of nuts and seeds has been validated in some EPIC cohorts, with correlations of 0.25 (men and women combined) in Germany (17) and 0.65 (men) and 0.38 (women) in Netherlands (18). In addition, we calculated the age-adjusted correlation coefficients between the mean intake of nuts and seeds from the EPIC diet questionnaires and the mean intake estimated from EPIC 24-hour dietary recalls, by sex and study center, in a subset of 36,000 EPIC subjects, to be 0.51 for men and 0.62 for women.

The EPIC diet questionnaires contain a range of questions on general intake of nuts and seeds, with most asking at least a general question about overall nut and seed intake. The amount of nut and seed intake and the types of nuts and seeds consumed throughout the EPIC centers vary widely. This wide range includes peanuts (and peanut butter), which are actually legumes but often identified by consumers as part of the nuts and seeds food group, and chestnuts, which are more starchy and have less fat content than most other nuts. Details of the dietary questionnaire items regarding nut and seed intake for each country (or center, if questionnaire questions were different for centers within a given country) are shown in Table 1. Questionnaires in the Malmö center of Sweden and in Spain were partly open ended (13), so specific items that appear in Table 1 for these centers represent details about nut and seed intake developed after asking a general question on overall consumption of nuts and seeds. Consumers were determined from the diet questionnaire and defined as those with an intake of nuts and seeds greater than zero.

Statistical Methods. The analyses were done using a multivariate Cox proportional hazards model (SAS statistical software, version 8e, SAS Institute, Cary, NC). To control for center effects such as follow-up procedures and questionnaire design, all analyses were stratified by center. Because the UK Oxford Center includes two very different populations (a health-conscious population and a general population), each was treated as a separate center.

Nut and seed intake data were divided into five categories with all nonconsumers (23.7% for men and 25.8% for women) placed in the first (reference) category. The consumers were divided by quartiles into the remaining four categories of intake (categories 2-5). Category cut points were defined across the entire cohort for men and women combined (grams of nut and seed intake per day): category 1 (nonconsumers; reference): 0.0 g/d, category 2: >0.0 to 0.8 g/d, category 3: >0.8 to 2.3 g/d, category 4: >2.3 to 6.2 g/d, and category 5: >6.2 g/d. The number of cases and noncases in each category of intake are listed in Table 2. Analyses were also done using gender-specific and gender and country–specific categories of intake, but as results were similar to those provided by non–gender and country–specific categories, the latter were used for all analyses presented. Analyses were also conducted by country and for the whole EPIC cohort, with nut and seed intake treated as a log-transformed (original value +1) continuous variable. Tests for interaction of nut and seed intake with country and by gender were also done.

In all models, age was used as the primary time variable. Risk estimates were computed using Cox regression by considering two different sets of adjusting variables and confounders: (*a*) energy-adjusted model: adjusted for gender, age, and intake of energy from fat, energy from alcohol, and energy from carbohydrates and proteins and (*b*) fully adjusted model: the same variables as in the energy-adjusted model plus additional inclusion of height, weight, total intake of fruits (without nuts and seeds), intake of total dietary fiber, physical activity at work, and duration of smoking (variable with separate categories of years of smoking for smokers and ex-smokers, including a category for nonsmokers). Because dietary folate may be a modulator of CRC risk, to take into account any potential confounding from this variable, dietary folate was additionally included as an adjustment variable in a large subset of EPIC subjects for whom dietary folate intake is available (1,182 cases, 412,085 noncases).

Total energy intake was partitioned into its components from fat, alcohol, and carbohydrate plus protein sources in all models used to improve the error correction with consideration of the high-fat content of nuts and seeds in general and the potential of both their fat and nonfat components to contribute to any possible cancer protective effects. Because alcohol is a rich source of energy and because its effect as a covariable was shown to be similar when it was included in the model either as a separate variable based on classes of amount of alcohol consumption, as a continuous variable, or as a component of total energy, it was deemed best to correct for alcohol consumption as a component of total energy.

Because previous methodologic studies indicate that weight is an important predictor of energy intake (19) and to control for body size, height and weight were adjusted for in all models. In these analyses, no differences were observed in the results when adjusting for height and weight in comparison with the body mass index alone or in combination with height or weight.

The same covariates were used in analysis models based on nut and seed intake as a continuous or categorical variable. In the categorical variable model, all covariates were categorical, whereas in the continuous variable models, all dietary intake variables were log transformed and continuous except for height, weight, physical activity, and smoking duration, which were modeled as categorical variables. For all models, linear trend tests were also done using a continuous variable scored from 1 to 5 according to the category interval in which an observation lay. To take into account possible deviations from linearity, an indicator variable (0 = nonconsumer, 1 = consumer) of nut intake was also included in models with nut and seed intake included as a continuous variable. However, this variable was not significantly associated with risk and the association between nut and seed intake and CRC incidence was not altered. Thus, only results without the indicator variable are presented.

The same set of models was run for colorectal, colon (whole, proximal, and distal sections), and rectal cancers. Due to potential differences between men and women in nut and seed intake patterns, variation in types of nuts and seeds consumed, and with regard to purported gender differences in incidence of CRC (20, 21), all analyses were also conducted by gender.

As described previously (22), 24-hour diet recalls have been collected from a subset (8% random sample; total of 36,000 subjects) of all EPIC cohorts for the purpose of calibrating dietary questionnaire data to counter estimation errors and differences in questionnaire design and application among centers, allowing for estimation of food intakes on a common scale, hence enabling better comparisons of cancer risk among the EPIC centers. A common strategy of calibration procedures is to adjust for center-specific errors in estimating the true mean intake of the food group of interest (22). This was done in this study by centering the mean intake for nuts and

Table 1. Listing of items for nuts and seeds from the list of items in the EPIC baseline dietary questionnaires, percentage of nut and seed consumers, average daily intake from both diet questionnaire and 24-hour recalls by country, HR and 95% CI for association of nuts and seeds for men and women combined, by country, and contribution of each country to the entire EPIC cohort and CRC cases

EPIC country and/or study center	Questionnaire food item concerning intake of nuts and/or seeds	Percentage of consumers*	Mean intake (g/d)†		24-hour recall mean intake (g/d)‡		HR of nut and seed intake§	Total person years	CRC cases (*n*)
			Males	Females	Males	Females			
Denmark	Peanuts	74.2	2.0 ± 4.4	1.5 ± 3.7	1.4 ± 9.2	1.2 ± 8.1	1.21 (0.97-1.51)	182,176	177
France	Nuts, nonspecific‖	71.7	—	5.5 ± 8.4	—	3.6 ± 15.6	0.85 (0.72-1.00)	437,420	174
Germany	Nuts, nonspecific‖; seeds, nonspecific‖; peanut butter	91.3	6.3 ± 11.0	5.2 ± 9.0	2.5 ± 11.6	2.7 ± 12.0	0.97 (0.78-1.17)	209,262	114
Greece	Nuts, nonspecific,‖ with salt; nuts, nonspecific,‖ without salt	92.9	7.2 ± 9.4	4.9 ± 7.3	3.5 ± 15.9	3.2 ± 14.3	0.98 (0.64-1.50)	92,661	24
Italy		89.0	0.9 ± 1.8	0.9 ± 2.1	3.0 ± 16.5	3.4 ± 18.1	0.92 (0.61-1.39)	186,919	101
Italy North (Florence, Turin, and Varese)	Walnuts; hazelnuts; almonds; peanuts								
Ragusa	Nuts, nonspecific‖								
Naples	Walnut								
Norway	Peanuts	47.2	—	2.2 ± 3.6	—	2.8 ± 12.7	0.90 (0.52-1.55)	57,423	20
Netherlands	Nuts, nonspecific, eaten at dinner‖; peanut butter; peanuts, cocktail nuts, and other nuts	94.1	12.4 ± 16.6	7.5 ± 10.6	10.0 ± 26.7	5.5 ± 15.9	0.87 (0.72-1.11)	161,194	97
Spain	Almond; chestnut; coconut; hazelnut; other nuts, nonspecific‖; peanut; pine nut; pistachio nut; seed, sunflower/pumpkin; walnut	38.0	5.0 ± 13.0	3.9 ± 9.7	6.8 ± 24.9	4.4 ± 15.1	0.98 (0.81-1.18)	236,108	110
Sweden		62.6	1.1 ± 3.7	0.8 ± 3.1	1.0 ± 6.9	1.0 ± 6.0	0.89 (0.72-1.10)	368,594	272
Malmö	Almonds; cashew nuts; chestnuts, roasted; hazelnuts; nuts/almonds; peanut butter; peanuts; peanuts, roasted salted; pecan nuts; pistachio nuts; pumpkin kernels; pumpkin/squash seeds, dried; walnuts								
Umeå	Peanuts, salted								
United Kingdom	Peanut butter, peanuts, or other nuts	84.6	6.7 ± 13.8	5.6 ± 10.6	5.5 ± 20.4	4.6 ± 15.6	1.10 (0.97-1.26)	351,401	240

*Defined as those with nut and seed intakes >0 from diet questionnaire.
†Unadjusted means ± SD from EPIC diet questionnaires, including nonconsumers.
‡Unadjusted means ± SD from EPIC 24-hour recalls in 38,000 EPIC subjects, including nonconsumers.
§HR and 95% CI for association of nut and seed intake with CRC risk for men and women combined, by country, with intake presented as a log-transformed continuous variable, using a model stratified by center with age as the primary time variable and adjusted for age, gender, height (categorical), weight (categorical), energy from alcohol (log-transformed continuous), energy from fat (log-transformed continuous), energy from carbohydrates and proteins (log-transformed continuous), fruit intake (without nuts and seeds; log-transformed), dietary fiber intake (log-transformed), physical activity (categorical), and smoking duration (categorical).
‖"Nonspecific" implies intake of any kind of nuts.

seeds obtained from questionnaire data on nut and seed intake data obtained from the 24-hour recalls (values shown in Table 1) using an additive approach. Cox regression models were re-run using calibrated data. The calibrated intake of nuts and seeds was again divided into cohort-wide categories of intake with all nonconsumers in the first (reference) category and the consumers divided as equally as possible among the remaining four categories with cut points defined as follows: category 1 (nonconsumers; reference): 0.0 g/d, category 2: >0.0 to 1.2 g/d, category 3: >1.2 to 3.5 g/d, category 4: >3.5 to 7.1 g/d, and category 5: >7.1 g/d. Intake of total fruits (without nuts and seeds) and dietary fiber as well as intake of energy from fat, energy from alcohol, and energy from carbohydrates and proteins were also calibrated in the same manner described above and then

Table 2. Description of the study population

	Whole cohort	Category of nut and seed intake*				
		1 (Reference; nonconsumers)	2 (>0-2.8 g/d)	3 (>0.8-2.3 g/d)	4 (>2.3-6.2 g/d)	5 (>6.2 g/d)
Dietary variables						
Nuts and seeds (g/d)†						
All subjects	4.2 ± 8.8	0.0	0.3 ± 0.2	1.4 ± 0.6	4.0 ± 1.0	16.7 ± 14.6
Men	4.6 ± 10.4	0.0	0.3 ± 0.2	1.3 ± 0.5	4.1 ± 1.0	19.1 ± 17.6
Women	4.1 ± 8.1	0.0	0.3 ± 0.2	1.5 ± 0.6	4.0 ± 1.0	15.7 ± 13.0
Total energy (MJ)†,‡						
All subjects	8.8 ± 2.7	8.5 ± 2.7	8.5 ± 2.7	9.0 ± 2.6	8.7 ± 2.5	9.6 ± 2.7
Men	10.3 ± 2.9	10.4 ± 2.9	9.7 ± 2.9	10.4 ± 2.7	10.3 ± 2.8	10.9 ± 3.0
Women	8.2 ± 2.3	7.8 ± 2.2	7.8 ± 2.3	8.3 ± 2.2	8.2 ± 2.2	9.0 ± 2.4
Fruits (g/d)†,§						
All subjects	244.1 ± 196.4	231.3 ± 186.9	264.7 ± 211.0	216.9 ± 168.2	258.1 ± 206.7	255.5 ± 206.1
Men	222.4 ± 207.5	214.0 ± 196.5	236.1 ± 214.8	171.6 ± 150.4	257.9 ± 237.0	247.2 ± 230.5
Women	253.3 ± 190.8	237.9 ± 182.6	279.8 ± 207.5	239.3 ± 172.0	258.1 ± 196.1	259.1 ± 194.7
Dietary fiber intake (g/d)†						
All subjects	22.4 ± 8.0	21.8 ± 8.0	21.5 ± 8.8	21.3 ± 7.3	22.7 ± 7.4	25.2 ± 8.0
Men	23.9 ± 9.2	23.8 ± 9.2	23.1 ± 10.3	21.6 ± 7.7	24.2 ± 8.4	27.1 ± 9.0
Women	21.8 ± 7.4	21.1 ± 7.3	20.6 ± 7.8	21.1 ± 7.1	22.2 ± 7.0	24.3 ± 7.4
Nondietary variables						
Colon cancer cases (*n*)						
All subjects	855	299	148	181	124	103
Men	327	116	59	75	40	37
Women	528	183	89	106	84	66
Rectal cancer cases (*n*)						
All subjects	474	175	69	90	59	81
Men	215	76	40	38	28	33
Women	259	99	29	52	31	48
Subjects (*n*)						
All subjects	478,040	119,890	87,120	92,532	88,963	89,535
Men	141,988	33,276	30,171	30,536	21,324	26,681
Women	336,052	86,614	56,949	61,996	67,639	62,854
Age (y)†						
All subjects	51.2 ± 9.9	54.0 ± 8.4	49.5 ± 10.9	52.6 ± 9.2	49.9 ± 9.8	49.0 ± 10.4
Men	52.2 ± 10.1	56.0 ± 8.4	49.7 ± 10.6	54.0 ± 8.3	51.2 ± 10.5	48.9 ± 11.2
Women	50.8 ± 9.8	53.2 ± 8.3	49.3 ± 11.1	51.9 ± 9.5	49.5 ± 9.6	49.0 ± 10.1
Height (cm)†						
All subjects	166.0 ± 9.0	164.9 ± 8.5	165.9 ± 9.5	167.1 ± 9.1	165.6 ± 8.7	166.9 ± 9.1
Men	174.8 ± 7.4	173.1 ± 7.2	174.7 ± 7.5	176.0 ± 6.8	174.8 ± 7.6	175.7 ± 7.7
Women	162.3 ± 6.8	161.8 ± 6.8	161.2 ± 6.9	162.7 ± 6.5	162.8 ± 6.8	163.2 ± 6.8
Weight (kg)†						
All subjects	70.6 ± 13.7	70.9 ± 13.7	71.3 ± 13.5	71.4 ± 14.1	69.5 ± 13.5	69.8 ± 13.5
Men	81.3 ± 12.0	81.4 ± 12.0	80.4 ± 11.7	82.1 ± 12.1	81.7 ± 12.0	80.9 ± 12.0
Women	66.1 ± 11.8	66.9 ± 12.1	66.4 ± 11.9	66.1 ± 11.8	65.6 ± 11.6	65.1 ± 11.2

NOTE: Average no. years of follow-up: category 1: all subjects $n = 5.1$, men $n = 5.5$, women $n = 4.9$; category 2: all subjects $n = 5.1$, men $n = 5.3$, women $n = 5.1$; category 3: all subjects $n = 4.4$, men $n = 4.0$, women $n = 4.6$; category 4: all subjects $n = 4.5$, men $n = 4.3$, women $n = 4.5$; and category 5: all subjects $n = 4.7$, men $n = 4.4$, women $n = 4.9$. Average no. proximal colon cancer cases (not including overlapping or unspecified tumors): category 1: all subjects $n = 117$, men $n = 46$, women $n = 71$; category 2: all subjects $n = 57$, men $n = 18$, women $n = 39$; category 3: all subjects $n = 84$, men $n = 36$, women $n = 48$; category 4: all subjects $n = 55$, men $n = 15$, women $n = 40$; and category 5: all subjects $n = 38$, men $n = 12$, women $n = 26$. Average no. distal colon cancer cases (not including overlapping or unspecified tumors): category 1: all subjects $n = 175$, men $n = 76$, women $n = 99$; category 2: all subjects $n = 69$, men $n = 40$, women $n = 29$; category 3: all subjects $n = 90$, men $n = 38$, women $n = 52$; category 4: all subjects $n = 59$, men $n = 28$, women $n = 31$; and category 5: all subjects $n = 81$, men $n = 33$, women $n = 48$.

*Non–sex-specific category cut points were defined across the entire cohort.

†Values are means ± SD and are not adjusted by any variable.

‡In men, 35.5% of total energy are from fats, 58.5% from carbohydrates and proteins, and 5.9% from alcohol. In women, 35.9% of total energy are from fats, 61.3% from carbohydrates and proteins, and 2.8% from alcohol.

§Not including intake of nuts and seeds.

redivided into EPIC-wide quintiles of intake. Analysis models for calibrated data were similar to the fully adjusted model described above for the noncalibrated (original) data in categories of intake.

Results

Table 1 lists the percentage of nut and seed consumers and the average amount of nut and seed intake by country as well as the extent of the contribution, in terms of the number of subjects, of each country to the entire cohort. It shows a wide degree of variation in intake of nuts and seeds, as derived from dietary questionnaires, among the EPIC countries, with the mean intake ranging from a low of 0.8 g/d (women in Sweden) to a high of 12.4 g/d (men in Netherlands). The total intake of nuts and seeds ranged from 0 to 300.2 g/d for men and 0 to 265.5 g/d for women. From Table 1, it is clear that a large percentage of subjects in most countries consume some nuts and seeds with the highest in Netherlands (94.1%). Table 1 also lists the mean intake of nuts and seeds derived from 24-hour recalls taken from a subset of the EPIC cohort and used in the calibration of the intake data, as described in more detail in Methods. The average percentage of consumers in all EPIC cohorts together was 76.6% and 74.2% for men and women, respectively, and 74.9% for all subjects combined. Table 1 also shows the hazard ratios (HR) for the association of nut and seed intake with CRC in each individual country. The results show variations in direction and magnitude of the risk by country. In addition, tests for interaction of nut and seed intake with country did not show any significant interactions.

A description of the study population is given in Table 2. Follow-up consisted of 2,294,592 person-years of follow-up (average 4.8 years per subject) with 478,040 subjects and a total of 1,329 cases of CRC.

Table 2 shows that the average unadjusted daily intake of nuts and seeds was 4.6 g for men and 4.1 g for women in the whole cohort. Table 2 also shows the average amount of nut and seed consumption per category of intake. Comparing the second category to the highest category, the intake of nuts and seeds showed over a 55-fold increase across category means (Table 2). Average total intakes in the whole cohort adjusted for energy were much higher for women (4.44 g/d) than for men (3.63 g/d).

The HRs for risk of colorectal, colon, and rectal cancers associated with total intake of nuts and seeds for categorical and continuous data for both energy-adjusted and fully adjusted analysis models are shown in Table 3. No meaningful differences were observed between energy-adjusted and fully adjusted analysis models. No associations between nut and seed intake and incidence of colorectal, colon, or rectal cancers were observed in men and women combined or in men alone in any of the analysis models (Table 3). However, subgroup analyses by gender show that in women, but not men, the highest category of nut and seed intake was associated with a reduced incidence of colon cancer (HR, 0.69; 95% CI, 0.50-0.95; fully adjusted model), particularly distal colon cancer (HR, 0.52; 95% CI, 0.32-0.85; fully adjusted model), with a mean intake of 15.7 g/d compared with nonconsumers. This is supported by P's for trend (P = 0.03; Table 3). No statistical significant association was observed in men. The same pattern is observed when the nut and seed intake data are presented as a log-transformed continuous variable (Table 3), with a reduced incidence of colon cancer (HR, 0.89; 95% CI, 0.80-0.98; fully adjusted model), particularly distal colon cancer (HR, 0.83; 95% CI, 0.70-0.97; fully adjusted model), in women and no associations in men. However, tests for interaction of nut and seed intake with gender did not show a significant difference in effect for CRC by gender (P = 0.18). No significant association of nut and seed intake with incidence of rectal cancer was observed for either gender (Table 3).

To fully explore the confounding effect of energy, models were also run with total energy or with energy partitioned into its components, as described in Methods. There was little difference in the results, however, and so the components of energy were used as confounders. No meaningful differences in results were observed when energy variables were adjusted for as either categorical or log-transformed continuous variables in the analysis models.

No relevant changes in results were observed with adjustments for dietary folate (Table 3). Furthermore, intakes of vegetables, legumes, meat and meat products, cereals and cereal products, dairy products, fish/shellfish, and vitamin E were also tested as potential covariates in the analysis models. However, the results were not modified after their inclusion. Because nuts and seeds are rich in polyunsaturated and monounsaturated fatty acids, the models were also tested correcting for ratios of either (*a*) polyunsaturated to saturated, (*b*) monounsaturated to saturated, or (*c*) monounsaturated to polyunsaturated fatty acids. However, none of these altered the results obtained.

Figure 1 shows the HRs for CRC risk of increased nut and seed intake for men and women based on calibrated compared with noncalibrated (original) data of categories of nut and seed intake from Table 3 (fully adjusted model). Calibrated results for men are very similar to the original data, with no significant effects at any level of intake. However, for women, calibration heightens the strength of the inverse association between increased nut and seed intake and CRC with a significant reduction in the two highest categories of intake.

Discussion

EPIC is one of the largest prospective cohort studies ever conducted on diet and cancer. The results of this present study show no significant protective associations on CRC risk for men and women combined. However, the results of subgroup analyses by gender suggest that a modest intake of an average of ~16 g of nuts and seeds daily is associated with reduced incidence of colon cancer in women relative to nonconsumers, with no observable effects in men, or rectal cancer for either gender. Adjusting for measurement error of nut and seed intake by calibrating diet questionnaire values against values obtained from a 24-hour intake measurement in a subset of the EPIC cohort (23) shows a heightened inverse association with CRC risk of increased nut and seed intake in women (Fig. 1), suggesting a potential improvement of intake estimation with calibration.

It is not clear why this study suggests an association for women but not for men, although the same has been observed for the intake of pulses, nuts, and seeds combined (10) in a case-control study. Nuts and seeds have been suggested to be rich sources of many phytochemicals (1, 24-26) and it is possible to speculate that hormonally active components may affect colon cancer risk differently in women versus men. Indeed,

Table 3. Multivariate-adjusted HR of categories of nut and seed intake and risk of colorectal, colon, and rectal cancers

	HR and 95% CI for category of nut and seed intake*					*P* for trend†	Continuous (Log)‡	Continuous (Log) with folate adjusted§
	1 (Reference)	2	3	4	5			
CRC								
Energy-adjusted model‖								
All subjects	1.00	0.95 (0.77-1.18)	0.98 (0.83-1.16)	0.93 (0.77-1.12)	0.89 (0.73-1.08)	0.23	0.97 (0.91-1.03)	0.98 (0.92-1.05)
Men	1.00	1.10 (0.79-1.55)	1.02 (0.78-1.32)	1.12 (0.83-1.51)	1.06 (0.78-1.43)	0.60	1.04 (0.94-1.14)	1.06 (0.95-1.18)
Women	1.00	0.87 (0.65-1.15)	0.95 (0.77-1.18)	0.83 (0.65-1.05)	0.79 (0.61-1.01)	0.04	0.92 (0.84-0.99)	0.93 (0.84-1.02)
Fully adjusted model¶								
All subjects	1.00	0.95 (0.76-1.18)	0.99 (0.84-1.17)	0.94 (0.78-1.14)	0.91 (0.75-1.11)	0.39	0.98 (0.92-1.05)	0.99 (0.92-1.07)
Men	1.00	1.10 (0.78-1.54)	1.03 (0.79-1.34)	1.13 (0.84-1.53)	1.09 (0.81-1.49)	0.42	1.06 (0.95-1.17)	1.07 0.96-1.19)
Women	1.00	0.87 (0.64-1.15)	0.96 (0.77-1.20)	0.84 (0.67-1.07)	0.81 (0.63-1.04)	0.07	0.93 (0.85-1.01)	0.94 (0.85-1.03)
Colon cancer								
Energy-adjusted model‖								
All subjects	1.00	1.04 (0.80-1.36)	1.07 (0.87-1.32)	1.01 (0.80-1.27)	0.80 (0.62-1.02)	0.18	0.94 (0.87-1.03)	0.93 (0.85-1.02)
Men	1.00	1.09 (0.70-1.67)	1.17 (0.84-1.62)	1.17 (0.80-1.72)	1.00 (0.67-1.50)	0.63	1.04 (0.92-1.18)	1.03 (0.90-1.18)
Women	1.00	1.01 (0.72-1.41)	1.00 (0.77-1.31)	0.91 (0.69-1.21)	0.68 (0.50-0.93)	0.03	0.88 (0.79-0.97)	0.86 (0.76-0.96)
Fully adjusted model¶								
All subjects	1.00	1.03 (0.79-1.35)	1.07 (0.87-1.32)	1.01 (0.80-1.27)	0.81 (0.63-1.04)	0.28	0.96 (0.88-1.04)	0.94 (0.86-1.03)
Men	1.00	1.09 (0.70-1.69)	1.17 (0.84-1.63)	1.17 (0.80-1.73)	1.01 (0.67-1.53)	0.50	1.06 (0.93-1.21)	1.05 (0.91-1.20)
Women	1.00	1.01 (0.72-1.41)	1.01 (0.77-1.32)	0.92 (0.70-1.23)	0.69 (0.50-0.95)	0.04	0.89 (0.80-0.98)	0.87 (0.77-0.98)
Proximal colon cancer								
Energy-adjusted model‖								
All subjects	1.00	1.23 (0.80-1.88)	1.36 (1.00-1.87)	1.22 (0.86-1.73)	0.79 (0.52-1.18)	0.74	0.95 (0.84-1.08)	0.94 (0.82-1.08)
Men	1.00	0.96 (0.44-2.07)	1.58 (0.96-2.59)	1.13 (0.61-2.11)	0.78 (0.39-1.56)	0.96	0.96 (0.78-1.19)	0.96 (0.77-1.19)
Women	1.00	1.39 (0.83-2.33)	1.24 (0.82-1.85)	1.24 (0.81-1.90)	0.76 (0.46-1.26)	0.59	0.93 (0.791.09)	0.92 (0.77-1.10)
Fully adjusted model¶								
All subjects	1.00	1.21 (0.79-1.86)	1.35 (0.99-1.86)	1.22 (0.86-1.73)	0.80 (0.53-1.21)	0.87	0.96 (0.85-1.10)	0.95 (0.82-1.10)
Men	1.00	0.95 (0.44-2.06)	1.55 (0.94-2.56)	1.11 (0.60-2.08)	0.77 (0.38-1.56)	0.91	0.97 (0.78-1.20)	0.96 (0.77-1.20)
Women	1.00	1.38 (0.83-2.31)	1.24 (0.82-1.86)	1.26 (0.82-1.93)	0.80 (0.48-1.33)	0.73	0.95 (0.81-1.12)	0.93 (0.78-1.12)
Distal colon cancer								
Energy-adjusted model‖								
All subjects	1.00	0.88 (0.59-1.30)	0.94 (0.69-1.27)	0.90 (0.64-1.26)	0.71 (0.49-1.03)	0.13	0.93 (0.82-1.05)	0.91 (0.80-1.05)
Men	1.00	1.09 (0.58-2.05)	0.99 (0.62-1.60)	1.01 (0.57-1.79)	1.05 (0.59-1.87)	0.87	1.08 (0.90-1.30)	1.09 (0.89-1.33)
Women	1.00	0.74 (0.44-1.24)	0.88 (0.60-1.30)	0.81 (0.53-1.24)	0.53 (0.33-0.87)	0.03	0.82 (0.70-0.97)	0.79 (0.66-0.95)
Fully adjusted model¶								
All subjects	1.00	0.88 (0.60-1.31)	0.94 (0.70-1.28)	0.91 (0.65-1.28)	0.70 (0.48-1.02)	0.17	0.94 (0.83-1.06)	0.92 (0.81-1.06)
Men	1.00	1.11 (0.59-2.10)	1.01 (0.63-1.64)	1.04 (0.58-1.85)	1.07 (0.60-1.92)	0.69	1.11 (0.92-1.34)	1.11 (0.90-1.36)
Women	1.00	0.74 (0.44-1.24)	0.88 (0.59-1.30)	0.82 (0.54-1.25)	0.52 (0.32-0.85)	0.03	0.83 (0.70-0.97)	0.80 (0.67-0.96)
Rectal cancer								
Energy-adjusted model‖								
All subjects	1.00	0.81 (0.59-1.18)	0.84 (0.63-1.11)	0.81 (0.59-1.11)	1.04 (0.77-1.42)	0.83	1.01 (0.91-1.12)	1.07 (0.96-1.20)
Men	1.00	1.13 (0.67-1.93)	0.81 (0.52-1.25)	1.06 (0.66-1.68)	1.17 (0.71-1.77)	0.80	1.03 (0.88-1.20)	1.10 (0.93-1.30)
Women	1.00	0.60 (0.35-1.02)	0.86 (0.59-1.25)	0.67 (0.43-1.04)	1.01 (0.67-1.52)	0.70	1.00 (0.87-1.16)	1.06 (0.91-1.24)
Fully adjusted model¶								
All subjects	1.00	0.82 (0.56-1.18)	0.86 (0.65-1.14)	0.83 (0.61-1.15)	1.10 (0.81-1.50)	0.95	1.03 (0.92-1.14)	1.08 (0.97-1.21)
Men	1.00	1.13 (0.67-1.92)	0.82 (0.53-1.27)	1.08 (0.68-1.72)	1.20 (0.75-1.90)	0.63	1.05 (0.90-1.23)	1.11 (0.94-1.31)
Women	1.00	0.61 (0.36-1.03)	0.88 (0.61-1.28)	0.69 (0.44-1.07)	1.05 (0.70-1.58)	0.99	1.01 (0.88-1.17)	1.07 (0.92-1.25)

*Values are HRs derived from models as described above, with all covariates presented as categorical variables. Non–sex-specific category cut points were defined across the entire cohort: category 1 (reference): 0.0 g/d, category 2: 0.0-0.8 g/d, category 3: >0.8-2.3 g/d, category 4: >2.3-6.2 g/d, and category 5: >6.2 g/d.

†*P* of χ^2 test for trend using a continuous variable with 1 *df*.

‡Values are HRs derived from models as described above, with all covariates presented as log-transformed continuous variables, except height, weight, physical activity, and duration of smoking (categorical).

§Values are HRs derived from models as described above with further adjustments for intake of folate, with all covariates presented as log-transformed variables, except height, weight, physical activity, and duration of smoking (categorical), from a large subset of EPIC subjects (men: cases n = 496, noncases n = 119,876; women: cases n = 686, noncases n = 292,209).

‖Energy-adjusted model: stratified by center with age as the primary time variable and adjusted for age, gender, energy from alcohol, energy from fat, and energy from carbohydrates and proteins.

¶Fully adjusted model: stratified by center with age as the primary time variable and adjusted for all the same variables as the energy-adjusted model plus adjustments for height, weight, intake of fruits (without nuts and seeds), intake of dietary fiber, physical activity, and duration of smoking.

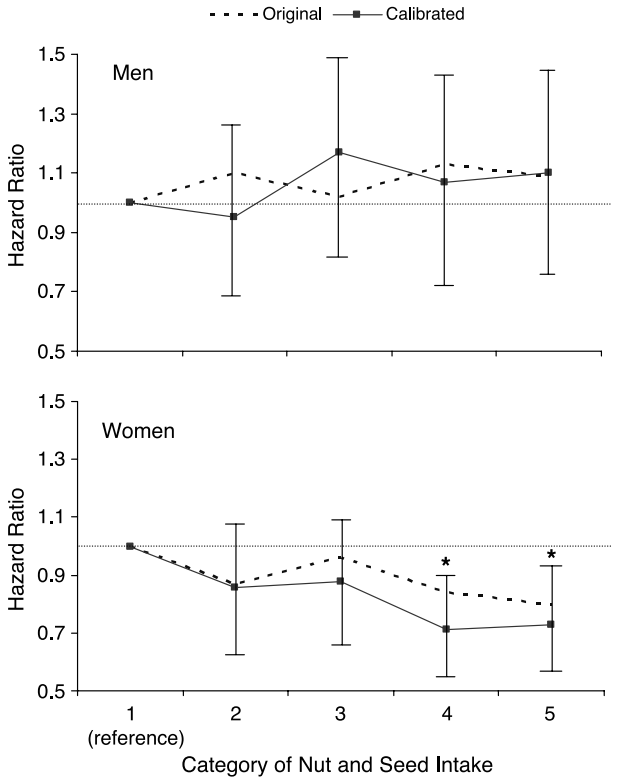


Figure 1. HRs for the risk of CRC in men and women associated with intake of nuts and seeds, original versus calibrated data. *Dotted line,* original HRs for noncalibrated dietary data and CRC risk, which are given and described in Table 3. *Solid line,* calibrated HRs with 95% CIs (*bars*). Calibrated HRs were derived from dietary questionnaire nut and seed intake values centered on 24-hour recall means for nut and seed intake, stratified by center with age as the primary variable and adjusted for age, gender, height (categorical), weight (categorical), energy from alcohol (categorical), energy from fat (categorical), energy from carbohydrates and proteins (categorical), fruit intake (without nuts and seeds; categorical), dietary fiber intake (categorical), physical activity (categorical), and duration of smoking (categorical). Cut points for the noncalibrated data are described in Tables 2 and 3. For calibrated data, non–sex-specific category cut points were redefined after calibration across the entire cohort: category 1 (nonconsumers; reference): 0.0 g/d, category 2: >0.0 to 1.2 g/d, category 3: >1.2 to 3.5 g/d, category 4: >3.5 to 7.1 g/d, and category 5: >7.1 g/d; *, $P < 0.05$, significant difference from reference category.

gender differences in colon cancer risk factors are thought to possibly have a hormonal basis (27) and cancerous and normal bowel tissue may express both types of estrogen receptors (28), suggesting that dietary phytoestrogens may be able to influence colon cancer development. That nuts and seeds, as a food group, may be capable of exerting a possible physiologic hormonal effect is suggested by the observation that increased nut and seed intake can delay the age of menarche in Spanish teenage girls (29). In the present study, to determine if varying hormonal milieus possibly play a role in determining a stronger effect in women, attempts were made to separate women based on their menopausal status, but this did not show any meaningful differences between premenopausal and menopausal women.

Different patterns of nut and seed consumption between men and women may also be a determining factor in the results of this study, although little descriptive information exists. It must also be noted that this study contained much more women subjects and cancer cases, which may strengthen the observation of associations in women versus men. Nonetheless, the apparent differences in effect between men and women observed in this study should, at this stage, only provide a basis for further research and requires clear confirmation.

In this study, it is also interesting that the protective association observed in women was observed in the distal (left) colon. This is not an unusual observation because other epidemiologic studies have also shown a differing effect of various dietary variables on proximal versus distal colon cancers (30, 31). Additionally, in this study, no effect of increased nut and seed intake was observed on the incidence of rectal cancer in either gender. Colon and rectal cancers may have different etiologies, and components of diet have been shown to affect colon cancer but not rectal cancer (32-34), whereas some environmental factors may affect colon and rectal cancers differently in men and women (35). It may, however, also be argued that a limitation of this study is the smaller number of rectal versus colon cancer cases for each gender and hence a reduced statistical power to observe any effects. Nonetheless, the site-specific associations observed in this study require further confirmation.

It may be suggested that nuts and seeds are acting as a marker of consumption of fruits and vegetables or just a generally healthier diet and that the observations of this study may be attributable to these factors instead of an effect of nuts and seeds as a food group. However, this does not seem to be the case because (*a*) adjustments for vegetables, legumes, meats and meat products, and cereals and cereal products, either individually or in combination, did not alter the results and (*b*) total intake of nuts and seeds was not highly correlated with intake of either dietary fiber, vegetables, legumes, fruits, cereals, vitamin E, or any other major food component.

As has been suggested to be the case for their effects on heart disease (2-4), part of the potential cancer protective effects of nuts and seeds may be attributable to their high content of unsaturated fatty acids. In this study, the persistence of a protective effect when intakes of energy from fats were included as covariates in the analysis model suggests that the fat profile of nuts and seeds as a whole may play a role in the observed associations. However, these remained unchanged after adjusting for the ratio of polyunsaturated or monounsaturated to saturated fatty acids, suggesting that the observations of this study may not be entirely due to the unsaturated fatty acid components of nuts and seeds.

From the EPIC dietary questionnaires, it is not possible to determine the exact types of nuts or seeds that are contributing to the overall intakes determined in this analysis; thus, further research is necessary to determine the exact type of nuts and seeds consumed by Europeans. Likewise, due to the small amount of information currently available regarding the phytochemical contents

of different varieties of nuts and seeds, much further research is necessary to detail the bioactive profiles of different varieties and to establish their contribution to the observations of this study.

The total amount of nut and seed intake used in this study does not include foods such as turron (a nut candy popular in Spain), marzipan (almond paste), nut-chocolate spreads, or other products that contain nuts. The contribution of these foods to overall nut and seed consumption in the EPIC database is very low and the testing of their inclusion as part of the nuts and seeds group did not at all affect the results of this study. However, chestnuts are included in this database as part of total nut and seed intake for Spain and the Malmö center of Sweden, whose partly open-ended questionnaires picked up chestnut consumption, and they may also be included for those countries whose questionnaires asked about nonspecific nut and seed intake (Table 1). Although chestnuts are tree nuts by definition, they do not have the same fat and phytochemical profile as other nuts; hence, their inclusion in this study may have reduced the power of the analyses. However, removal of chestnuts as part of the total nut and seed intake values from Spain and the Malmö center of Sweden, where their intake is readily identifiable, did not change the results of this study in any way.

In conclusion, the EPIC study has shown that higher intake of nuts and seeds is not associated with a CRC protective effect in men and women combined. However, subgroup analyses by gender suggest that it may be associated with a reduced incidence of colon cancer in women with no observed effects on rectal cancer for either gender. The potential gender and site–specific associations observed in this study require further confirmation before any recommendations can be made about increasing intake of nuts and seeds in the general population.

References

1. Dreher ML, Maher CV, Kearney P. The traditional and emerging role of nuts in healthful diets. Nutr Rev 1996;54:241–5.
2. Hu FB, Stampfer MJ, Manson JE, et al. Frequent nut consumption and risk of coronary heart disease in women: prospective cohort study. Br Med J 1996;317:1341–5.
3. Fraser GE, Sabate J, Beeson WL, Strahan TM. A possible protective effect of nut consumption on risk of coronary heart disease. The Adventist Health Study. Arch Intern Med 1992;152:1416–24.
4. Prineas RJ, Kushi LH, Folsom AR, Bostick RM, Wu Y. Walnuts and serum lipids. N Engl J Med 1993;328:603–7.
5. Jain MG, Hislop GT, Howe GR, Ghadirian P. Plant foods, antioxidants and prostate cancer risk: findings from case-control studies in Canada. Nutr Cancer 1999;34:173–84.
6. Mills PK, Beeson WL, Phillips RL, Fraser GE. Cohort study of diet, lifestyle, and prostate cancer in Adventist men. Cancer 1989;64: 598–604.
7. Hebert JR, Hurley TG, Olendzki BC, Teas J, Ma Y, Hampl S. Nutritional and socioeconomic factors in relation to prostate cancer mortality: a cross-national study. J Natl Cancer Inst 1998;90:1637–47.
8. Davis PA, Iwahashi CK. Whole almonds and almond fractions reduce aberrant crypt foci in a rat model of colon carcinogenesis. Cancer Lett 2001;165:27–33.
9. Pickle LW, Greene MH, Ziegler RG, et al. Colorectal cancer in rural Nebraska. Cancer Res 1984;44:363–9.
10. Peters RK, Pike MC, Garabrant D, Mack TM. Diet and colon cancer in Los Angeles County, California. Cancer Causes Control 1992;3: 457–73.
11. Kune S, Kune GA, Watson LF. Case-control study of dietary etiological factors: the Melbourne Colorectal Cancer Study. Nutr Cancer 1987;9:21–42.
12. Singh PN, Fraser GE. Dietary risk factors for colon cancer in a low risk population. Am J Epidemiol 1998;148:761–74.
13. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113–24.
14. Riboli E, Kaaks R. The EPIC project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26:S6–14.
15. Bingham SA, Day NE, Luben R, et al. Dietary fiber in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. Lancet 2003;361:1496–501.
16. Kaaks R, Slimani N, Riboli E. Pilot phase studies on the accuracy of dietary intake measurements in the EPIC project: overall evaluation of results. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26:S26–36.
17. Bohlscheid-Thomas S, Hoting I, Boeing H, Wahrendorf J. Reproducibility and relative validity of food group intake in a food frequency questionnaire developed for the German part of the EPIC project. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26:S59–70.
18. Ocke MC, Bueno-de-Mesquita HB, Goddijn HE, et al. The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int J Epidemiol 1997;26:S37–48.
19. Day NE, Ferrari P. Some methodological issues in nutritional epidemiology. IARC Sci Publ 2002;156:5–10.
20. McCashland TM, Brand R, Lyden E, de Garmo P. Gender differences in colorectal polyps and tumors. Am J Gastroenterol 2001;96:882–6.
21. dos Santos Silva I, Swerdlow AJ. Sex differences in the risks of hormone-dependent cancers. Am J Epidemiol 1993;138:10–28.
22. Kaaks R, Riboli E. Validation and calibration of dietary measurements in the EPIC project: methodological consideration. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26:S15–25.
23. Slimani N, Kaaks R, Ferrari P, et al. European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study: rationale, design and population characteristics. Public Health Nutr 2002;5:1125–45.
24. Bocker LK, Van der Schouw YT, De Kleijn MJJ, Jacques PF, Grobbee DE, Peeters PHM. Intake of dietary phytoestrogens by Dutch women. J Nutr 2002;132:1319–28.
25. Kris-Etherton PM, Hecker KD, Bonanome A, et al. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 2002;113:71–88S.
26. Liggins J, Bluck LJC, Runswick S, Atkinson C, Coward WA, Bingham SA. Daidzein and genistein content of fruits and nuts. J Nutr Biochem 2000;11:326–31.
27. Potter JD, Slattery ML, Bostick RM, Gasptur SM. Colon cancer: a review of the epidemiology. Epidemiol Rev 1993;15:499–545.
28. Di Leo A, Messa C, Cavallini A, Linsalata M. Estrogens and colorectal cancer. Curr Drug Targets Immune Endocr Metabol Disord 2001;1:1–12.
29. Soriguer FJ, Gonzalez-Romero S, Esteva I, et al. Does the intake of nuts and seeds alter the appearance of menarche? Acta Obstet Gynecol Scand 1995;74:455–61.
30. Borugian MJ, Sheps SB, Whittemore AS, Wu AH, Potter JD, Gallagher RP. Carbohydrates and colorectal cancer risk among Chinese in North America. Cancer Epidemiol Biomarkers Prev 2002;11:187–93.
31. Wu K, Willett WC, Fuchs CS, Colditz GA, Giovannucci EL. Calcium intake and risk of colon cancer in women and men. J Natl Cancer Inst 2002;94:347–6.
32. Hu JF, Liu YY, Yu YK, Zhao TZ, Liu SD, Wang QQ. Diet and cancer of colon and rectum: a case-control study in China. Int J Epidemiol 1991;20:362–7.
33. Graham S, Dayal H, Swanson M, Mittleman A, Wilkinson G. Diet in the epidemiology of cancer of the colon and rectum. J Natl Cancer Inst 1978;61:709–14.
34. Phillips RL, Snowdon DA. Dietary relationships with fatal colorectal cancer among Seventh-Day Adventists. J Natl Cancer Inst 1985;74: 307–17.
35. Nakaji S, Umeda T, Shimoyama T, et al. Environmental factors affect colon carcinoma and rectal carcinoma in men and women differently. Int J Colorectal Dis 2003;18:481–6.

Short Communication

Is the Association with Fiber from Foods in Colorectal Cancer Confounded by Folate Intake?

Sheila A. Bingham,[1] Teresa Norat,[3] Aurelie Moskal,[3] Pietro Ferrari,[3] Nadia Slimani,[3] Françoise Clavel-Chapelon,[4] Emmanuelle Kesse,[4] Alexandra Nieters,[5] Heiner Boeing,[6] Anne Tjønneland,[7] Kim Overvad,[8] Carmen Martinez,[9] Miren Dorronsoro,[10] Carlos A. González,[11] Eva Ardanaz,[12] Carmen Navarro,[13] José R. Quirós,[14] Timothy J. Key,[15] Nicholas E. Day,[2] Antonia Trichopoulou,[16] Androniki Naska,[16] Vittorio Krogh,[17] Rosario Tumino,[18] Domenico Palli,[19] Salvatore Panico,[20] Paolo Vineis,[21,22] H.B. Bueno-de-Mesquita,[23] Marga C. Ocké,[23] Petra H.M. Peeters,[24] Göran Berglund,[25] Göran Hallmans,[26] Eiliv Lund,[27] Guri Skeie,[27] Rudolf Kaaks,[3] and Elio Riboli[3]

[1]Medical Research Council Dunn Human Nutrition Unit; [2]Strangeways Research Laboratory, University of Cambridge, Cambridge, United Kingdom; [3]International Agency for Research on Cancer (IARC-WHO), Lyon, France; [4]Institut National de la Sante et de la Recherche Medicale, Institut Gustave Roussy, Villejuif, France; [5]German Cancer Research Center, Heidelberg, Germany; [6]German Institute of Human Nutrition, Potsdam-Rehbücke, Germany; [7]Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark; [8]Institute of Epidemiology and Social Medicine, University of Aarhus, Aarhus, Denmark; [9]Andalusian School of Public Health, Granada, Spain; [10]Department of Public Health of Guipuzkoa, San Sebastian, Spain; [11]Catalan Institute of Oncology, Barcelona, Spain; [12]Public Health Institute of Navarra, Pamplona, Spain; [13]Epidemiology Department, Health Council of Murcia, Murcia, Spain; [14]Public Health Directorate for Health and Social Services of Asturias, Oviedo, Spain; [15]Epidemiology Unit, Cancer Research UK, University of Oxford, Oxford, United Kingdom; [16]University of Athens Medical School, Athens, Greece; [17]Department of Epidemiology, National Cancer Institute, Milan, Italy; [18]Cancer Registry, Azienda Ospedaliera ''Civile M.P. Arezzo,'' Ragusa, Italy; [19]Molecular and Nutritional Epidemiology Unit, Centro per lo Studio e la Prevenzione Oncologica, Scientific Institute of Tuscany, Florence, Italy; [20]Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy; [21]Cancer Epidemiology Department, University of Turin, Turin, Italy; [22]Department of Epidemiology and Public Health, Imperial College of Science, Technology and Medicine, London, United Kingdom; [23]National Institute of Public Health and the Environment, Bilthoven, the Netherlands; [24]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands; [25]Malmö Diet and Cancer Study, Lund University, Malmö, Sweden; [26]Department of Nutritional Research, University of Umeå, Umeå, Sweden; and [27]Institute of Community Medicine, University of Tromsø, Tromsø, Norway

Abstract

The effect of multivariate adjustment including folate on the strong protective effect of fiber in foods on colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition was investigated in 1,721 cases identified in the latest follow-up. The inclusion of an additional 656 cases confirmed our previously published results, with a strong and significant reduction in colorectal cancer risk of ~9% for each uncalibrated quintile increase in fiber ($P_{\text{linear trend}} < 0.001$) compared with an 8% reduction in our previous report, which had not been adjusted for folate. Inclusion of the other covariates (physical activity, alcohol, smoking, and red and processed meat) confirmed this significant inverse association for colon cancer and strengthened the association with left-sided colon cancer ($P < 0.001$). After maximum adjustment, the association between fiber and rectal cancer was not significant, as in our previous analysis. The association with fiber from different food sources was analyzed, but again, there were no significance trends after maximum adjustment. (Cancer Epidemiol Biomarkers Prev 2005;14(6):1552–6)

Introduction

In the largest prospective study on diet and cancer thus far conducted, the European Prospective Investigation into Cancer and Nutrition (EPIC), dietary fiber from foods was inversely related to incidence of large bowel cancer (1). However, it has been argued that studies in European populations are more prone to confounding by folate intake because folic acid fortification of cereals is not mandatory (2). A further contention is that the reason for the discrepancy between the results from this large European study and those from intervention and prospective studies elsewhere was that no adjustment for folate intake was made (2). Therefore, we investigated this supposition in the EPIC study, utilizing our data from a more recent follow-up of 1,721 cases for whom data on intake of folate from foods was available. In addition, we have investigated the suggestion that it is fiber from fruit, rather than total fiber, that is protective in colorectal cancer.

Materials and Methods

Outline. The total EPIC cohort consists of subcohorts recruited in 22 centers in 10 European countries: Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, United Kingdom, allowing comparisons between areas with very different rates of cancer occurrence and distribution of lifestyle and food habits. Food-related

Received 12/6/04; revised 3/15/05; accepted 4/8/05.

Grant support: ''Europe Against Cancer'' Programme of the European Commission (SANCO); Ligue contre le Cancer (France); Société 3M (France); Mutuelle Générale de l'Education Nationale; Institut National de la Santé et de la Recherche Médicale; German Cancer Aid; German Cancer Research Center; German Federal Ministry of Education and Research; Danish Cancer Society; Health Research Fund (FIS) of the Spanish Ministry of Health; ISCIII, Red de Centros RCESP, C03/09; the participating regional governments and institutions of Spain; Cancer Research UK; Medical Research Council, United Kingdom; the Stroke Association, United Kingdom; British Heart Foundation; Department of Health, United Kingdom; Food Standards Agency, United Kingdom; the Wellcome Trust, United Kingdom; Greek Ministry of Health; Greek Ministry of Education; Italian Association for Research on Cancer; Italian National Research Council; Dutch Ministry of Public Health, Welfare and Sports; Dutch Ministry of Health; Dutch Prevention Funds; LK Research Funds; Dutch Zorg Onderzoek Nederland; World Cancer Research Fund; Swedish Cancer Society; Swedish Scientific Council; Regional Government of Skane, Sweden; Norwegian Cancer Society, Norwegian Research Council and Norwegian Foundation for Health and Rehabilitation.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Request for reprints: Elio Riboli, Nutrition and Hormones Group, IARC-WHO, 150 cours Albert-Thomas, 69372 Lyon cedex 08, France. Phone: 33-472-73-84-11; Fax: 33-472-73-83-61.

questionnaires and lifestyle and personal questionnaires, as well as anthropometric measurements, were collected from all subjects at the time of enrollment in the cohort. Methods were reported in full by Riboli et al. (3).

Study Subjects. The 519,978 eligible study subjects were mostly aged 25 to 70 years and recruited from the general population residing in a given geographic area, a town or a province. Exceptions were the French cohort (based on female members of the health insurance for state school employees), the Utrecht cohort (based on women attending breast cancer screening), the Ragusa cohort (based on blood donors and their spouses), and most of the Oxford cohort (based on vegetarian volunteers and healthy eaters). Eligible subjects were invited to participate in the study by mail or by personal contact. Those who accepted gave informed consent, and diet and lifestyle questionnaires were mailed to them to be filled in. Anthropometric measurements, including height, weight, waist, hip, and sitting height, were obtained as described elsewhere (3).

Diet and Lifestyle Questionnaires. Following the results of several methodologic studies conducted in the early 1990s, diet was measured by country-specific questionnaires designed to capture local dietary habits and to provide high compliance. Seven countries adopted an extensive self-administered dietary questionnaire, which provided data on up to 300- to 350-food items per country. In Spain and Sicily, a dietary questionnaire, very similar in content to the above but administered by direct interview, was used. A food frequency questionnaire and a 7-day record were adopted in the United Kingdom. The food frequency questionnaire was used in this analysis. The lifestyle questionnaires included questions on education, occupation, leisure and job-related physical activity, history of previous illness and disorders or surgical operations, and lifetime history of consumption of tobacco and alcoholic beverages.

End Points. The follow-up was based on population cancer registries in seven of the participating countries: Denmark, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom. A combination of methods, including health insurance records, cancer and pathology registries, and active follow-up through study subjects and their next-of-kin, was used in three countries—France, Germany, and Greece. Mortality data are also collected from either the cancer registry or mortality registries at the regional or national level. By April 2004, for all centers using cancer registry data, reports to the IARC represented complete follow-up until December 2000 (Asturias, Murcia, Bilthoven, and Cambridge), 2001 (Italy, Granada, Navarra, San Sebastian, Oxford, Norway, and Malmo), 2002 (Umea, Denmark, and France), or 2003 (Utrecht). In Turin, the follow-up was completed until December 1999. For the three countries using individually based follow-up, the end of the follow-up was considered to be the last known contact, or date of diagnosis, or death.

The 10th Revision of the International Statistical Classification of Diseases, Injuries, and Causes of Death was used. Mortality data were coded following the rules of ICD-10, and cancer incidence data following ICD-0-2. Cancer of the rectum included tumors occurring at the rectosigmoid junction (C19) and rectum (C20). Anal canal tumors were excluded. Right colon tumors included the cecum, appendix, ascending colon, hepatic flexure, transverse colon, and splenic flexure (C18.0-18.5). Left colon tumors included the descending and sigmoid colon (C18.6-18.7). All colorectal incident cases (ICD-0-2 C18, C19, and C20) with dietary data were included, but prevalent cases were excluded.

Statistical Methods. Detailed descriptions of the statistical methods used are described in the original publication (1). For this analysis, sex-specific cohort-wide quintiles of total dietary fiber and fiber from different sources were used. Data from individuals in the top and bottom 1% of the ratio of energy intake to estimated energy requirement (calculated from age, sex, and body weight) and from the top 1% of sex-specific fiber intakes were excluded from the analysis to reduce the impact of implausible extreme values. Results are reported using Cox regression, stratified by center to control for different methods of fiber analysis used in European food

Table 1. Baseline characteristics by quintile of fiber intake in EPIC participants

Quintile of dietary fiber	Men					Women					*P*
	Q_1	Q_2	Q_3	Q_4	Q_5	Q_1	Q_2	Q_3	Q_4	Q_5	
Dietary fiber (mean g/d)	18.2	21.0	23.2	25.6	30.1	15.9	17.8	19.4	21.3	24.3	
Colorectal cancer cases	198	167	156	136	107	238	231	197	199	197	
Age (y)	52.0	52.5	52.4	52.1	51.2	50.8	51.2	51.4	51.3	51.2	<0.001
Weight (kg)	81.1	81.3	81.2	81.3	81.4	66.9	67.4	67.4	67.6	68.1	0.01
Height (m)	1.74	1.75	1.75	1.75	1.76	1.62	1.62	1.63	1.63	1.63	<0.001
Body mass index (kg/m^2)	26.8	26.7	26.5	26.4	26.4	25.6	25.6	25.5	25.5	25.6	<0.001
Physical activity (%)											<0.001*
Inactive	33.1	28.9	26.4	22.6	17.2	31.0	28.3	26.4	25.0	23.7	
Moderately inactive	27.9	32.1	33.0	32.3	30.3	22.6	24.1	24.9	25.4	26.9	
Moderately active	18.4	19.4	19.9	21.6	23.9	23.8	25.7	26.6	28.7	32.2	
Active	17.7	16.8	17.6	19.8	24.2	7.7	7.1	7.0	7.0	7.3	
Unknown	2.9	2.7	3.2	3.7	4.4	14.9	14.8	15.1	13.9	9.9	
Smoking status (%)											<0.001*
Never	29.7	31.5	32.8	33.9	35.3	48.1	53.7	55.8	58.3	61.2	
Former	34.8	38.2	37.8	36.9	35.2	21.7	23.3	23.4	23.0	21.9	
Smoker	33.6	28.8	27.9	27.8	28.1	28.2	20.8	18.5	16.2	14.2	
Unknown	1.9	1.5	1.5	1.5	1.4	2.0	2.2	2.3	2.5	2.7	
Energy from fat (kcal/d)	694	791	862	938	1,060	561	637	690	748	833	<0.001
Energy from nonfat (kcal/d)	1,195	1,397	1,547	1,712	1,975	930	1,109	1,233	1,359	1,565	<0.001
Red and processed meat (g/d)	123.3	111.8	104.4	95.9	79.7	83.4	76.1	70.6	64.6	53.6	<0.001
Folate (μg/d)	243.8	282.2	308.9	336.5	389.6	209.6	247.1	271.2	297.3	350.8	<0.001
Alcohol (g/d)	33.5	25.6	20.3	15.3	7.8	11.8	9.3	7.8	6.2	3.8	<0.001

NOTE: Values are means adjusted for center (all variables) and age at enrollment (all variables except age). Quintiles of fiber are based on the food frequency questionnaires, and means were estimated from 24-hour recall data. Means of other dietary variables are based on food frequency questionnaires and adjusted for energy intake.

*P values for trend and association were the same for men and women. All values are significant due to large size of the cohort.

Table 2. Multivariate hazard ratios and 95% confidence intervals of colorectal cancer by quintile of dietary fiber using different variables for adjustment

	Study-wide quintiles*										P_{trend}	HR (95% CI) for one quintile of increase
	1		2		3		4		5			
Fiber (g/d)	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean		
Men	<16.0	18.2	16-20	21.0	20.1-24	23.2	24.1-29.4	25.6	>29.4	30.1		
Women	<15.5	15.9	15.5-19	17.8	19.1-22.4	19.4	22.5-26.7	21.3	>26.7	24.3		
Colorectum												
Base (n =1,065)†		1	0.94 (0.78-1.13)		0.77 (0.63-0.95)		0.76 (0.61-0.95)		0.75 (0.59-0.96)		0.005	0.92 (0.87-0.97)
Base (n = 1,721)‡		1	0.90 (0.78-1.04)		0.77 (0.66-0.90)		0.73 (0.62-0.86)		0.70 (0.58-0.85)		<0.001	0.91 (0.87-0.95)
Base + folate (n = 1,721)§		1	0.89 (0.77-1.03)		0.76 (0.64-0.89)		0.71 (0.60-0.85)		0.68 (0.55-0.84)		<0.01	0.90 (0.86-0.95)
Maximally adjusted (n = 1,721)‖		1	0.93 (0.80-1.08)		0.82 (0.69-0.97)		0.79 (0.66-0.96)		0.79 (0.63-0.99)		0.01	0.93 (0.89-0.99)
Colon												
Base (n = 706)†		1	0.95 (0.75-1.19)		0.75 (0.58-0.96)		0.71 (0.55-0.94)		0.72 (0.54-0.97)		0.006	0.91 (0.85-0.97)
Base (n = 1,118)‡		1	0.88 (0.74-1.05)		0.71 (0.58-0.86)		0.68 (0.55-0.84)		0.74 (0.58-0.93)		<0.001	0.91 (0.86-0.96)
Base + folate (n = 1,118)§		1	0.87 (0.73-1.04)		0.70 (0.57-0.85)		0.66 (0.53-0.83)		0.71 (0.55-0.92)		<0.001	0.90 (0.85-0.96)
Maximally adjusted (n = 1,118)‖		1	0.89 (0.74-1.07)		0.72 (0.59-0.89)		0.70 (0.55-0.88)		0.77 (0.58-1.02)		0.01	0.92 (0.86-0.98)
Colon left												
Base (n = 286)†		1	0.66 (0.46-0.93)		0.55 (0.37-0.80)		0.51 (0.34-0.77)		0.65 (0.43-0.99)		0.006	0.89 (0.80-0.99)
Base (n = 496)‡		1	0.68 (0.53-0.89)		0.60 (0.45-0.80)		0.49 (0.36-0.67)		0.65 (0.46-0.91)		0.001	0.87 (0.80-0.95)
Base + folate (n = 496)§		1	0.67 (0.51-0.88)		0.58 (0.43-0.77)		0.47 (0.33-0.65)		0.60 (0.42-0.86)		<0.001	0.86 (0.78-0.93)
Maximally adjusted (n = 496)‖		1	0.66 (0.50-0.86)		0.56 (0.42-0.76)		0.45 (0.32-0.64)		0.58 (0.39-0.86)		<0.001	0.85 (0.77-0.93)
Colon right												
Base (n = 287)†		1	1.21 (0.84-1.71)		0.93 (0.63-1.37)		0.89 (0.59-1.35)		0.73 (0.46-1.19)		0.09	0.91 (0.82-1.05)
Base (n = 476)‡		1	1.19 (0.90-1.58)		0.94 (0.68-1.28)		0.95 (0.68-1.34)		0.88 (0.59-1.29)		0.09	0.95 (0.87-1.04)
Base + folate (n = 452)§		1	1.18 (0.88-1.57)		0.92 (0.66-1.27)		0.93 (0.65-1.32)		0.85 (0.54-1.28)		0.21	0.94 (0.85-1.03)
Maximally adjusted (n = 452)‖		1	1.22 (0.91-1.64)		0.97 (0.69-1.35)		1.00 (0.69-1.45)		0.93 (0.59-1.47)		0.47	0.96 (0.87-1.07)
Rectum												
Base (n = 359)†		1	0.92 (0.66-1.27)		0.83 (0.59-1.18)		0.85 (0.59-1.24)		0.80 (0.53-1.22)		0.22	0.95 (0.85-1.05)
Base (n = 603)‡		1	0.94 (0.73-1.19)		0.88 (0.68-1.15)		0.84 (0.63-1.10)		0.62 (0.44-0.87)		0.01	0.91 (0.84-0.98)
Base + folate (n = 603)§		1	0.92 (0.72-1.18)		0.87 (0.67-1.14)		0.81 (0.60-1.10)		0.60 (0.41-0.87)		0.01	0.90 (0.83-0.98)
Maximally adjusted (n = 603)‖		1	1.02 (0.79-1.31)		1.01 (0.77-1.34)		1.01 (0.74-1.38)		0.81 (0.55-1.21)		0.50	0.97 (0.89-1.06)

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.
*The ranges are based on the food frequency questionnaires, and the means are estimated from 24-hour recall data from participants in the calibration study.
†From published results (1).
‡Base: Cox regression using age, sex, energy from nonfat sources (continuous variable), energy from fat sources (continuous variable), height, and weight (tertiles defined for each sex and center), and stratified for center (same adjustment as in ref. 1).
§Base + folate: Same covariates as base and folate.
‖Maximally adjusted: Same covariates as base + folate and physical activity (five categories), alcohol consumption (g/d), smoking status (never, former, current smoker, missing), educational level, and intake of red and processed meat.

tables and other center effects, such as follow-up procedures and questionnaire design. Age was used as the primary time variable in all Cox regression models. Age at colorectal cancer incidence or at censoring date was used as time variable of end of the study. The analysis focused on dietary fiber, with some other dietary and lifestyle variables included as covariates. Analyses were run using variables both as categorical and as continuous scored from 1 to 5 according to the interquintile interval in which an observation lay. Trend tests were computed using these quintile-based scores. Categorical relative risks were calculated from the hazard ratio. Estimated energy intake was divided into energy from fat and energy from nonfat sources as described elsewhere (1). Models were run first using the same model as previously published with age, sex, energy from nonfat sources and fat energy (continuous variable), height, and weight (tertiles defined for each sex and center; base model). Analyses were then run including folate from food as a continuous variable in the model (base model plus folate). Third, risks were adjusted in addition for physical activity (five categories of leisure and occupational activities), smoking status (four categories), alcohol consumption (grams per day), and red and processed meat (grams per day; maximally adjusted model).

Results

There were 2,279,075 person-years in 6.2 average years of follow-up (3-8.4 years) since 1992, and 1,826 colorectal cancer cases; 1,178 tumors were located in the colon and 648 were rectal tumors; 523 colon cancers were located on the right side, and 476 on the left side of the colon. The analyses presented here are based on 1,721 cases because folate intake in participants from Greece and Heidelberg was not available in the central data set.

Baseline characteristics by quintile of fiber intake are shown in Table 1. Age was positively associated with fiber intake in women and inversely in men. Body mass index was inversely related with fiber intake in men only. Physical activity was positively related with fiber intake, whereas smoking, alcohol, and red meat intakes were inversely related to fiber intake. Trends for folate by quintile of dietary fiber were significant because of a significant correlation between the two (Spearman partial correlation coefficient adjusted for age, energy intake, and center, r = 0.35 men, r = 0.28 women). Partial correlation coefficients between fiber from vegetables and folate intake were also positive (0.55 men, 0.61 women), as were fiber from fruits (0.25 men, 0.27 women) and from legumes (0.21 men, 0.34 women). The partial correlation

coefficients between cereal fiber and folate were heterogeneous (0.09 men; −0.21 women overall EPIC; negative or close to zero in France, Italy, and Spain; and of similar value to the correlation with fiber from fruits and legumes in the remaining countries).

Table 2 shows hazard ratios for 1,721 cases adjusted as in the previously published report. The inclusion of an additional 656 cases confirmed our previously published results, with a strong and significant reduction in colorectal cancer risk of ~9% for each quintile increase of fiber ($P_{\text{linear trend}} < 0.001$) compared with an 8% reduction in our previous report (1). As before, the reduction in risk was apparent at the third quintile of fiber intake of approximately >20 g of fiber per day compared with <16 g/d. Adjustment for folate, in addition (base model plus folate), did not materially alter the results for colon cancer but the inverse association with left-sided colon cancer was slightly strengthened. Results for right-sided colon cancer were not significant, as before. Adjustment for folate did not materially affect results for rectal cancer. Results were not changed when use of educational levels (five categories) or multivitamins (yes/no) was also included; for example, the hazard ratio for colon cancer for the highest versus lowest quintile of fiber was 0.74 (confidence interval, 0.56-0.98). Results were consistent across countries ($P_{\text{heterogeneity}} = 0.72$; Fig. 1).

In the maximally adjusted model, inclusion of the other covariates (physical activity, alcohol, smoking, and red and processed meat) with folate strengthened the results for left-sided colon cancer ($P < 0.001$). After maximum adjustment, the association between fiber and rectal cancer was not significant, as in our previous analysis.

Table 3 shows hazard ratios for colorectal cancer by different types of fiber. With more cases, the hazard ratios remained essentially the same for all types of fiber as before (1), although the trends became significant for fiber from cereals ($P = 0.01$) and from fruit ($P = 0.04$). Adjustment for folate (base model plus folate) did not materially affect categorical results, although the trend for fiber from fruit became nonsignificant. In the maximally adjusted model, the hazard ratios and the trend for fiber from cereals also became nonsignificant. In the maximally adjusted model, hazard ratios for fruit fiber were statistically significant for the 2nd, 3rd, and 5th quintile of intake compared with the 1st quintile, but the trend for fiber from fruits was not significant.

Discussion

Our original publication was the largest prospective study published to date on fiber in food in colorectal cancer prevention (1). The size of the study allowed analysis by subsite and, furthermore, the detailed dietary analysis of heterogeneous populations allowed some correction for measurement error in dietary intake, a problem that has increasingly caused concern in nutritional epidemiology in relation to cancer (4, 5). These results have, however, been questioned because no adjustment was made for dietary folate (2). The present report in which an even larger number of cases was included confirms our original results of a strong protective association between fiber intake in food and risk of colon cancer. Furthermore, hazard ratios were somewhat strengthened for left-sided colon cancer. Contrary to the suggestion that results for colorectal cancer would be confounded by folate intake in this European population, adjustment for folate did not modify our findings.

A recent editorial (6) on the finding of a null association of fruits and vegetables with cancer risk in two cohort studies raises the problem of multivariate modeling in the presence of measurement error and weak associations. Although prospective epidemiologic evidence to date does not provide strong support for a protective association between fruit and vegetable intake and cancer, ''... it is important to be alert to the possibility that findings emerging from new, large cohort studies could shift the preponderance of the evidence, as may be occurring with the dietary fiber-colorectal cancer association'' (6). In our report, we showed that the protective association of fiber with colon cancer is observed in both less and more adjusted models. As stated in the editorial, efforts should be made to study diet and cancer in populations with a wide range of dietary intake, because it is the ratio of interindividual variation to intraindividual measurement error that determines the magnitude of relative risk distortion. Such was the approach behind the EPIC study (7).

Although calibration was previously shown to considerably strengthen associations with fiber and colorectal cancer, in this report, which specifically addresses the issue of confounding factors, results were essentially the same as previously published; therefore, we have not calibrated our results. We have presented more detailed results of our previous findings on the effect of other suggested confounders, which were reported before. In our previous report, there was no effect of

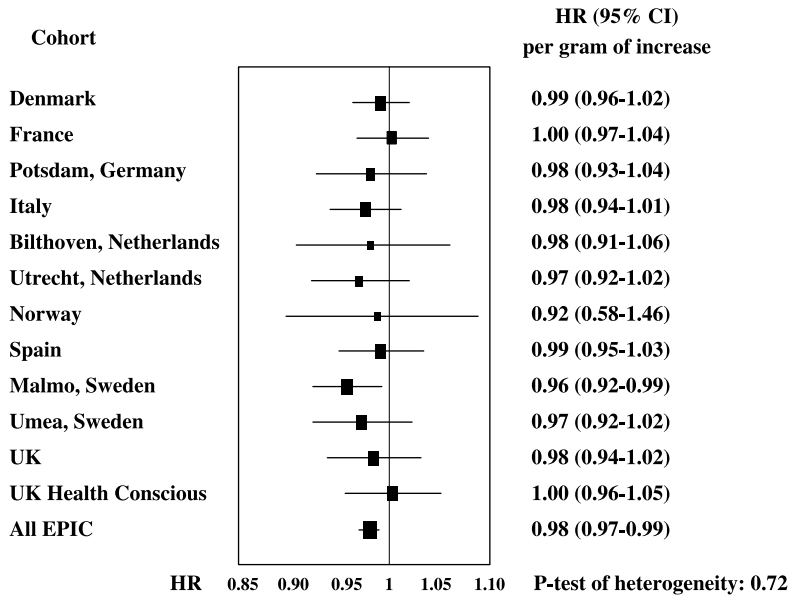


Figure 1. Multivariate hazard ratio (*HR*) and 95% confidence intervals (*95% CI*) of colorectal cancer for dietary fiber in EPIC cohorts. Cox regression using age, sex, energy from nonfat sources (continuous variable), energy from fat sources (continuous variable), height and weight (tertiles defined for each sex and center), folate, physical activity (five categories), alcohol consumption (g/d), smoking status (never, former, current smoker, missing), and intake of red meat and processed meat.

Table 3. Multivariate hazard ratios of colorectal cancer and 95% confidence intervals for quintiles of fiber intake by source of fiber

Source of fiber	Study-wide quintiles*										*P* trend
	1		2		3		4		5		
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	
Fiber from fruits (g/d)											
Men	<1.4	2.7	1.4-2.3	3.1	2.4-3.5	3.8	3.5-5.2	4.3	>5.2	5.3	
Women	<2.1	2.8	2.1-3.2	3.4	3.3-4.5	3.9	4.6-6.6	4.4	>6.6	5.4	
Base (n = 1,065)†	1		0.69 (0.57-0.85)		0.76 (0.63-0.92)		0.82 (0.66-0.99)		0.78 (0.64-0.97)		0.17
Base (n = 1,721)‡	1		0.73 (0.62-0.85)		0.74 (0.63-0.87)		0.84 (0.71-0.98)		0.74 (0.62-0.87)		0.04
Base + folate (n = 1,721)§	1		0.73 (0.62-0.86)		0.74 (0.63-0.87)		0.84 (0.72-0.99)		0.75 (0.63-0.89)		0.07
Maximally adjusted (n = 1,721)‖	1		0.75 (0.64-0.89)		0.78 (0.67-0.92)		0.90 (0.76-1.06)		0.81 (0.68-0.97)		0.42
Fiber from cereals (g/d)											
Men	<4.6	6.6	4.6-7.4	8.1	7.5-10.1	9.5	10.2-13.5	10.5	>13.5	13.1	
Women	<3.9	4.9	3.9-5.8	5.9	5.9-7.7	6.8	7.8-10.6	7.5	>10.6	9.2	
Base (n = 1,065)†	1		0.89 (0.74-1.08)		0.85 (0.69-1.03)		0.88 (0.71-1.08)		0.78 (0.62-0.98)		0.06
Base (n = 1,721)‡	1		1.01 (0.87-1.18)		0.88 (0.74-1.04)		0.90 (0.75-1.07)		0.81 (0.66-0.99)		0.02
Base + folate (n = 1,721)§	1		0.99 (0.84-1.16)		0.88 (0.74-1.05)		0.90 (0.75-1.07)		0.82 (0.67-1.00)		0.03
Maximally adjusted (n = 1,721)‖	1		1.02 (0.87-1.20)		0.94 (0.79-1.11)		0.98 (0.82-1.17)		0.93 (0.76-1.15)		0.44
Fiber from vegetables (g/d)											
Men	<1.4	2.7	1.4-2.3	3.1	2.4-3.4	3.8	3.5-5.2	4.3	>5.2	5.3	
Women	<2.1	2.8	2.1-3.2	3.4	3.3-4.6	3.9	4.6-6.6	4.4	>6.6	5.4	
Base (n = 1,065)†	1		0.94 (0.77-1.15)		0.95 (0.77-1.16)		1.00 (0.81-1.24)		0.88 (0.70-1.11)		0.52
Base (n = 1,721)‡	1		0.98 (0.83-1.16)		0.96 (0.80-1.13)		0.94 (0.79-1.12)		0.92 (0.76-1.10)		0.60
Base + folate (n = 1,721)§	1		0.99 (0.84-1.17)		0.97 (0.82-1.15)		0.96 (0.80-1.16)		0.96 (0.77-1.18)		0.63
Maximally adjusted (n = 1,721)‖	1		0.99 (0.84-1.17)		0.97 (0.82-1.16)		0.96 (0.80-1.16)		0.94 (0.76-1.16)		0.52
Fiber from legumes (g/d)											
Men	0	NC	0-0.1	NC	0.1-0.4	0.5	0.5-1.3	0.9	>1.3	1.9	
Women	0	NC	0-0.1	NC	0.1-0.5	0.4	0.6-1.3	0.7	>1.3	1.0	
Base (n = 1,065)†	1		1.02 (0.83-1.26)		1.10 (0.91-1.34)		1.18 (0.97-1.43)		1.04 (0.84-1.30)		0.31
Base (n = 1,721)‡	1		0.98 (0.83-1.16)		0.96 (0.82-1.12)		1.04 (0.89-1.23)		0.94 (0.79-1.12)		0.77
Base + folate (n = 1,721)§	1		0.98 (0.83-1.16)		0.96 (0.82-1.13)		1.05 (0.89-1.24)		0.95 (0.80-1.14)		0.90
Maximally adjusted (n = 1,719)‖	1		1.00 (0.84-1.18)		0.97 (0.83-1.14)		1.08 (0.91-1.27)		0.98 (0.82-1.17)		0.86

Abbreviation: NC, not computed.

*The ranges are based on the food frequency questionnaires, and the means are estimated from 24-hour recall data from participants in the calibration study. Mean fiber from legumes from 24-hour recall was not computed for the 1st and 2nd quintile because of many zero or extreme values.

†From published results (1).

‡Base: Cox regression using age, sex, energy from nonfat sources (continuous variable), energy from fat sources (continuous variable), height and weight (tertiles defined for each sex and center), and stratified for center (same adjustment as in ref. 1).

§Base + folate: Same covariates as base and folate.

‖Maximally adjusted: Same covariates as base + folate and physical activity (five categories), alcohol consumption (g/d), smoking status (never, former, current smoker, missing), educational level, and intake of meat and processed meat.

adjustment for physical activity, alcohol, smoking status, and red and processed meat in colon cancer (1), whereas in the current analysis this adjustment has minor effects. Further investigation of the use of multivitamin tablets in this European population did not modify our conclusions either. Our former results for rectal cancer were weaker than for colon cancer results and in this report, when fully adjusted, were substantially weakened.

In our first report, we were unable to attribute the effects of fiber to any particular food source. It has been suggested that fiber from fruit is more strongly associated with protection from colorectal cancer than fiber from all sources (2). However, in this EPIC population, trends with fiber intake from fruits were not significant. The effect of fiber from cereals was statistically significant but the significance was lost in the maximally adjusted model. Any mechanism whereby fruit fiber should protect against colorectal cancer is not established but is unlikely to be folate because adjustment for folate had little effect on our results.

References

1. Bingham SA, Day NE, Luben R, et al. European Prospective Investigation into Cancer and Nutrition. Dietary fiber in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. Lancet 2003;361:1496–501.
2. Papas MA, Giovannucci E, Platz E. Fiber from fruit and colorectal neoplasia. Cancer Epidemiol Biomarkers Prev 2004;13:1267–70.
3. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113–24.
4. Day NE, McKeown N, Wong MY, Welch A, Bingham S. Epidemiological assessment of diet: a comparison of a 7-day diary with a food frequency questionnaire using urinary markers of nitrogen, potassium and sodium. Int J Epidemiol 2001;30:309–17.
5. Kipnis V, Midthune D, Freedman LS, et al. Empirical evidence of correlated biases in dietary assessment instruments and its implications. Am J Epidemiol 2001;153:394–403.
6. Schatzkin A, Kipnis V. Could exposure assessment problems give us wrong answers to nutrition and cancer questions? J Natl Cancer Inst 2004;96:1564–5.
7. Riboli E, Kaaks R. The EPIC project: rationale and study design. Int J Epidemiol 1997;26:S6–14.

Body Size and Risk of Colon and Rectal Cancer in the European Prospective Investigation Into Cancer and Nutrition (EPIC)

Tobias Pischon, Petra H. Lahmann, Heiner Boeing, Christine Friedenreich, Teresa Norat, Anne Tjønneland, Jytte Halkjaer, Kim Overvad, Françoise Clavel-Chapelon, Marie-Christine Boutron-Ruault, Gregory Guernec, Manuela M. Bergmann, Jakob Linseisen, Nikolaus Becker, Antonia Trichopoulou, Dimitrios Trichopoulos, Sabina Sieri, Domenico Palli, Rosario Tumino, Paolo Vineis, Salvatore Panico, Petra H. M. Peeters, H. Bas Bueno-de-Mesquita, Hendriek C. Boshuizen, Bethany Van Guelpen, Richard Palmqvist, Göran Berglund, Carlos Alberto Gonzalez, Miren Dorronsoro, Aurelio Barricarte, Carmen Navarro, Carmen Martinez, J. Ramón Quirós, Andrew Roddam, Naomi Allen, Sheila Bingham, Kay-Tee Khaw, Pietro Ferrari, Rudolf Kaaks, Nadia Slimani, Elio Riboli

Background: **Body weight and body mass index (BMI) are positively related to risk of colon cancer in men, whereas weak or no associations exist in women. This discrepancy may be related to differences in fat distribution between sexes or to the use of hormone replacement therapy (HRT) in women.** ***Methods:*** **We used multivariable adjusted Cox proportional hazards models to examine the association between anthropometric measures and risks of colon and rectal cancer among 368 277 men and women who were free of cancer at baseline from nine countries of the European Prospective Investigation Into Cancer and Nutrition. All statistical tests were two-sided.** ***Results:*** **During 6.1 years of follow-up, we identified 984 and 586 patients with colon and rectal cancer, respectively. Body weight and BMI were statistically significantly associated with colon cancer risk in men (highest versus lowest quintile of BMI, relative risk [RR] = 1.55, 95% confidence interval [CI] = 1.12 to 2.15; P_{trend} = .006) but not in women. In contrast, comparisons of the highest to the lowest quintile showed that several anthropometric measures, including waist circumference (men, RR = 1.39, 95% CI = 1.01 to 1.93; P_{trend} = .001; women, RR = 1.48, 95% CI = 1.08 to 2.03; P_{trend} = .008), waist-to-hip ratio (WHR; men, RR = 1.51, 95% CI = 1.06 to 2.15; P_{trend} = .006; women, RR = 1.52, 95% CI = 1.12 to 2.05; P_{trend} = .002), and height (men, RR = 1.40, 95% CI = 0.99 to 1.98; P_{trend} = .04; women, RR = 1.79, 95% CI = 1.30 to 2.46; P_{trend}<.001) were related to colon cancer risk in both sexes. The estimated absolute risk of developing colon cancer within 5 years was 203 and 131 cases per 100 000 men and 129 and 86 cases per 100 000 women in the highest and lowest quintiles of WHR, respectively. Upon further stratification, no association of waist circumference and WHR with risk of colon cancer was observed among postmenopausal women who used HRT. None of the anthropometric measures was statistically significantly related to rectal cancer.** ***Conclusions:*** **Waist circumference and WHR, indicators of abdominal obesity, were strongly associated with colon cancer risk in men and women in this population. The association of abdominal obesity with colon cancer risk may vary depending on HRT use in postmenopausal women; however, these findings require confirmation in future studies. [J Natl Cancer Inst 2006;98:920–31]**

A possible association between body size and risk of colorectal cancer has been examined in many epidemiologic studies *(1–30)*. In general, body weight and body mass index (BMI) have been found to be positively related to risk of colon cancer in men, whereas weaker or no associations have been reported for women *(1–30)*. Among the smaller number of studies that examined associations with rectal cancer, most found no association with

Affiliations of authors: Department of Epidemiology, German Institute of Human Nutrition, Potsdam-Rehbruecke, Germany (TP, PHL, HB, MMB); Division of Population Health and Information, Alberta Cancer Board, Calgary, Alberta, Canada (CF); International Agency for Research on Cancer, Lyon, France (TN, PF, RK, NS, ER); Danish Cancer Society, Institute of Cancer Epidemiology, Copenhagen, Denmark (A. Tjønneland, JH); Department of Clinical Epidemiology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark (KO); INSERM U 521, Institut Gustave Roussy, Villejuif, France (FCC, MCBR, GG); Division of Clinical Epidemiology, German Cancer Research Center, Heidelberg, Germany (JL, NB); Department of Hygiene and Epidemiology, School of Medicine, University of Athens, Athens, Greece (A. Trichopoulou, DT); Epidemiology Unit, National Cancer Institute, Milan, Italy (SS); Molecular and Nutritional Epidemiology Unit, CSPO-Scientific Institute of Tuscany, Florence, Italy (DP); Cancer Registry, Azienda Ospedaliera "Civile M.P. Arezzo," Ragusa, Italy (RT); Imperial College London, UK, and University of Torino, Turin, Italy (PV); Dipartimento di Medicina Clinica e Sperimentale, Università di Napoli, Naples, Italy (SP); Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands (PHMP); Center for Nutrition and Health (HBBdM), Center for Information Technology and Informatics (HCB), National Institute of Public Health and the Environment, Bilthoven, The Netherlands; Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden (BVG, RP); Department of Medicine, Lund University, Malmö, Sweden (GB); Department of Epidemiology, Catalan Institute of Oncology, IDIBELL, Barcelona, Spain (CAG); Department of Public Health of Guipuzkoa, San Sebastian, Spain (MD); Public Health Institute of Navarra, Pamplona, Spain (AB); Epidemiology Department, Murcia Health Council, Murcia, Spain (CN); Escuela Andaluza de Salud Publica, Granada, Spain (CM); Health Information Unit, Public Health and Health Planning Directorate, Asturias, Spain (JRQ); Cancer Research UK Epidemiology Unit, University of Oxford, Oxford, UK (AR, NA); Dunn Human Nutrition Unit, Medical Research Council, Cambridge, UK (SB); Department of Public Health and Primary Care, School of Clinical Medicine, University of Cambridge, Cambridge, UK (KTK).

Correspondence to: Tobias Pischon, MD, MPH, Department of Epidemiology, German Institute of Human Nutrition (DIfE), Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114–116, 14558 Nuthetal, Germany (e-mail: pischon@mail.dife.de).

See "Notes" following "References."

DOI: 10.1093/jnci/djj246

body weight or BMI *(2–5,12,17,27,30)*. The reasons for the apparent discrepancy in the association of body weight with colon cancer risk between men and women are unclear.

One potential reason for the discrepancy is that men and women have different body compositions. Fat makes up a lower percentage of the body mass of men (approximately 20%) than of women (approximately 30%). The relationship of body weight to fat distribution also differs between men and women. Higher body weight is more closely related to abdominal obesity than lower body obesity in men and more closely related to gluteofemoral obesity than to abdominal obesity in women. Furthermore, upper-body fat has been shown to be more strongly associated with metabolic abnormalities than lower-body obesity *(31,32)*. However, only a few prospective studies have examined the association of body fat distribution—as reflected by waist and hip circumference—and colon cancer risk *(11,13,14,19,21)*. Also, in most of these studies *(11,13,14)*, waist and hip circumference were self-reported rather than measured.

Other reasons for sex differences in the association between adiposity and colon cancer risk may be related to use of hormone replacement therapy (HRT) in postmenopausal women. Postmenopausal HRT use has been associated with reduced risk of colon cancer in observational and intervention studies *(33–36)* and has been shown to affect the association between body weight and postmenopausal breast cancer *(37)*; however, little is known about associations with colon cancer *(20)*.

The aim of this study was to examine the association between anthropometric measures, including waist and hip circumference, and risk of colon and rectal cancer in participants of the European Prospective Investigation Into Cancer and Nutrition (EPIC), a large European cohort study. In particular, we examined whether body fat distribution is related to risk of colon and rectal cancer. Furthermore, we aimed to examine whether the associations differ among postmenopausal women who were HRT users and those who were not.

Subjects and Methods

Study Population

The EPIC is an ongoing multicenter prospective cohort study designed primarily to investigate the relationship between nutrition and cancer. The EPIC study consists of subcohorts recruited in 23 administrative centers in 10 European countries—Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden, and the United Kingdom. The 519 978 eligible male and female participants were aged between 25 and 70 years at enrollment (1992–2000) and were recruited from the general population residing in a given geographic area (i.e., town or province). Exceptions were the French cohort (based on female members of a health insurance plan for school employees), the Utrecht cohort in The Netherlands (based on women attending breast cancer screening), the Ragusa cohort in Italy (based on blood donors and their spouses), and the Oxford cohort in the United Kingdom (including mainly vegetarian volunteers and healthy eaters). Eligible subjects were invited to participate in the study, and those who accepted gave written informed consent and completed questionnaires on their diet, lifestyle, and medical history. Subjects were then invited to a center to provide a blood sample and to have anthropometric measurements taken. The methods have been reported in full by Riboli et al. *(38,39)*. Approval for this study was obtained from the ethical review boards of the International Agency for Research on Cancer and from all local institutions where subjects had been recruited for the EPIC study.

This study is based on 495 417 participants without prevalent cancer at any site at baseline, as reported on the lifestyle questionnaire or based on information from the cancer registries. We excluded the Umea, Sweden, cohort (n = 24 811) because participants did not provide information on leisure time physical activity that was compatible with the other EPIC questionnaires. We also excluded subjects without measured body height or weight and thus excluded the cohorts from Norway (n = 35 956), 48 960 participants from the French cohorts, and 7903 participants from the other cohorts. For the "health-conscious" group based in Oxford (UK), linear regression models were used to predict sex- and age-specific values from subjects with both measured and self-reported body measures, as previously described *(40,41)*. We further excluded 2166 participants with missing questionnaire data or with missing dates of diagnosis or follow-up and, to reduce the impact on the analysis of implausible extreme values, the 7344 participants who were in the top or bottom 1% of the ratio of energy intake to estimated energy requirement that was calculated from body weight, height, and age *(42)*. Therefore, the study included a total of 368 277 participants.

Assessment of Endpoints

Incident colorectal cancer case patients were identified by population cancer registries (Denmark, Italy, The Netherlands, Spain, Sweden, the United Kingdom) or by active follow-up (France, Germany, Greece), depending on the follow-up system in each of the participating centers. Active follow-up used a combination of methods, including health insurance records, cancer and pathology registries, and direct contact with participants or next of kin. Mortality data were also obtained from cancer or mortality registries at the regional or national level. Follow-up began at the date of enrollment and ended at either the date of diagnosis of colorectal cancer, death, or last complete follow-up. By April 30, 2004, for the centers using record linkage with cancer registry data, complete follow-up was available through December 31, 1999 (Turin, Italy); June 30, 2000 (Bilthoven, The Netherlands); December 31, 2000 (Asturias and Murcia, Spain; Cambridge, UK); December 31, 2001 (Oxford, UK; Malmö, Sweden; Florence, Naples, Ragusa, and Varese, Italy); December 31, 2002 (Granada, Navarra, and San Sebastian, Spain; Aarhus and Copenhagen, Denmark); and June 30, 2003 (Utrecht, The Netherlands). For the centers using active follow-up, the last contact dates were June 30, 2002 (France); November 19, 2002 (Greece); December 16, 2003 (Heidelberg, Germany); and March 11, 2004 (Postdam, Germany). Mortality data were coded following the rules of the 10th revision of the International Statistical Classification of Diseases, Injuries and Causes of Death (ICD-10), and cancer incidence data were coded according to the 2nd revision of the International Classification of Diseases for Oncology (ICD-O-2). We included all patients with colon (C18) and rectal (C19, C20) cancer.

Assessment of Anthropometric Data, Diet, and Lifestyle Factors

Weight and height were measured with subjects wearing no shoes to the nearest 0.1 kg, and—depending on study center—to

the nearest 0.1, 0.5, or 1.0 cm, respectively *(40)*. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Waist circumference was measured either at the narrowest torso circumference (France; Italy; Utrecht, The Netherlands; Denmark) or at the midpoint between the lower ribs and iliac crest (Bilthoven, The Netherlands; Potsdam, Germany; Malmö, Sweden; Oxford, UK, general population). In Spain; Greece; Heidelberg, Germany; and Cambridge, UK, a combination of methods was used, although most participants were measured at the narrowest torso circumference. Hip circumference was measured at the widest circumference (France; Italy; Spain; Bilthoven, The Netherlands; Greece; Malmö, Sweden) or over the buttocks (the United Kingdom; Utrecht, The Netherlands; Germany; Denmark). Results of the present analyses for waist and hip circumference were similar for the different assessment methods. Waist and hip circumference measurements were missing for 3869 (1.05%) and 6399 (1.74%) participants, respectively, who were excluded for analyses on these variables. For this study, body weight and waist and hip circumference were corrected, as described in detail elsewhere *(40)*, to reduce heterogeneity due to protocol differences in clothing worn during measurement.

Diet during the 12 months before enrollment was measured by country-specific validated questionnaires *(43)*. Most centers adopted a self-administered dietary questionnaire covering 88–266 food items. In Greece, Spain, and Ragusa, Italy, the questionnaire was administered at a personal interview. In Malmö, Sweden, a questionnaire combined with a food record was used. Country-specific food composition tables were used to calculate nutrient intakes *(44)*.

Recreational and household activity was computed as average metabolic equivalent-hours (MET-hr), based on the types and durations of activities reported separately for summer and winter on the baseline questionnaires. The reported activities included walking, cycling, gardening, sports and exercise, housework, home repair (do-it-yourself activities), stair climbing, and vigorous recreational activity. Each type of activity was assigned a specific MET value according to Ainsworth et al. *(45)*. Occupational activity was coded as sedentary occupation, standing occupation, manual work, heavy manual work, unemployed, or missing, as reported on the questionnaire. To create a variable for total physical activity, subjects were cross-classified on the basis of sex-specific quartiles of recreational and household activity and on categories of occupational work and were coded as inactive, moderately inactive, moderately active, active, and missing.

Information on sociodemographic and lifestyle characteristics and medical history was obtained from standardized questionnaires at study entry *(38)*. Women were classified according to menopausal status at enrollment on the basis of an algorithm that accounts for complete and combined information on menstrual status/history, type of menopause (natural, bi-/unilateral oophorectomy, hysterectomy), and use of oral contraceptives and menopausal hormones *(37)*. Current HRT use refers to the use of menopausal hormones at the time of recruitment as derived from the country-specific questionnaires or during interviews, and includes estrogen alone and combined estrogen–progestin preparations. The prevalence of HRT use within EPIC has been described in detail elsewhere *(46)*.

Statistical Analyses

We analyzed the association between anthropometric variables and risks of colon and rectal cancer separately for men and women by calculating relative risks (RRs) as incident rate ratios using Cox proportional hazards models. Age was used as the underlying time variable, with entry and exit time defined as the subject's age at recruitment and age at colorectal cancer diagnosis or censoring, respectively. Subjects were grouped into quintiles on the basis of the anthropometric variables of the entire male or female cohorts, respectively. We also performed additional analyses by grouping participants into predefined well-established categories for BMI (<25, 25–<30, or ≥30 kg/m^2), waist circumference (<102 or ≥102 cm in men, and <88 or ≥ 88 in women), and waist-to-hip ratio (WHR; <0.95 or ≥0.95 in men; and <0.80 or ≥0.80 in women) *(47,48)*. Models were stratified by age at recruitment and by study center to reduce sensitivity to any violations of the proportional hazards assumption. We further adjusted the analysis for smoking status (never, past, current, or unknown), education (no school degree or primary school, technical or professional school, secondary school, university degree, or unknown), alcohol consumption (grams/day, continuous), physical activity (inactive, moderately inactive, moderately active, active, missing), fiber intake (grams/day, continuous), and consumption of red and processed meat, fish and shellfish, and fruits and vegetables (all grams/day, continuous). Analyses of weight, waist and hip circumference, and WHR were also adjusted for body height, and, in additional models, for body weight. We also performed additional analyses that adjusted for total energy intake; however, because the overall results did not change substantially, we did not include energy intake in our analysis. To test for linear trend across categories, we used the median anthropometric variable within quintiles as a continuous variable. In separate analyses we included body size measures as continuous variables in the models to estimate the relative risk of colon and rectal cancer per unit increase in each anthropometric variable. Differences in the associations across study centers were assessed with the chi-square test using heterogeneity statistics that are based on the inverse variance method *(49)*. To test for differences between sexes, we performed the analysis with men and women combined and added an interaction term to the model. Among postmenopausal women we further stratified the analysis by HRT use and tested for differences between HRT and non-HRT users by adding an interaction term. The proportional hazards assumption was checked by adding an interaction term of the main exposure variable with time to each model. The interaction term was not statistically significant (at the 5% level) in any model. Absolute risks were estimated from the survivor function with covariates (including age) set to sex-specific mean levels.

All P values presented are two-tailed, and $P<.05$ was considered statistically significant. Analyses were performed using SAS 9.1 (SAS Institute, Cary, NC).

Results

A total of 368 277 participants were monitored for an average 6.1 ± 1.7 years, for a total of 2 254 727 person-years (Table 1). During follow-up, 1570 members of the cohort were diagnosed with colorectal cancer (984 colon, 586 rectum). Mean age at baseline was 51.7 years; 64.8% of participants were female.

We compared the age-standardized characteristics of the EPIC participants at baseline by BMI quintile for men and women, respectively (men and women in higher BMI categories were older than those in the lower BMI categories; Table 2). Alcohol

Table 1. Cohort characteristics, the European Prospective Investigation into Cancer and Nutrition

	Cohort size, n		Mean age, y		Person-years		Colon cancer, n		Rectal cancer, n		Mean BMI, kg/m^2*		Mean WHR*	
Country	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
France	0	19752	0	52.8	0	167895	0	53	0	8	0	23.2	0	0.777
Italy	13895	30325	50.2	50.7	74170	187029	44	64	17	26	26.5	25.7	0.936	0.797
Spain	14986	24616	50.7	48.3	102413	162305	39	39	26	15	28.5	28.4	0.951	0.829
United Kingdom	22542	50998	53.1	47.7	118468	277133	92	91	33	54	25.4	24.5	0.914	0.769
The Netherlands	9890	27484	43.2	51.0	50057	181701	11	100	11	46	26.0	25.2	0.926	0.789
Greece	10529	14922	52.9	53.3	38776	55514	7	6	7	5	27.9	28.4	0.955	0.814
Germany	21340	27712	52.4	49.1	124150	162055	58	44	53	16	27.0	25.8	0.944	0.800
Sweden	10263	14010	59.0	57.3	79008	105781	48	58	46	42	25.6	24.4	0.933	0.782
Denmark	26286	28727	56.6	56.7	174439	193832	122	108	102	79	26.4	25.0	0.949	0.796
Total	129731	238546	52.8	51.1	761482	1493245	421	563	295	291	26.6	25.5	0.939	0.792

*Values are age adjusted. BMI = body mass index; WHR = waist-to-hip ratio.

consumption was positively related to BMI in men but inversely related to BMI in women. Both men and women in the higher BMI categories were less likely than those in the lower BMI categories to be current smokers and more likely to have a lower education level. Men in the higher BMI categories were less likely to be never smokers, whereas women in the higher BMI categories were more likely to be never smokers. Among postmenopausal women, HRT use was more common among leaner women. Subjects in the higher BMI categories had higher intake of all food groups analyzed (fruits and vegetables, fish and shellfish, and meat and meat products) than subjects in the lower BMI categories. WHR was more closely related to BMI in men than in women. Age-adjusted Pearson correlation coefficients for the association of BMI with waist and hip circumference and WHR were $r = 0.86$, $r = 0.77$, and $r = 0.56$, respectively, for men, and $r = 0.85$, $r = 0.86$, and $r = 0.43$, respectively, for women (all $P<.001$).

We examined relative risks of colon cancer by quintile of anthropometric variables in men and women (Table 3). In both sexes, there was a statistically significant trend of increasing relative risks of colon cancer across quintile of height (for men [≥180.5 cm versus < 168.0 cm], RR = 1.40, 95% CI = 0.99 to 1.98; $P_{trend} = 0.04$; for women [≥167.5 cm versus <156.0 cm], RR = 1.79, 95% CI = 1.30 to 2.46; $P_{trend}<.001$; Table 3). On a continuous scale, a 5-cm higher body height was related to an increased risk of colon cancer in both men (RR = 1.09, 95% CI = 1.01 to 1.18; $P = .02$) and women (RR = 1.12, 95% CI = 1.04 to 1.20; $P = .003$) ($P = .33$ for difference in the association between men and women).

Among men, weight and BMI were associated with a higher risk of colon cancer (weight ≥ 90.0 kg versus <71 kg, RR = 1.43, 95% CI = 1.02 to 2.02; $P_{trend} = .007$; BMI ≥ 29.4 kg/m^2 versus <23.6 kg/m^2, RR = 1.55, 95% CI = 1.12 to 2.15; $P_{trend} = .006$; Table 3) but not among women. Per unit increase, higher body weight and BMI were associated with colon cancer risk in men (per 5 kg of higher body weight, RR = 1.09, 95% CI = 1.04 to 1.13; $P<.001$; per kg/m^2 higher BMI, RR = 1.05, 95% CI = 1.02 to 1.08; $P<.001$). However, among women the association was weaker and only marginally statistically significant (per 5 kg higher body weight, RR = 1.04, 95% CI = 1.00 to 1.08; $P = .03$; $P = .35$ for difference to men; per kg/m^2 higher BMI, RR = 1.02, 95% CI = 1.00 to 1.04; $P = .07$; $P = .04$ for difference to men).

In both sexes, waist circumference was positively related to risk of colon cancer (for men [≥103.0 cm versus <86.0 cm], RR = 1.39, 95% CI = 1.01 to 1.93; $P_{trend} = .001$; for women [≥89.0 cm versus <70.2 cm], RR = 1.48, 95% CI = 1.08 to 2.03; $P_{trend} = .008$)). Per 5 cm higher waist circumference, the relative risk for men was 1.10 (95% CI = 1.05 to 1.56; $P<.001$) and for women was 1.07 (95% CI = 1.03 to 1.12; $P<.001$; $P = .48$ for difference between men and women). In contrast, hip circumference was statistically significantly positively related to colon cancer in men (per 5 cm higher hip circumference, RR = 1.12, 95% CI = 1.05 to 1.21; $P = .002$) but not in women (RR = 1.04, 95% CI = 0.99 to 1.09; $P = .10$; $P = .15$ for difference between men and women).

WHR was positively related to colon cancer risk in both men (RR per 0.1 higher WHR, RR = 1.24, 95% CI = 1.05 to 1.46; $P = .01$) and women (per 0.1 higher WHR, RR = 1.24, 95% CI = 1.10 to 1.39; $P<.001$) ($P = .92$ for difference between men and women). In fact, of all anthropometric parameters, WHR showed the strongest association with colon cancer (highest versus lowest quintile for men, RR = 1.51, 95% CI = 1.06 to 2.15; $P_{trend} = .006$; for women, RR = 1.52, 95% CI = 1.12 to 2.05; $P_{trend} = .002$). The estimated absolute 5-year rate of developing colon cancer per 100 000 subjects were, for men, 203 cases (95% CI = 155 to 250) in the highest quintile of WHR and 131 cases (95% CI = 91 to 170) in the lowest and, for women, 129 cases (95% CI = 102 to 156) in the highest quintile of WHR and 86 (95% CI = 63 to 108) in the lowest. We found no statistically significant heterogeneity across study centers for any of the associations of the anthropometric measures with colon cancer risk in men or women ($P_{heterogeneity} = 0.34–0.92$). We examined the consistency of our findings by excluding 239 patients diagnosed during the first 2 years of follow-up, to eliminate the possible effects of changes in body weight and fat distribution in the prediagnostic disease phase. After exclusion, the relative risk of colon cancer per 0.1 higher WHR was 1.26 (95% CI = 1.04 to 1.52; $P = .02$) in men, and 1.25 (95% CI = 1.09 to 1.42; $P = .001$) in women. Thus, these patients did not bias the results.

There was no statistically significant association of any of the anthropometric measures with rectal cancer (Table 4). The apparent nonlinear relationship for WHR in men may be due to the low number of case patients in the reference category.

We further divided participants into groups based on well-established risk categories for BMI, waist circumference, and

Table 2. Characteristics of study participants in the European Prospective Investigation into Cancer and Nutrition by body mass index (BMI)*

	Men, quintile of BMI, kg/m^2						Women, quintile of BMI, kg/m^2				
	1	2	3	4	5	1	2	3	4	5
	Range	<23.6	23.6–25.3	25.4–27.0	27.1–29.3	≥29.4	<21.7	21.7–23.6	23.7–25.7	25.8–28.8	≥28.9
Characteristic	Mean	22.0	24.6	26.3	28.2	32.0	20.2	22.7	24.7	27.2	32.7
N		25 946	25 948	25 947	25 956	25 934	47 736	47 683	47 706	47 714	47 707
Mean age, y		50.3	52.6	53.3	53.8	54.0	46.7	49.7	51.7	53.3	54.1
Mean alcohol intake, g/day		20.0	21.3	22.2	23.3	24.7	9.6	9.6	8.9	7.9	6.0
Smoking status†, %											
Never smoker		33.9	32.0	30.3	27.9	26.5	52.7	52.9	53.5	56.8	62.6
Past smoker		29.9	36.1	39.1	41.1	42.1	23.0	25.0	24.6	22.7	19.8
Current smoker		34.9	30.4	29.0	29.5	29.9	23.1	21.2	21.1	19.7	16.6
Education†, %											
No school degree or primary school		21.7	24.8	28.5	35.4	42.5	14.6	19.4	26.5	35.7	47.1
Technical or professional school		24.3	25.2	25.8	25.3	23.7	24.2	26.2	26.0	24.6	21.3
Secondary school		15.7	16.0	15.2	13.8	12.9	24.7	23.2	21.6	19.0	15.3
University degree		34.7	30.8	27.4	22.6	17.8	31.6	26.2	21.3	16.2	11.2
Total physical activity†, %											
Inactive		19.8	20.4	20.2	19.9	19.9	19.0	17.8	16.2	14.0	11.4
Moderately inactive		28.9	28.2	28.4	28.9	29.1	37.1	33.4	30.9	28.5	26.6
Moderately active		35.7	34.9	35.5	35.3	36.2	34.8	38.5	42.2	46.9	51.5
Active		13.1	13.9	13.3	13.6	13.1	8.0	9.0	9.2	9.2	9.2
Menopausal status†, %											
Premenopausal		—	—	—	—	—	33.3	33.6	33.8	33.8	33.9
Perimenopausal		—	—	—	—	—	13.1	13.0	12.6	12.4	11.9
Postmenopausal		—	—	—	—	—	45.9	45.6	45.6	45.0	44.6
Surgical postmenopausal		—	—	—	—	—	2.4	2.8	3.1	3.8	4.7
HRT use among postmenopausal women†, %											
No		—	—	—	—	—	69.6	71.4	73.7	78.1	84.4
Yes		—	—	—	—	—	28.3	26.4	24.2	20.2	14.2
Mean weight, kg		68.2	75.5	80.1	85.2	95.7	54.1	60.3	64.8	70.5	83.1
Mean height, cm		175.8	175.2	174.5	173.8	172.7	163.3	162.8	162.0	160.9	159.4
Mean waist circumference, cm		83.7	89.7	93.8	98.4	107.4	69.2	74.0	78.3	83.9	95.1
Mean hip circumference, cm		94.2	97.9	100.3	103.0	108.8	91.9	96.3	99.7	104.0	113.4
Mean waist-to-hip ratio		0.89	0.92	0.94	0.96	0.99	0.75	0.77	0.79	0.80	0.84
Mean fiber intake, g/day		24.6	24.4	24.4	24.7	25.1	22.9	22.8	22.7	22.7	23.0
Mean fruit and vegetable intake, g/day		393.0	417.2	434.3	465.2	496.5	466.2	477.2	485.9	507.8	538.3
Mean fish and shellfish intake, g/day		34.9	37.1	38.9	41.1	43.4	29.2	30.7	31.7	33.2	34.2
Mean red and processed meat intake, g/day		95.5	102.1	105.7	110.1	116.4	59.8	63.6	67.5	70.6	73.6

*All values except age, BMI, and number of subjects are age standardized. HRT = hormone replacement therapy.

†Numbers do not add up to 100% because of missing values; — = not applicable.

WHR, respectively. Compared with nonoverweight subjects (BMI < 25 kg/m^2), the relative risk for colon cancer was 1.00 (95% CI = 0.80 to 1.26) for overweight men (BMI = 25–29.9 kg/m^2) and 1.16 (95% CI = 0.96 to 1.40) for overweight women, and 1.41 (95% CI = 1.06 to 1.88; P_{trend} = .03) for obese men (BMI ≥ 30 kg/m^2) and 1.07 (95% CI = 0.82 to 1.38; P_{trend} = .41) for obese women. Among men, those with a waist circumference of at least 102 cm had a higher risk for colon cancer than those with a waist circumference of less than 102 cm (RR = 1.37, 95% CI = 1.10 to 1.70; P = .004), whereas among women, risk did not differ between those with a waist circumference at least 88 cm versus less than 88 cm (RR = 1.18; 95% CI = 0.97 to 1.43; P = .10). However, men in the higher WHR category had a higher risk of colon cancer (≥0.95 versus <0.95, RR = 1.44, 95% CI = 1.17 to 1.76; P<.001), and the same was true for women (≥0.80 versus <0.80, RR = 1.27, 95% CI = 1.06 to 1.51; P = .008). The absolute 5-year rate of colon cancer per 100 000 individuals was 206 cases (95% CI = 167 to 245) for men with a WHR of at least 0.95 and 144 cases (95% CI = 117 to 171) for men with a WHR less than 0.95, 115 cases (95% CI = 95 to 135) for women with a WHR of at least 0.80, and 91 cases (95% CI = 76 to 106) for women with a WHR less than 0.80.

In analyses that also adjusted for body weight, waist and hip circumference and WHR were not statistically significantly related to risk of colon cancer in men, whereas WHR remained statistically significant in women (Table 5). Results were similar when we adjusted these analyses for BMI instead of for weight and height (highest versus lowest WHR quintile adjusted

Table 3. Relative risks (RRs) and 95% confidence intervals (CIs) of colon cancer across quintiles of anthropometric measures in the European Prospective Investigation into Cancer and Nutrition

Men				Women			
Measure	N*	Crude RR (95% CI)†	Multivariable RR (95% CI)‡	Measure	N*	Crude RR (95% CI)†	Multivariable RR (95% CI)‡
Height, cm				Height, cm			
<168.0	79	1 (Referent)	1 (Referent)	<156.0	80	1 (Referent)	1 (Referent)
168.0–172.4	84	1.09 (0.79 to 1.50)	1.10 (0.80 to 1.52)	156.0–159.9	106	1.34 (0.99 to 1.80)	1.33 (0.99 to 1.80)
172.5–176.1	86	1.14 (0.82 to 1.57)	1.16 (0.84 to 1.60)	160.0–163.2	141	1.72 (1.29 to 2.30)	1.71 (1.28 to 2.28)
176.2–180.4	91	1.26 (0.91 to 1.74)	1.29 (0.93 to 1.79)	163.3–167.4	128	1.68 (1.25 to 2.27)	1.66 (1.23 to 2.24)
≥180.5	81	1.33 (0.95 to 1.87)	1.40 (0.99 to 1.98)	≥167.5	108	1.82 (1.33 to 2.50)	1.79 (1.30 to 2.46)
P_{trend}§		.06	.04	P_{trend}§		<.001	<.001
Weight, kg				Weight, kg			
<71.0	72	1 (Referent)	1 (Referent)	<56.9	83	1 (Referent)	1 (Referent)
71.0–76.9	68	0.94 (0.67 to 1.31)	0.91 (0.65 to 1.28)	56.9–62.0	100	1.20 (0.89 to 1.60)	1.14 (0.84 to 1.53)
77.0–82.7	79	1.12 (0.81 to 1.54)	1.06 (0.76 to 1.48)	62.1–67.4	108	1.19 (0.89 to 1.59)	1.10 (0.82 to 1.49)
82.8–89.9	93	1.33 (0.97 to 1.82)	1.24 (0.89 to 1.73)	67.5–74.9	137	1.35 (1.02 to 1.78)	1.23 (0.91 to 1.64)
≥90.0	109	1.57 (1.16 to 2.13)	1.43 (1.02 to 2.02)	≥75.0	135	1.40 (1.06 to 1.86)	1.25 (0.93 to 1.70)
P_{trend}§		<.001	.007	P_{trend}§		.02	.14
BMI, kg/m^2				BMI, kg/m^2			
<23.6	64	1 (Referent)	1 (Referent)	<21.7	87	1 (Referent)	1 (Referent)
23.6–25.3	85	1.20 (0.86 to 1.66)	1.18 (0.85 to 1.63)	21.7–23.5	96	0.92 (0.69 to 1.23)	0.92 (0.68 to 1.23)
25.4–27.0	74	1.03 (0.74 to 1.45)	1.00 (0.71 to 1.41)	23.6–25.7	120	1.02 (0.77 to 1.35)	1.02 (0.77 to 1.35)
27.1–29.3	88	1.24 (0.89 to 1.72)	1.19 (0.85 to 1.66)	25.8–28.8	137	1.09 (0.83 to 1.44)	1.09 (0.83 to 1.45)
≥29.4	110	1.64 (1.19 to 2.25)	1.55 (1.12 to 2.15)	≥28.9	123	1.04 (0.78 to 1.39)	1.06 (0.79 to 1.42)
P_{trend}§		.002	.006	P_{trend}§		.46	.40
Waist circumference, cm				Waist circumference, cm			
<86.0	63	1 (Referent)	1 (Referent)	<70.2	62	1 (Referent)	1 (Referent)
86.0–91.8	57	0.75 (0.53 to 1.08)	0.73 (0.50 to 1.04)	70.2–75.8	91	1.13 (0.81 to 1.56)	1.10 (0.80 to 1.52)
91.9–96.5	78	1.03 (0.74 to 1.44)	0.97 (0.69 to 1.36)	75.9–80.9	125	1.27 (0.93 to 1.73)	1.23 (0.90 to 1.68)
96.6–102.9	95	1.20 (0.87 to 1.66)	1.10 (0.79 to 1.53)	81.0–88.9	135	1.29 (0.95 to 1.76)	1.25 (0.91 to 1.70)
≥103.0	125	1.56 (1.14 to 2.14)	1.39 (1.01 to 1.93)	≥89.0	149	1.53 (1.12 to 2.09)	1.48 (1.08 to 2.03)
P_{trend}§		<.001	.001	P_{trend}§		.004	.008
Hip circumference, cm				Hip circumference, cm			
<95.2	71	1 (Referent)	1 (Referent)	<93.7	83	1 (Referent)	1 (Referent)
95.2–98.9	62	0.93 (0.66 to 1.31)	0.90 (0.64 to 1.27)	93.7–97.9	90	1.03 (0.76 to 1.39)	0.99 (0.73 to 1.34)
99.0–101.9	97	1.13 (0.83 to 1.55)	1.08 (0.78 to 1.48)	98.0–101.9	137	1.16 (0.88 to 1.52)	1.09 (0.82 to 1.44)
102.0–105.9	76	1.36 (0.98 to 1.89)	1.27 (0.90 to 1.78)	102.0–107.9	108	1.10 (0.82 to 1.47)	1.02 (0.76 to 1.38)
≥106.0	110	1.51 (1.11 to 2.06)	1.37 (0.99 to 1.90)	≥108.0	142	1.28 (0.97 to 1.70)	1.20 (0.89 to 1.60)
P_{trend}§		<.001	.01	P_{trend}§		.07	.19
WHR				WHR			
<0.887	48	1 (Referent)	1 (Referent)	<0.734	68	1 (Referent)	1 (Referent)
0.887–0.922	72	1.19 (0.83 to 1.72)	1.16 (0.80 to 1.68)	0.734–0.768	94	1.06 (0.78 to 1.46)	1.07 (0.78 to 1.47)
0.923–0.952	77	1.19 (0.83 to 1.72)	1.15 (0.79 to 1.65)	0.769–0.802	113	1.13 (0.83 to 1.53)	1.15 (0.84 to 1.56)
0.953–0.989	109	1.63 (1.15 to 2.31)	1.54 (1.08 to 2.19)	0.803–0.845	125	1.17 (0.86 to 1.58)	1.19 (0.88 to 1.61)
≥0.990	110	1.63 (1.15 to 2.31)	1.51 (1.06 to 2.15)	≥0.846	160	1.48 (1.10 to 2.00)	1.52 (1.12 to 2.05)
P_{trend}§		<.001	.006	P_{trend}§		.003	.002

*Number of colon cancer patients. BMI = body mass index; WHR = waist-to-hip ratio.

†Crude model is derived from Cox regression using age as the underlying time variable and stratified by center and age at recruitment.

‡Multivariable models for height and BMI were based on the crude model with additional adjustment for smoking status (never, past, current, or unknown), education (no school degree or primary school, technical or professional school, secondary school, university degree, or unknown), alcohol intake (continuous), physical activity (inactive, moderately inactive, moderately active, active, or missing), fiber intake (continuous), and consumption of red and processed meat (continuous), fish and shellfish (continuous), and fruits and vegetables (continuous). Multivariable model for weight, waist, hip, and WHR were further adjusted for height (continuous).

§P_{trend} (two-sided) across categories is based on the median anthropometric variable within quintiles as a continuous variable and was calculated using the Wald chi-square statistic.

for BMI in men, RR = 1.19, 95% CI = 0.80 to 1.77; P_{trend} = .26; in women, RR = 1.46, 95% CI = 1.06 to 2.01; P_{trend} = .01). Conversely, no statistically significant association was observed for BMI after adjustment for WHR among men or women.

When we restricted the analysis to women who were postmenopausal at baseline (including 424 postmenopausal women who developed colon cancer), the results were similar to those in women overall (data not shown). Among postmenopausal women, 336 colon cancer patients (75.1%) reported no HRT use at baseline, and 81 patients (23.3%) reported HRT use; for the remaining 7 patients, information on HRT use was unavailable. The positive associations for waist circumference and WHR with risk of colon cancer were restricted to postmenopausal women who did not use HRT at baseline (Table 6) (for difference between postmenopausal women with and without HRT use for the association of colon cancer with waist circumference, P = .05; and for the association with WHR, P = .19). HRT alone was not statistically significantly related to colon cancer risk in postmenopausal women.

Discussion

In this large prospective cohort study, we found that body weight and BMI were statistically significantly related to colon

Table 4. Relative risks (RRs) and 95% confidence intervals (CIs) of rectal cancer across quintiles of anthropometric measures in the European Prospective Investigation into Cancer and Nutrition

Men				Women			
Measure	N*	Crude RR (95% CI)†	Multivariable RR (95% CI)‡	Measure	N*	Crude RR (95% CI)†	Multivariable RR (95% CI)‡
Height, cm				Height, cm			
<168.0	53	1 (Referent)	1 (Referent)	<156.0	50	1 (Referent)	1 (Referent)
168.0–172.4	74	1.32 (0.91 to 1.90)	1.30 (0.90 to 1.87)	156.0–159.9	61	1.03 (0.70 to 1.51)	1.03 (0.70 to 1.52)
172.5–176.1	57	0.99 (0.67 to 1.47)	0.97 (0.65 to 1.44)	160.0–163.2	83	1.24 (0.86 to 1.80)	1.25 (0.86 to 1.81)
176.2–180.4	59	1.03 (0.69 to 1.52)	1.00 (0.67 to 1.49)	163.3–167.4	53	0.80 (0.53 to 1.21)	0.81 (0.54 to 1.23)
≥180.5	52	1.03 (0.68 to 1.56)	1.00 (0.66 to 1.52)	≥167.5	44	0.77 (0.49 to 1.18)	0.78 (0.50 to 1.21)
P_{trend}§		.66	.55	P_{trend}§		.09	.12
Weight, kg				Weight, kg			
<71.0	49	1 (Referent)	1 (Referent)	<56.9	53	1 (Referent)	1 (Referent)
71.0–76.9	59	1.15 (0.78 to 1.68)	1.17 (0.80 to 1.73)	56.9–62.0	45	0.76 (0.51 to 1.14)	0.81 (0.54 to 1.21)
77.0–82.7	57	1.10 (0.75 to 1.62)	1.14 (0.76 to 1.70)	62.1–67.4	60	0.92 (0.63 to 1.34)	1.01 (0.69 to 1.49)
82.8–89.9	67	1.26 (0.87 to 1.83)	1.30 (0.87 to 1.94)	67.5– 74.9	67	0.93 (0.64 to 1.34)	1.04 (0.71 to 1.53)
≥90.0	63	1.18 (0.81 to 1.73)	1.22 (0.80 to 1.86)	≥75.0	66	0.92 (0.63 to 1.33)	1.06 (0.71 to 1.57)
P_{trend}§		.36	.36	P_{trend}§		.94	.44
BMI, kg/m^2				BMI, kg/m^2			
<23.6	52	1 (Referent)	1 (Referent)	<21.7	47	1 (Referent)	1 (Referent)
23.6–25.3	52	0.89 (0.60 to 1.31)	0.88 (0.60 to 1.30)	21.7–23.5	44	0.77 (0.51 to 1.16)	0.78 (0.51 to 1.18)
25.4–27.0	58	0.98 (0.67 to 1.44)	0.96 (0.66 to 1.40)	23.6–25.7	72	1.11 (0.77 to 1.62)	1.14 (0.78 to 1.66)
27.1–29.3	69	1.15 (0.80 to 1.67)	1.11 (0.77 to 1.62)	25.8–28.8	63	0.92 (0.63 to 1.36)	0.95 (0.64 to 1.41)
≥29.4	64	1.12 (0.77 to 1.62)	1.05 (0.72 to 1.55)	≥28.9	65	1.03 (0.70 to 1.52)	1.06 (0.71 to 1.58)
P_{trend}§		.28	.47	P_{trend}§		.58	.51
Waist circumference, cm				Waist circumference, cm			
<86.0	40	1 (Referent)	1 (Referent)	<70.2	40	1 (Referent)	1 (Referent)
86.0–91.8	52	1.09 (0.72 to 1.65)	1.06 (0.70 to 1.61)	70.2–75.8	54	1.08 (0.71 to 1.63)	1.10 (0.73 to 1.66)
91.9–96.5	60	1.18 (0.79 to 1.77)	1.15 (0.76 to 1.73)	75.9–80.9	55	0.91 (0.60 to 1.37)	0.94 (0.62 to 1.42)
96.6–102.9	65	1.23 (0.82 to 1.84)	1.18 (0.78 to 1.77)	81.0–88.9	72	1.18 (0.79 to 1.76)	1.22 (0.82 to 1.83)
≥103.0	76	1.37 (0.93 to 2.04)	1.27 (0.84 to 1.91)	≥89.0	70	1.18 (0.79 to 1.78)	1.23 (0.81 to 1.86)
P_{trend}§		.08	.21	P_{trend}§		.28	.22
Hip circumference, cm				Hip circumference, cm			
<95.2	53	1 (Referent)	1 (Referent)	<93.7	49	1 (Referent)	1 (Referent)
95.2–98.9	53	1.07 (0.73 to 1.57)	1.07 (0.73 to 1.58)	93.7–97.9	46	0.92 (0.61 to 1.38)	0.97 (0.65 to 1.46)
99.0–101.9	73	1.12 (0.78 to 1.60)	1.12 (0.78 to 1.61)	98.0–101.9	70	1.05 (0.72 to 1.52)	1.14 (0.78 to 1.66)
102.0–105.9	52	1.18 (0.80 to 1.74)	1.19 (0.79 to 1.78)	102.0–107.9	64	1.16 (0.79 to 1.71)	1.27 (0.86 to 1.88)
≥106.0	61	1.07 (0.73 to 1.56)	1.05 (0.70 to 1.56)	≥108.0	62	1.00 (0.67 to 1.47)	1.10 (0.74 to 1.64)
P_{trend}§		.66	.77	P_{trend}§		.75	.44
WHR				WHR			
<0.887	23	1 (Referent)	1 (Referent)	<0.734	41	1 (Referent)	1 (Referent)
0.887–0.922	64	2.15 (1.33 to 3.47)	2.07 (1.28 to 3.35)	0.734–0.768	47	0.90 (0.59 to 1.37)	0.88 (0.58 to 1.34)
0.923–0.952	71	2.17 (1.35 to 3.49)	2.06 (1.28 to 3.32)	0.769–0.802	60	1.03 (0.69 to 1.55)	1.01 (0.68 to 1.52)
0.953–0.989	56	1.62 (0.99 to 2.64)	1.49 (0.91 to 2.45)	0.803–0.845	65	1.07 (0.72 to 1.60)	1.04 (0.69 to 1.56)
≥0.990	78	2.17 (1.35 to 3.49)	1.93 (1.19 to 3.13)	≥0.846	78	1.26 (0.85 to 1.87)	1.20 (0.81 to 1.79)
P_{trend}§		.04	.16	P_{trend}§		.11	.17

*Number of rectal cancer patients. BMI = body mass index; WHR = waist-to-hip ratio.

†Crude model is derived from Cox regression using age as the underlying time variable and stratified by center and age at recruitment.

‡Multivariable models for height and BMI were based on the crude model with additional adjustment for smoking status (never, past, current, or unknown), education (no school degree or primary school, technical or professional school, secondary school, university degree, or unknown), alcohol intake (continuous), physical activity (inactive, moderately inactive, moderately active, active, or missing), fiber intake (continuous), and consumption of red and processed meat (continuous), fish and shellfish (continuous), and fruits and vegetables (continuous). Multivariable model for weight, waist, hip, and WHR were further adjusted for height (continuous).

§P_{trend} (two-sided) across categories is based on the median anthropometric variable within quintiles as a continuous variable using the Wald chi-square statistic.

cancer risk in men but only weakly related to risk in women. In contrast, both waist circumference and WHR were strongly related to colon cancer risk in both sexes. Thus, WHR conveyed statistically significant information beyond body weight for colon cancer risk in women, but not in men. These data support the hypothesis that abdominal obesity is a risk factor for colon cancer in both sexes and suggest that fat distribution is more important than body weight or BMI for disease risk in women. Further, our results indicate that the association of body fat accumulation and risk of colon cancer in postmenopausal women may be associated with HRT use. That is, waist circumference and WHR were statistically significantly related to risk of colon cancer among nonusers but not among users of HRT, although the difference was only marginally statistically significant. Finally, results from our study support the hypothesis that height is related to colon cancer risk in both sexes.

Previous studies have primarily used body weight or BMI to assess the association of obesity with colon cancer risk. Similar to our findings (Table 3), most of these studies found positive associations of these measurements for men but weaker or no associations for women *(1–30)*. However, these measurements may not be ideal because the changes in physiologic functions that accompany obesity depend to a certain extent on regional adipose tissue distribution. Intra-abdominal visceral obesity is related to elevated blood pressure and insulin levels, insulin resistance, and dyslipidemia, and several studies have shown that

Table 5. Relative risk (RRs) and 95% confidence intervals (CIs) of colon cancer across quintiles of waist and hip circumference and waist-to-hip ratio (WHR) after controlling for body weight in men and women of the European Prospective Investigation into Cancer and Nutrition*

Men		Women	
Measure	Multivariable RR (95% CI)	Measure	Multivariable RR (95% CI)
Waist circumference, cm		Waist circumference, cm	
<86.0	1 (Referent)	<70.2	1 (Referent)
86.0–91.8	0.67 (0.46 to 0.98)	70.2–75.8	1.10 (0.79 to 1.53)
91.9–96.5	0.85 (0.59 to 1.23)	75.9–80.9	1.22 (0.87 to 1.70)
96.6–102.9	0.91 (0.61 to 1.35)	81.0–88.9	1.23 (0.85 to 1.77)
≥103.0	1.01 (0.62 to 1.65)	≥89.0	1.44 (0.92 to 2.26)
P_{trend}†	.50	P_{trend}†	.12
Hip circumference, cm		Hip circumference, cm	
<95.2	1 (Referent)	<93.7	1 (Referent)
95.2–98.9	0.81 (0.57 to 1.16)	93.7–97.9	0.95 (0.70 to 1.29)
99.0–101.9	0.89 (0.63 to 1.26)	98.0–101.9	1.00 (0.74 to 1.36)
102.0–105.9	0.97 (0.65 to 1.43)	102.0–107.9	0.89 (0.63 to 1.27)
≥106.0	0.88 (0.55 to 1.39)	≥108.0	0.94 (0.60 to 1.46)
P_{trend}†	.85	P_{trend}†	.72
WHR		WHR	
<0.887	1 (Referent)	<0.734	1 (Referent)
0.887–0.922	1.08 (0.75 to 1.57)	0.734–0.768	1.06 (0.78 to 1.46)
0.923–0.952	1.02 (0.70 to 1.49)	0.769–0.802	1.13 (0.83 to 1.54)
0.953–0.989	1.32 (0.91 to 1.91)	0.803–0.845	1.16 (0.85 to 1.58)
≥0.990	1.18 (0.79 to 1.76)	≥0.846	1.46 (1.06 to 2.00)
P_{trend}†	.27	P_{trend}†	.01

*Multivariable models were derived from Cox regression using age as the underlying time variable and stratified by center and age at recruitment with additional adjustment for height (continuous), weight (continuous), smoking status (never, past, current, or unknown), education (no school degree or primary school, technical or professional school, secondary school, university degree, or unknown), alcohol consumption (continuous), physical activity (inactive, moderately inactive, moderately active, active, or missing), fiber intake (continuous), and consumption of red and processed meat (continuous), fish and shellfish (continuous), and fruits and vegetables (continuous).

†P_{trend} (two-sided) across categories is based on the median anthropometric variable within quintiles as a continuous variable and was calculated using the Wald chi-square statistic.

upper-body fat distribution is independently associated with a higher risk of developing diabetes and cardiovascular disease *(50)*. Higher body weight is more closely related to abdominal obesity than to lower-body obesity in men but more closely related to gluteofemoral obesity in women *(31)*. Similarly, in our cohort WHR was more closely related to BMI in men than in women. Hence, assuming that it is primarily visceral and not nonvisceral adipose tissue that is involved in tumorigenic processes, body weight and BMI may not accurately reflect the colon cancer risk that is associated with abdominal fat accumulation, at least in women.

Few prospective studies have examined the association of body fat distribution—as reflected by waist and hip circumference—and colon cancer risk *(11,13,14,19,21,51)*. Among these studies, we are aware of only one report *(21)* that presented results for men and women separately. In this report, from the Framingham Study *(21)*, which included 306 colon cancer case patients, waist circumference was an equally strong risk factor for colon cancer in men and women, and it was a stronger risk factor than BMI in both sexes. A report from the Cardiovascular Health Study, which included 102 men and women with colorectal cancer, found that waist circumference and WHR were statistically significantly related to risk of colorectal cancer but that BMI was not; however, this analysis did not present sex-specific results *(51)*.

Our findings are in contrast with reports from the Melbourne Collaborative Cohort Study *(19)* and the Health Professionals Follow-up Study *(13)*, which found an association between WHR and colon cancer risk in men even after adjustment for BMI, and with reports from the Iowa Women's Health Study *(11)* and the Nurses' Health Study *(14)*, which found statistically significantly positive associations in women between BMI but not WHR and risk of colon cancer. However, most of these studies *(11,13,14)* relied on self-reported anthropometric data, which limits the interpretability of these results.

The pathophysiology underlying the association between obesity and increased colon cancer risk is unclear. Some authors have suggested that components of the metabolic syndrome, particularly insulin resistance and subsequent hyperinsulinemia, are the underlying link, which may reflect the growth-promoting effects of insulin *(52–54)*. These speculations are also supported by studies that found subjects with type 2 diabetes to be at increased risk of colon cancer *(55,56)*. Hyperinsulinemia is also related to increased levels of bioavailable insulin-like growth factor 1, which is known to have cancer-promoting effects *(57–60)*. Further potential mediators include leptin, which stimulates growth of colonic epithelial cells *(61–63)*, and adiponectin, which has antiangiogenic and antitumor activities *(64,65)*. We are now analyzing the relationship of these and other biomarkers with risk of colon and rectal cancer in EPIC.

Postmenopausal HRT has been associated with reduced risk of colon cancer in observational studies *(33)*, a finding that has been supported by the results of the Women's Health Initiative intervention trial of estrogen plus progestin use *(34–36)*. HRT has been hypothesized to reduce the risk of colon cancer by reducing the likelihood of estrogen receptor methylation *(66,67)*. In our study we found that, among postmenopausal women, the positive association of waist circumference and WHR with risk of colon cancer was not apparent in women who used HRT at baseline (Table 6). We are aware of only one case–control study that has examined the interaction between HRT use and adiposity on colon cancer risk, with body weight assessed in colon cancer

Table 6. Relative risks (RRs) and 95% confidence intervals (CIs) of colon cancer across quintiles of anthropometric measures in postmenopausal women stratified by hormone replacement therapy (HRT) use at baseline in the European Prospective Investigation into Cancer and Nutrition*

	No HRT use		HRT use	
Measure	N†	RR (95% CI)	N†	RR (95% CI)
Weight‡, kg				
<56.9	48	1 (Referent)	15	1 (Referent)
56.9–62.0	56	1.05 (0.71 to 1.56)	16	1.04 (0.50 to 2.18)
62.1–67.4	61	0.99 (0.67 to 1.46)	15	0.95 (0.44 to 2.03)
67.5–74.9	82	1.08 (0.74 to 1.57)	23	1.55 (0.74 to 3.22)
≥75.0	89	1.13 (0.77 to 1.67)	12	1.01 (0.43 to 2.37)
P_{trend}§		.49		.69
BMI, kg/m^2				
<21.7	40	1 (Referent)	21	1 (Referent)
21.7–23.5	50	0.96 (0.63 to 1.45)	15	0.69 (0.35 to 1.35)
23.6–25.7	77	1.21 (0.82 to 1.78)	17	0.80 (0.41 to 1.56)
25.8–28.8	83	1.11 (0.75 to 1.64)	20	1.10 (0.57 to 2.10)
≥28.9	86	1.12 (0.75 to 1.67)	8	0.72 (0.31 to 1.70)
P_{trend}§		.52		.88
Waist circumference‡, cm				
<70.2	25	1 (Referent)	17	1 (Referent)
70.2–75.8	48	1.30 (0.80 to 2.11)	21	1.07 (0.55 to 2.06)
75.9–80.9	71	1.35 (0.85 to 2.14)	17	0.79 (0.39 to 1.57)
81.0–88.9	85	1.39 (0.88 to 2.19)	17	0.90 (0.44 to 1.83)
≥89.0	106	1.68 (1.06 to 2.64)	9	0.76 (0.32 to 1.80)
P_{trend}§		.02		.46
Hip circumference‡, cm				
<93.7	43	1 (Referent)	23	1 (Referent)
93.7–97.9	46	0.93 (0.61 to 1.41)	17	0.71 (0.37 to 1.36)
98.0–101.9	88	1.21 (0.83 to 1.76)	13	0.43 (0.21 to 0.88)
102.0–107.9	68	1.04 (0.70 to 1.54)	14	0.70 (0.34 to 1.44)
≥108.0	89	1.08 (0.74 to 1.60)	13	0.77 (0.37 to 1.61)
P_{trend}§		.69		.54
WHR‡				
<0.734	27	1 (Referent)	19	1 (Referent)
0.734–0.768	40	1.02 (0.62 to 1.66)	19	0.79 (0.41 to 1.53)
0.769–0.802	75	1.55 (1.00 to 2.42)	11	0.46 (0.21 to 0.99)
0.803–0.845	85	1.51 (0.97 to 2.34)	14	0.69 (0.34 to 1.43)
≥0.846	107	1.76 (1.14 to 2.72)	17	0.96 (0.47 to 1.94)
P_{trend}§		.002		.89

*Relative risks derived from multivariable Cox regression models using age as the underlying time variable and stratified by center and age at recruitment with additional adjustment for smoking status (never, past, current, or unknown), education (no school degree or primary school, technical or professional school, secondary school, university degree, or unknown), alcohol consumption (continuous), physical activity (inactive, moderately inactive, moderately active, active, or missing), fiber intake (continuous), and consumption of red and processed meat (continuous), fish and shellfish (continuous), and fruits and vegetables (continuous). BMI = body mass index; WHR = waist-to-hip ratio.

†Number of colon cancer patients.

‡Relative risks for weight, waist and hip circumference, and WHR are also adjusted for height (continuous).

§P_{trend} (two-sided) across categories is based on the median anthropometric variable within quintiles as a continuous variable and was calculated using the Wald chi-square statistic.

patients and population-based control subjects *(20)*. Contrary to our findings, in that report *(20)* a positive association between BMI and colon cancer risk was observed for postmenopausal women on HRT only. The reasons for this discrepancy are unclear; however, differences between HRT users and nonusers in the association of obesity with postmenopausal breast cancer risk similar to our observations for colon cancer have previously been reported *(37)*. Because the differences between HRT users and non users were only marginally statistically significant in our study, we cannot rule out a role for chance. Clearly, our findings need to be confirmed in future studies and to be put in context with the complex effects observed in interventional studies of HRT use on women's health, e.g., their detrimental effects on the cardiovascular system *(34)*.

In our analysis, height was related to risk of colon cancer (Table 3), a finding that is in agreement with previous studies *(68)*. The magnitude of this association was similar in men and women. Height has been related to several types of cancer, including colon, breast, pancreatic, and prostate cancer *(37,69)*, and it was recently estimated that 18% of total cancers are attributable to factors related to tallness *(69)*. Tallness is related to having more cells in the body structure, which may increase the probability of malignant transformation *(70)*. Postnatal growth depends largely on a complex interaction between nutrition, growth hormones (GH, insulin-like growth factor), and sex hormones, all of which have been suggested to be involved in cancer development *(71)*. For example, evidence from animal and human studies suggests that restricted caloric intake in early life is related to lower adult cancer risk *(72–74)*. Adult height may also reflect increased exposure to GH, insulin-like growth factor 1, and insulin in preadulthood that may predispose to cancer development in later life *(57,58,75,76)*. These speculations are supported by studies that found patients with GH excess (e.g., acromegaly) to be at increased cancer risk, particularly for colon cancer *(77,78)*.

In agreement with most previous reports, we found no statistically significant association between body size and risk of rectal

cancer (Table 4) *(2–5,12,17,27,30)*. This finding suggests differences in tumor susceptibility between colon and rectum, although the potential mechanisms accounting for these differences need further investigation.

Our study has strengths and limitations. Among the strengths are its prospective design and the large sample size, which included several European countries. Also, all body measures were assessed directly at baseline, in contrast to self-reported data used in most previous studies. Among the limitations were slight differences in the method of assessment of waist and hip circumference between centers in EPIC; however, we found similar results for the associations of body size with risk of colon and rectal cancer across centers, which also reduces the possibility of residual confounding by geographic region. The combination of related measures in our analysis may lead to imprecision and instability of the risk estimates; however, the width of the confidence intervals did not substantially change when we combined body weight with waist or hip circumference or WHR, therefore indicating that these combinations did not substantially decrease precision. Nevertheless, the anthropometric parameters we used—although being standard, well established, and routinely used for disease risk assessment—may be imperfect measures of body size or underlying true biologic risk factors, and this situation may complicate the interpretation of our findings when considering these measures simultaneously.

Within EPIC we currently have standardized information about exposure variables available at baseline only, which neglects modifications in subjects' exposure status during follow-up. However, any potential misclassification should be nondifferential and, if anything, is expected to bias our results toward the null.

Information on menopausal status was available at baseline only; we were therefore unable to stratify our analysis by menopausal status at time of cancer diagnosis. Previous studies have suggested that the association of BMI with risk of colon cancer may be stronger in, or even limited to, premenopausal women *(20,23,79)*. In our analysis, only 33% of women were premenopausal at baseline; it is therefore reasonable to assume that most women were postmenopausal (or at least perimenopausal) at the time of cancer diagnosis. In line with this hypothesis, when we restricted the analysis to women who were postmenopausal at baseline, our findings were almost identical to those using all women. Although our study included many colon cancer case patients, stratification may have limited the power to detect statistically significant associations of waist circumference or WHR with risk of colon cancer among postmenopausal women with HRT use. However, in this group the point estimates of risk showed no substantial variation across quintiles of measures of obesity, arguing against a substantial association with colon cancer risk. Despite excluding participants with reported cancers at baseline, we cannot exclude the possibility that some subjects had underlying yet undiagnosed colon or rectal cancer. However, results did not appreciably change when we excluded subjects with a follow-up time of less than 2 years.

In conclusion, in this study we found that abdominal obesity is an equally strong risk factor for colon cancer in men and women, whereas body weight and BMI were associated with colon cancer risk in men but not in women. These data suggest that fat distribution is a more important risk factor than body weight and BMI for colon cancer in women. Also, our study suggests that the relationship between abdominal obesity and colon cancer risk may vary according to HRT use; however, these findings require confirmation in future studies. Our data give further credence to public health efforts aiming to reduce the prevalence of obesity to prevent cancer and other chronic diseases. Measurement of waist circumference or WHR should be included in current guidelines to maintain a healthful lifestyle for disease prevention *(80)*.

References

(1) Graham S, Marshall J, Haughey B, Mittelman A, Swanson M, Zielezny M, et al. Dietary epidemiology of cancer of the colon in western New York. Am J Epidemiol 1988;128:490–503.

(2) Gerhardsson de Verdier M, Hagman U, Steineck G, Rieger A, Norell SE. Diet, body mass and colorectal cancer: a case-referent study in Stockholm. Int J Cancer 1990;46:832–8.

(3) Kune GA, Kune S, Watson LF. Body weight and physical activity as predictors of colorectal cancer risk. Nutr Cancer 1990;13:9–17.

(4) Dietz AT, Newcomb PA, Marcus PM, Storer BE. The association of body size and large bowel cancer risk in Wisconsin (United States) women. Cancer Causes Control 1995;6:30–6.

(5) Le Marchand L, Wilkens LR, Kolonel LN, Hankin JH, Lyu LC. Associations of sedentary lifestyle, obesity, smoking, alcohol use, and diabetes with the risk of colorectal cancer. Cancer Res 1997;57:4787–94.

(6) Caan BJ, Coates AO, Slattery ML, Potter JD, Quesenberry CP Jr, Edwards SM. Body size and the risk of colon cancer in a large case-control study. Int J Obes Relat Metab Disord 1998;22:178–84.

(7) Russo A, Franceschi S, La Vecchia C, Dal Maso L, Montella M, Conti E, et al. Body size and colorectal-cancer risk. Int J Cancer 1998;78:161–5.

(8) Slattery ML, Potter J, Caan B, Edwards S, Coates A, Ma KN, et al. Energy balance and colon cancer—beyond physical activity. Cancer Res 1997; 57:75–80.

(9) West DW, Slattery ML, Robison LM, Schuman KL, Ford MH, Mahoney AW, et al. Dietary intake and colon cancer: sex- and anatomic site-specific associations. Am J Epidemiol 1989;130:883–94.

(10) Lee IM, Paffenbarger RS Jr. Quetelet's index and risk of colon cancer in college alumni. J Natl Cancer Inst 1992;84:1326–31.

(11) Bostick RM, Potter JD, Kushi LH, Sellers TA, Steinmetz KA, McKenzie DR, et al. Sugar, meat, and fat intake, and non-dietary risk factors for colon cancer incidence in Iowa women (United States). Cancer Causes Control 1994;5:38–52.

(12) Chyou PH, Nomura AM, Stemmermann GN. A prospective study of colon and rectal cancer among Hawaii Japanese men. Ann Epidemiol 1996;6: 276–82.

(13) Giovannucci E, Ascherio A, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Physical activity, obesity, and risk for colon cancer and adenoma in men. Ann Intern Med 1995;122:327–34.

(14) Martinez ME, Giovannucci E, Spiegelman D, Hunter DJ, Willett WC, Colditz GA. Leisure-time physical activity, body size, and colon cancer in women. Nurses' Health Study Research Group. J Natl Cancer Inst 1997;89: 948–55.

(15) Ford ES. Body mass index and colon cancer in a national sample of adult US men and women. Am J Epidemiol 1999;150:390–8.

(16) Murphy TK, Calle EE, Rodriguez C, Kahn HS, Thun MJ. Body mass index and colon cancer mortality in a large prospective study. Am J Epidemiol 2000;152:847–54.

(17) Le Marchand L, Wilkens LR, Mi MP. Obesity in youth and middle age and risk of colorectal cancer in men. Cancer Causes Control 1992;3: 349–54.

(18) Lin J, Zhang SM, Cook NR, Rexrode KM, Lee IM, Buring JE. Body mass index and risk of colorectal cancer in women (United States). Cancer Causes Control 2004;15:581–9.

(19) MacInnis RJ, English DR, Hopper JL, Haydon AM, Gertig DM, Giles GG. Body size and composition and colon cancer risk in men. Cancer Epidemiol Biomarkers Prev 2004;13:553–9.

(20) Slattery ML, Ballard-Barbash R, Edwards S, Caan BJ, Potter JD. Body mass index and colon cancer: an evaluation of the modifying effects of estrogen (United States). Cancer Causes Control 2003;14:75–84.

(21) Moore LL, Bradlee ML, Singer MR, Splansky GL, Proctor MH, Ellison RC, et al. BMI and waist circumference as predictors of lifetime colon cancer risk in Framingham Study adults. Int J Obes Relat Metab Disord 2004;28:559–67.
(22) Terry P, Giovannucci E, Bergkvist L, Holmberg L, Wolk A. Body weight and colorectal cancer risk in a cohort of Swedish women: relation varies by age and cancer site. Br J Cancer 2001;85:346–9.
(23) Terry PD, Miller AB, Rohan TE. Obesity and colorectal cancer risk in women. Gut 2002;51:191–4.
(24) Garfinkel L. Overweight and cancer. Ann Intern Med 1985;103:1034–6.
(25) Must A, Jacques PF, Dallal GE, Bajema CJ, Dietz WH. Long-term morbidity and mortality of overweight adolescents. A follow-up of the Harvard Growth Study of 1922 to 1935. N Engl J Med 1992;327:1350–5.
(26) Nomura A, Heilbrun LK, Stemmermann GN. Body mass index as a predictor of cancer in men. J Natl Cancer Inst 1985;74:319–23.
(27) Phillips RL, Snowdon DA. Dietary relationships with fatal colorectal cancer among Seventh-Day Adventists. J Natl Cancer Inst 1985;74:307–17.
(28) Thun MJ, Calle EE, Namboodiri MM, Flanders WD, Coates RJ, Byers T, et al. Risk factors for fatal colon cancer in a large prospective study. J Natl Cancer Inst 1992;84:1491–500.
(29) Wu AH, Paganini-Hill A, Ross RK, Henderson BE. Alcohol, physical activity and other risk factors for colorectal cancer: a prospective study. Br J Cancer 1987;55:687–94.
(30) Chute CG, Willett WC, Colditz GA, Stampfer MJ, Baron JA, Rosner B, et al. A prospective study of body mass, height, and smoking on the risk of colorectal cancer in women. Cancer Causes Control 1991;2:117–24.
(31) Krotkiewski M, Bjorntorp P, Sjostrom L, Smith U. Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. J Clin Invest 1983;72:1150–62.
(32) Lonnqvist F, Thorne A, Large V, Arner P. Sex differences in visceral fat lipolysis and metabolic complications of obesity. Arterioscler Thromb Vasc Biol 1997;17:1472–80.
(33) Nelson HD, Humphrey LL, Nygren P, Teutsch SM, Allan JD. Postmenopausal hormone replacement therapy: scientific review. JAMA 2002;288: 872–81.
(34) Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 2002;288:321–33.
(35) Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, Black H, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA 2004;291:1701–12.
(36) Chlebowski RT, Wactawski-Wende J, Ritenbaugh C, Hubbell FA, Ascensao J, Rodabough RJ, et al. Estrogen plus progestin and colorectal cancer in postmenopausal women. N Engl J Med 2004;350:991–1004.
(37) Lahmann PH, Hoffmann K, Allen N, Van Gils CH, Khaw KT, Tehard B, et al. Body size and breast cancer risk: Findings from the European prospective investigation into cancer and nutrition (EPIC). Int J Cancer 2004;111: 762–71.
(38) Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113–24.
(39) Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26:S6–14.
(40) Haftenberger M, Lahmann PH, Panico S, Gonzalez CA, Seidell JC, Boeing H, et al. Overweight, obesity and fat distribution in 50- to 64-year-old participants in the European Prospective Investigation into Cancer and Nutrition (EPIC). Public Health Nutr 2002;5:1147–62.
(41) Spencer EA, Appleby PN, Davey GK, Key TJ. Validity of self-reported height and weight in 4808 EPIC-Oxford participants. Public Health Nutr 2002;5:561–5.
(42) Bingham SA, Gill C, Welch A, Day K, Cassidy A, Khaw KT, et al. Comparison of dietary assessment methods in nutritional epidemiology: weighed records v. 24 h recalls, food-frequency questionnaires and estimated-diet records. Br J Nutr 1994;72:619–43.
(43) Margetts BM, Pietinen P. European Prospective Investigation into Cancer and Nutrition: validity studies on dietary assessment methods. Int J Epidemiol 1997;26 Suppl 1:S1–5.
(44) Deharveng G, Charrondiere UR, Slimani N, Southgate DA, Riboli E. Comparison of nutrients in the food composition tables available in the nine European countries participating in EPIC. European Prospective Investigation into Cancer and Nutrition. Eur J Clin Nutr 1999;53:60–79.
(45) Ainsworth BE, Haskell WL, Leon AS, Jacobs DR Jr, Montoye HJ, Sallis JF, et al. Compendium of physical activities: classification of energy costs of human physical activities. Med Sci Sports Exerc 1993;25:71–80.
(46) Banks E, Barnes I, Baker K, Key TJ. Use of hormonal therapy for menopause in nine European countries. IARC Sci Publ 2002;156:301–3.
(47) Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 2000;894:1–253.
(48) Lean ME, Han TS, Morrison CE. Waist circumference as a measure for indicating need for weight management. BMJ 1995;311:158–61.
(49) Deeks JJ, Altman DG, Bradburn MJ. Statistical methods for examining heterogeneity and combining results from several study in meta-analysis. In Egger M, Smith GD, Altman DG, editors. Systematic reviews in health care. Meta-Analysis in context. 2nd ed. London (UK): BMJ; 2001.
(50) Pi-Sunyer FX. The obesity epidemic: pathophysiology and consequences of obesity. Obes Res 2002;10 Suppl 2:97S–104S.
(51) Schoen RE, Tangen CM, Kuller LH, Burke GL, Cushman M, Tracy RP, et al. Increased blood glucose and insulin, body size, and incident colorectal cancer. J Natl Cancer Inst 1999;91:1147–54.
(52) Giovannucci E. Diet, body weight, and colorectal cancer: a summary of the epidemiologic evidence. J Womens Health (Larchmt) 2003;12: 173–82.
(53) Giovannucci E. Insulin, insulin-like growth factors and colon cancer: a review of the evidence. J Nutr 2001;131:3109S-20S.
(54) McKeown-Eyssen G. Epidemiology of colorectal cancer revisited: are serum triglycerides and/or plasma glucose associated with risk? Cancer Epidemiol Biomarkers Prev 1994;3:687–95.
(55) Hu FB, Manson JE, Liu S, Hunter D, Colditz GA, Michels KB, et al. Prospective study of adult onset diabetes mellitus (type 2) and risk of colorectal cancer in women. J Natl Cancer Inst 1999;91:542–7.
(56) Seow A, Yuan JM, Koh WP, Lee HP, Yu MC. Diabetes mellitus and risk of colorectal cancer in the Singapore Chinese Health Study. J Natl Cancer Inst 2006;98:135–8.
(57) Aaronson SA. Growth factors and cancer. Science 1991;254:1146–53.
(58) Kaaks R, Lukanova A. Energy balance and cancer: the role of insulin and insulin-like growth factor-I. Proc Nutr Soc 2001;60:91–106.
(59) Sandhu MS, Dunger DB, Giovannucci EL. Insulin, insulin-like growth factor-I (IGF-I), IGF binding proteins, their biologic interactions, and colorectal cancer. J Natl Cancer Inst 2002;94:972–80.
(60) Wu Y, Yakar S, Zhao L, Hennighausen L, LeRoith D. Circulating insulin-like growth factor-I levels regulate colon cancer growth and metastasis. Cancer Res 2002;62:1030–5.
(61) Hardwick JC, Van Den Brink GR, Offerhaus GJ, Van Deventer SJ, Peppelenbosch MP. Leptin is a growth factor for colonic epithelial cells. Gastroenterology 2001;121:79–90.
(62) Stattin P, Lukanova A, Biessy C, Soderberg S, Palmqvist R, Kaaks R, et al. Obesity and colon cancer: does leptin provide a link? Int J Cancer 2004;109:149–52.
(63) Stattin P, Palmqvist R, Soderberg S, Biessy C, Ardnor B, Hallmans G, et al. Plasma leptin and colorectal cancer risk: a prospective study in Northern Sweden. Oncol Rep 2003;10:2015–21.
(64) Brakenhielm E, Veitonmaki N, Cao R, Kihara S, Matsuzawa Y, Zhivotovsky B, et al. Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. Proc Natl Acad Sci U S A 2004; 101:2476–81.
(65) Wei EK, Giovannucci E, Fuchs CS, Willett WC, Mantzoros CS. Low Plasma Adiponectin Levels and Risk of Colorectal Cancer in Men: A Prospective Study. J Natl Cancer Inst 2005;97:1688–94.
(66) Slattery ML, Potter JD, Curtin K, Edwards S, Ma KN, Anderson K, et al. Estrogens reduce and withdrawal of estrogens increase risk of microsatellite instability-positive colon cancer. Cancer Res 2001;61:126–30.
(67) Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. Nat Genet 1994;7:536–40.
(68) Engeland A, Tretli S, Austad G, Bjorge T. Height and body mass index in relation to colorectal and gallbladder cancer in two million norwegian men and women. Cancer Causes Control 2005;16:987–96.

(69) Giovannucci E, Rimm EB, Liu Y, Willett WC. Height, predictors of C-peptide and cancer risk in men. Int J Epidemiol 2004;33:217–25.
(70) Albanes D, Winick M. Are cell number and cell proliferation risk factors for cancer? J Natl Cancer Inst 1988;80:772–4.
(71) Okasha M, Gunnell D, Holly J, Davey Smith G. Childhood growth and adult cancer. Best Pract Res Clin Endocrinol Metab 2002;16:225–41.
(72) Weindruch R, Sohal RS. Seminars in medicine of the Beth Israel Deaconess Medical Center. Caloric intake and aging. N Engl J Med 1997;337:986–94.
(73) Frankel S, Gunnell DJ, Peters TJ, Maynard M, Davey Smith G. Childhood energy intake and adult mortality from cancer: the Boyd Orr Cohort Study. BMJ 1998;316:499–504.
(74) Ross MH, Bras G. Lasting influence of early caloric restriction on prevalence of neoplasms in the rat. J Natl Cancer Inst 1971;47:1095–113.
(75) Juul A, Dalgaard P, Blum WF, Bang P, Hall K, Michaelsen KF, et al. Serum levels of insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) in healthy infants, children, and adolescents: the relation to IGF-I, IGF-II, IGFBP-1, IGFBP-2, age, sex, body mass index, and pubertal maturation. J Clin Endocrinol Metab 1995;80:2534–42.
(76) Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 2004;363:1346–53.
(77) Matano Y, Okada T, Suzuki A, Yoneda T, Takeda Y, Mabuchi H. Risk of colorectal neoplasm in patients with acromegaly and its relationship with serum growth hormone levels. Am J Gastroenterol 2005;100:1154–60.
(78) Renehan AG, O'Connell J, O'Halloran D, Shanahan F, Potten CS, O'Dwyer ST, et al. Acromegaly and colorectal cancer: a comprehensive review of epidemiology, biological mechanisms, and clinical implications. Horm Metab Res 2003;35:712–25.
(79) Giovannucci E. Obesity, gender, and colon cancer. Gut 2002;51:147.
(80) Byers T, Nestle M, McTiernan A, Doyle C, Currie-Williams A, Gansler T, et al. American Cancer Society guidelines on nutrition and physical activity for cancer prevention: Reducing the risk of cancer with healthy food choices and physical activity. CA Cancer J Clin 2002;52:92–119.

Notes

Supported by the "Europe Against Cancer" Programme of the European Commission (SANCO); Deutsche Krebshilfe; German Cancer Research Center; German Federal Ministry of Education and Research; Danish Cancer Society; Health Research Fund (FIS) of the Spanish Ministry of Health (Network RCESP C03/09); the Spanish Regional Governments of Andalucia, Asturia, Basque Country, Murcia and Navarra; Cancer Research UK; Medical Research Council, UK; the Stroke Association, UK; British Heart Foundation; Department of Health, UK; Food Standards Agency, UK; the Wellcome Trust, UK; Greek Ministry of Health; Greek Ministry of Education; Italian Association for Research on Cancer (AIRC); Dutch Ministry of Public Health, Welfare and Sports; National Cancer Registry and the Regional Cancer Registries Amsterdam, East and Maastricht of The Netherlands; World Cancer Research Fund (WCRF); Swedish Cancer Society; Swedish Scientific Council; Regional Government of Skåne, Sweden. The sponsors had no role in the study design, data collection, analysis, interpretation of results, or writing of the manuscript.

We thank Bertrand Hemon, Ellen Kohlsdorf, and Wolfgang Bernigau for data coding, as well as all participants in EPIC for their invaluable contribution to the study.

Present address: Elio Riboli, Department of Epidemiology & Public Health, Imperial College London, UK.

Manuscript received December 13, 2005; revised April 18, 2006; accepted May 16, 2006.

Physical Activity and Risk of Colon and Rectal Cancers: The European Prospective Investigation into Cancer and Nutrition

Christine Friedenreich,[1,2] Teresa Norat,[1] Karen Steindorf,[3] Marie-Christine Boutron-Ruault,[5] Tobias Pischon,[6] Mathieu Mazuir,[1] Françoise Clavel-Chapelon,[5] Jakob Linseisen,[4] Heiner Boeing,[6] Manuela Bergman,[6] Nina Fons Johnsen,[7] Anne Tjønneland,[7] Kim Overvad,[8] Michelle Mendez,[9] J. Ramón Quirós,[10] Carmen Martinez,[11] Miren Dorronsoro,[12] Carmen Navarro,[13] Aurelio Barricarte Gurrea,[14] Sheila Bingham,[15] Kay-Tee Khaw,[16] Naomi Allen,[17] Tim Key,[17] Antonia Trichopoulou,[18] Dimitrios Trichopoulos,[18] Natassa Orfanou,[18] Vittorio Krogh,[19] Domenico Palli,[20] Rosario Tumino,[21] Salvatore Panico,[22] Paolo Vineis,[23,24] H. Bas Bueno-de-Mesquita,[25] Petra H.M. Peeters,[26] Evelyn Monninkhof,[26] Göran Berglund,[27] Jonas Manjer,[28] Pietro Ferrari,[1] Nadia Slimani,[1] Rudolf Kaaks,[1] and Elio Riboli[1,22]

[1]Nutrition and Hormones Group, IARC, Lyon, France; [2]Division of Population Health and Information, Alberta Cancer Board, Calgary, Alberta, Canada; [3]Unit of Environmental Epidemiology, German Cancer Research Centre; [4]Division of Clinical Epidemiology, German Cancer Research Centre, Heidelberg, Germany; [5]Institut National de la Santé et de la Recherche Medicale U521, Institut Gustave Rouissy, Villejuif, France; [6]Department of Epidemiology, German Institute of Human Nutrition, Potsdam-Rehbruecke, Germany; [7]Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark; [8]Department of Clinical Epidemiology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark; [9]Department of Epidemiology, Catalan Institute of Oncology, Institut d'Investigació Biomèdica de Bellvitge, Barcelona, Spain; [10]Public Health and Health Planning Directorate, Oviedo, Spain; [11]Escuela Andaluza de Salud Publica, Granada, Spain; [12]Department of Public Health of Guipuzcoa, San Sebastian, Spain; [13]Department of Epidemiology, Health Council of Murcia, Murcia, Spain; [14]Public Health Institute of Navarra, Pamplona, Spain; [15]Dunn Human Nutrition Unit, Medical Research Council MRC Centre for Nutritional Epidemiology in Cancer Prevention and Survival, University of Cambridge, United Kingdom; [16]Department of Public Health and Primary Care, School of Clinical Medicine, University of Cambridge, Cambridge, United Kingdom; [17]Cancer Research UK Epidemiology Unit, University of Oxford, United Kingdom; [18]Department of Hygiene and Epidemiology, School of Medicine, University of Athens, Athens, Greece; [19]Epidemiology Unit, National Cancer Institute, Milan, Italy; [20]Molecular and Nutritional Epidemiology Unit, Centro per lo Studio e la Prevenzione Oncologica-Scientific Institute of Tuscany, Florence, Italy; [21]Cancer Registry, Azienda Ospedaliera ''Civile M.P. Arezzo,'' Ragusa, Italy; [22]Dipartimento di Medicina Clinica e Sperimentale, Università di Napoli, Naples, Italy; [23]University of Torino, Turin, Italy; [24]Department of Epidemiology and Public Health, Imperial College, London, United Kingdom; [25]National Institute of Public Health and the Environment, Bilthoven, the Netherlands; [26]Julius Centre for Health Sciences and Primary Care, University Medical Centre, Utrecht, the Netherlands; [27]Department of Clinical Sciences, Malmö University Hospital; and [28]Department of Surgery, Malmö University Hospital, Malmö, Sweden

Abstract

We investigated several aspects of the role of physical activity in colon and rectal cancer etiology that remain unclear in the European Prospective Investigation into Nutrition and Cancer. This cohort of 413,044 men and women had 1,094 cases of colon and 599 cases of rectal cancer diagnosed during an average of 6.4 years of follow-up. We analyzed baseline data on occupational, household, and recreational activity to examine associations by type of activity, tumor subsite, body mass index (BMI), and energy intake. The multivariate hazard ratio for colon cancer was 0.78 [95% confidence interval (95% CI), 0.59-1.03] among the most active participants when compared with the inactive, with evidence of a dose-response effect (P_{trend} = 0.04). For right-sided colon tumors, the risk was 0.65 (95% CI, 0.43-1.00) in the highest quartile of activity with evidence of a linear trend (P_{trend} = 0.004). Active participants with a BMI under 25 had a risk of 0.63 (95% CI, 0.39-1.01) for colon cancer compared with the inactive. Finally, an interaction between BMI and activity ($P_{interaction}$ = 0.03) was observed for right-sided colon cancers; among moderately active and active participants with a BMI under 25, a risk of 0.38 (95% CI, 0.21-0.68) was found as compared with inactive participants with BMI >30. No comparable decreased risks were observed for rectal cancer for any type of physical activity for any subgroup analyses or interactions considered. We found that physical activity reduced colon cancer risk, specifically for right-sided tumors and for lean participants, but not rectal cancer. (Cancer Epidemiol Biomarkers Prev 2006;15(12):2398–407)

Received 7/18/06; revised 9/14/06; accepted 10/5/06.

Grant support: ''Europe Against Cancer Program'' of the European Commission (SANCO); Danish Cancer Society; German Cancer Aid; Ligue Nationale contre le Cancer, 3M Company, Institut National de la Sante et de la Recherche Medicale; German Cancer Research Center; German Federal Ministry of Education and Research; Dutch Ministry of Public Health, Welfare and Sports; National Cancer Registry and the Regional Cancer Registries Amsterdam, East and Maastricht of the Netherlands; Norwegian Cancer Society; Norwegian Research Council; Health Research Fund (Fondo Investigación Sanitaria) of the Spanish Ministry of Health; Greek Ministry of Health; Greek Ministry of Education; Italian Association for Research on Cancer; Spanish Regional Governments of Andalucia, Asturias, Basque Country, Murcia and Navarra and ISCIII, Red de Centros RCESP, C03/09; Swedish Cancer Society; Swedish Scientific Council; Regional Government of Skane, Sweden; Cancer Research UK; Medical Research Council, UK; Stroke Association, UK; British Heart Foundation; Department of Health, UK; Food Standards Agency, UK; Wellcome Trust, UK.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: This work was done while Christine Friedenreich was a Visiting Scientist at the IARC. Heather Neilson and Marla Orenstein assisted with the literature review for this article.

Requests for reprints: Christine Friedenreich, Division of Population Health and Information, Alberta Cancer Board, Calgary, Alberta, Canada T2N 4N2. Phone: 403-521-3841; Fax: 403-270-8003; E-mail: chrisf@cancerboard.ab.ca

doi:10.1158/1055-9965.EPI-06-0595

Introduction

There is convincing evidence that physical activity reduces colon cancer risk; however, the evidence for rectal cancer is unclear (1). Of the 58 studies conducted to date on colon, rectal, or colorectal cancer and physical activity (2-59), 46 studies have found a risk reduction for colon cancer among the most physically active as compared with the least active study subjects despite many different physical activity assessment methods used in these studies (3, 4, 6, 9-24, 26, 27, 31-37, 40, 42-44, 47-52, 54-61). The risk reduction observed ranged from 10% to >50%, with 27 studies (3, 6, 9, 12, 17, 19-24, 27, 31, 32, 34, 35, 37, 44, 45, 47-50, 52, 56, 58, 59) finding an average risk reduction of at least 40% for colon cancer. Very few studies have had detailed measurements of physical activity and ~30 studies (2, 6, 8, 9, 12, 14-18, 20, 22, 24, 27, 31, 32, 34, 42, 44, 45, 51, 52, 55, 56, 58-60, 62-64) have been able to examine the risk by colon tumor subsite. Some evidence also suggests that the etiology of colon cancer may differ by subsite (65, 66);

however, the evidence regarding the effect of physical activity on colon tumor subsite remains inconsistent. In addition, none of the large prospective cohort studies that examined these associations (10, 11, 18, 36, 57) has been conducted in a heterogeneous study population drawn from numerous different countries. We are conducting a large multinational cohort study in Europe in which data about physical activity were collected at baseline and with detailed data on confounders, effect modifiers, and tumor location. Given the important public health significance of physical activity for cancer risk reduction and the need for more definitive evidence on this topic, we examined these associations in the European Prospective Investigation into Cancer and Nutrition (EPIC).

Materials and Methods

Study Cohort. The EPIC study is a prospective cohort originally established to investigate the associations between dietary, lifestyle, genetic, and environmental factors and risk of specific cancers. The design and baseline data collection methods have previously been described (67). There were 366,521 women and 153,457 men enrolled between 1992 and 1998 in 23 regional or national centers in 10 European countries (Denmark, France, Germany, Greece, Italy, Norway, Spain, Sweden, the Netherlands, and United Kingdom; ref. 67). These participants were recruited from the general population from defined areas in each country in most subcohorts with some exceptions: women who were members of a health insurance scheme for state school employees in France; women attending breast cancer screening in Utrecht, the Netherlands; blood donors in some components of the Italian and Spanish subcohorts; and a high number of vegans and vegetarians in the Oxford ''Health conscious'' cohort. Participants were mainly between 35 and 70 years of age at enrollment and provided written informed consent at the time they completed the baseline questionnaires on diet, lifestyle, and medical history. Approval for this study was obtained from the ethical review boards of the IARC and from all local institutions where subjects had been recruited for the EPIC study.

For this analysis, we excluded 26,040 cohort members with prevalent cancer at any site at enrollment based on the self-reported lifestyle questionnaire or based on information from the cancer registries; 65,648 members who had no physical activity questionnaire data including all study subjects from Norway and Umeå, Sweden, ~25% of the participants in Bilthoven, the Netherlands, and a few in the two UK centers; and 16,725 members with missing questionnaire data or missing dates of diagnosis or follow-up. We also excluded participants who were in the lowest and the highest 1% of the distribution of the ratio of reported total energy intake to energy requirement (68). The number of subjects included in this analysis was 413,044.

Identification of Colorectal Cancer Patients. Cases were identified through population-based cancer registries, except in France, Germany, and Greece, where a combination of methods, including health insurance records, cancer and pathology registries, and active follow-up through study subjects and their next-of-kin was used. Follow-up began at the date of enrollment and ended at either the date of diagnosis of colorectal cancer, death, or last complete follow-up. By April 2004, for the centers using record linkage with cancer registry data (Denmark, Italy, United Kingdom, the Netherlands, Spain, and Sweden), complete follow-up was available between December 31, 1999 and June 30, 2003, and for the centers using active follow-up (France, Germany, Greece), the last contact dates ranged between June 30, 2002 and March 11, 2004. The International Classification of Diseases for Oncology, 2nd version, was used to classify all incident cases of colon (C18) and rectal cancer (C19 and C20). Tumors of the anal canal were not included. For some analyses, colon cancers were subdivided into right colon tumors (codes C18.0-18.5 corresponding to tumors of the cecum, appendix, ascending colon, hepatic flexure, transverse colon, and splenic flexure) and left colon tumors (C18.6-18.7 including the descending and sigmoid colon).

Physical Activity Data. A description of the physical activity ascertainment used in the EPIC study has been described in detail elsewhere (69). The baseline questions on physical activity were derived from the more extensive modified Baecke questionnaire (70). An assessment of the relative validity and reproducibility of the nonoccupational physical activity questions was undertaken in a sample of men and women from the Netherlands and the short version of the questionnaire, similar to that used in EPIC, was found to be satisfactory for the ranking of subjects for their physical activity levels although less suitable for the estimation of energy expenditure (71). Physical activity data were obtained in either in-person interviews or self-administered using a standardized questionnaire in all centers included in this analysis.

Data on current occupational activity included employment status and the level of physical activity done at work (nonworker, sedentary, standing, manual, heavy manual. and unknown). In the Danish centers, the question focused on type of work activity done within the last year, and participants who did not answer this question were categorized as nonworking. Housewives were categorized as nonworkers except in the Spanish centers where housewives were categorized as ''standing'' most of the time. For comparability purposes, Spanish women who reported >35 h/wk of household activity were considered as housewives and their occupational physical activity data recoded to ''nonworker.''

The frequency and duration of nonoccupational physical activity data that were captured in all centers comprised household activities, including housework, home repair (do-it-yourself activities), gardening, and stair climbing, and recreational activities, including walking, cycling, and sports combined as done in winter and summer separately. Because the intensity of recreational and household activities was not directly recorded, a metabolic equivalent (MET) value was assigned to each reported activity according to the Compendium of Physical Activities (72). A MET is defined as the ratio of work metabolic rate to a standard metabolic rate of 1.0 (4.184 kJ) kg^{-1} h^{-1}; 1 MET is considered a resting metabolic rate obtained during quiet sitting. The MET values assigned to the nonoccupational data were 3.0 for walking, 6.0 for cycling, 4.0 for gardening, 6.0 for sports, 4.5 for home repair (do-it-yourself work), 3.0 for housework, and 8.0 for stair climbing. These mean MET values were obtained by estimating the average of all comparable activities in the Compendium. The mean numbers of hours per week of summer and winter household and recreational activities were estimated and then multiplied by the appropriate MET values to obtain MET-hours per week of activity.

Household and recreational activities in MET-hours per week were combined and cohort participants classified according to sex-specific EPIC-wide quartiles of total nonoccupational physical activity (low, medium, high, and very high). To derive an index of physical activity, quartiles of nonoccupational physical activity were cross-classified with the categories of occupational activity (Appendix Table 1). This index was developed based on a previous index constructed by Wareham and colleagues for the EPIC physical activity questionnaire data, which cross-classified occupational activity with hours spent doing cycling and sports. They validated the index against energy expenditure assessed by heart rate

monitoring in 173 men and women ages 40 to 65 years from Ely, Cambridgeshire (73). In this validation study, the index was found to be appropriate for ranking participants in large epidemiologic studies. To make the index more comprehensive, we cross-classified all household and recreational activity combined with occupational activity. In so doing, more information on each individual's actual activity done at baseline was included into the assessment of overall physical activity. We compared the results obtained using Wareham's index with those obtained with this new total physical activity index, and they were very similar.

As a way of indirectly assessing the validity of the total physical activity index derived by us, we also examined the means for each category of the index with the ratio of energy intake and basal metabolic rate adjusted for age, center, and BMI. The estimates of energy intake were taken from the dietary data collected in EPIC and the basal metabolic rate was estimated using prediction equations based on age, sex, height, and weight (74). We found that for men and women, there was a positive relationship between energy intake/basal metabolic rate and total activity, indicating that this index appropriately ranked the subjects according to their energy intake and requirements for their activity levels.

Statistical Methods. The analyses were conducted separately for colon and rectal cancers and tumor subsite within the colon because our a priori knowledge was that the association between physical activity and colon cancer differs according to site. Analyses were conducted using Cox proportional hazards regression. Attained age was used as the primary time variable. The analyses were stratified by center to control for differences in questionnaire design, follow-up procedures, and other center effects. Sex was included as a covariate when the analyses were conducted for the entire study population. In all models, age was used as the primary time variable, with time at entry and time when participants were diagnosed with cancer, died, lost to follow-up, or were censored at the end of the follow-up period, whichever came first, as the time at entry and exit, respectively. For descriptive purposes, mean values were computed after adjustment for age and center.

Physical activity was analyzed using categorical variables. For recreational and household activity, quartile cut points based on the cohort population distribution were used. Trend tests were estimated on scores (1-4) applied to the categories/quartiles of the physical activity variables and entered as a continuous term in the regression models. Relative risks were estimated from the hazard ratio within each category. Two sets of models are presented for each physical activity variable considered. The first are stratified for age and center and adjusted for the other types of physical activity (i.e., occupational, household, or recreational) and the second are adjusted for these factors and several other confounders.

A full examination of confounding was undertaken with the data on physical activity and cancer. Variables that were considered as potential confounders included the following dietary variables: total energy intake, intakes of red and processed meat, fish, fiber, fruits and vegetables, dairy products, current and lifelong alcohol, dietary calcium, folate, and the following lifestyle and demographic variables: education (none, primary school completed, technical/professional school, secondary school, university degree), marital status, smoking status (never, former, current, and unknown), ever use of hormone replacement therapy (for women only), height, weight, body mass index [BMI; weight (kg)/height $(cm)^2$], waist and hip circumference, and waist-hip ratio. The variables that were chosen as confounders either influenced the goodness-of-fit of the model (as assessed by examining the log likelihood) or were considered to be biologically relevant or important to control for in the final multivariate model. The final models for colon cancer were adjusted for education, smoking status, current alcohol intake (in grams per day, categorized into quartiles), height (in centimeters, categorized into sex- and center-specific tertiles), weight (in kilograms, categorized into sex- and center-specific tertiles), energy intake (in kilocalories, categorized into quartiles), and fiber intake (in grams per day, categorized into quartiles). The final models for rectal cancer were also adjusted for fish intake (in grams per day, categorized into quartiles). The confounders that were retained because they influenced the goodness-of-fit of the model were education, height, weight, alcohol intake, smoking, and fish intake (rectal cancer models only); energy and fiber intakes were retained because of their biological relevance in colorectal cancer etiology.

We also examined the possibility of effect modification by stratifying the population on BMI (<25, ≥25-<30, ≥30) and on energy intake (in tertiles) and by including an interaction term in our models. These factors were chosen as they are all considered independent risk factors for colon cancer and were considered a priori as effect modifiers of the relation between physical activity and colon cancer. Finally, we examined the heterogeneity of the results by country within the EPIC study by including country as a main effect and including interaction terms in the Cox models with dummy variables for each country. All analyses were done using SAS Statistical Software, version 8 (75); all statistical tests were two sided. To test hazard ratios for overall significance, P values for Wald χ^2 were computed with degrees of freedom equal to the number of categories minus one.

Results

We included 413,044 study participants who contributed 2,635,075 person-years for the mean follow-up of 6.38 years available for this analysis (Table 1). During the follow-up to 2003, there were 1,693 colorectal cancers, of which 1,094 were colon cancers and 599 rectal cancers. Histologic confirmation of these cancers was available for 1,376 of the tumors. The remaining tumors were confirmed with a variety of diagnostic methods and 22 (1.3%) were self-reported. The mean age at recruitment into this cohort was 51.9 years and 69.1% of the participants were female.

The demographic and lifestyle characteristics of the colon and rectal cancer cases and noncases were compared (Table 2). The case patients were older than the noncase participants, had slightly greater BMIs (weight/$height^2$), but had comparable mean total energy intake (kcal/d) and mean physical activity levels to the noncases. They also had similar smoking habits, education, and type of occupational activity. Differences were found between the cases and noncases in their dietary intakes, with cases having higher red meat and fish consumption, lower fruit and vegetable intakes, and higher alcohol intakes, particularly for the rectal cancer cases.

The first set of analyses examined the risk of colon cancer by type of physical activity. All analyses were initially conducted for men and women separately because our a priori hypothesis was that the associations differ by gender. No heterogeneity between sexes was observed and we present only the results for the total study population (for colon cancer, P values of the heterogeneity test for sex differences were 0.92, 0.83, 0.13, and 0.51 for total physical activity index, occupational, household, and recreational physical activities, respectively; for rectal cancer, the corresponding P values were 0.40, 0.48, 0.95, and 0.96, respectively).

For total physical activity, a statistically significant trend of decreasing relative risk estimates with increasing activity category was observed for colon cancer (P_{trend} = 0.04) in multivariate adjusted models (Table 3). Active study participants had a hazard ratio of 0.78 [95% confidence interval (95% CI), 0.59-1.03] as compared with the inactive participants.

Table 1. Size of the EPIC cohort for the analyses of physical activity and colon and rectal cancers, by country

Country	Cohort size	Age at recruitment (mean ± SD), y	Years of follow-up (mean ± SD)	Person-years	Female, %	No. colon cancer cases	No. rectal cancer cases	%Active*		%Inactive*	
								M	F	M	F
France	67,654	52.7 ± 6.6	8.41 ± 0.92	569,258	100	164	21	NA	2.0	NA	15.5
Italy	44,567	50.5 ± 7.9	5.91 ± 1.54	263,550	68.5	110	44	11.0	9.1	28.1	14.3
Spain	39,992	49.2 ± 8.0	6.68 ± 1.05	267,346	62.1	80	41	14.7	5.8	21.5	5.8
United Kingdom—general population	28,211	57.68 ± 9.3	5.47 ± 1.39	154,221	58.4	118	58	14.9	11.0	12.5	10.5
United Kingdom—health conscious	45,880	43.9 ± 14.4	5.38 ± 1.20	246,960	77.1	62	33	13.3	9.5	18.2	21.3
The Netherlands	32,394	49.8 ± 11.8	6.09 ± 2.03	197,235	76.3	107	52	26.0	19.0	12.5	8.5
Greece	25,574	53.1 ± 12.6	3.71 ± 0.76	94,809	58.6	13	12	9.8	12.7	18.1	5.5
Germany	49,498	50.6 ± 8.6	5.83 ± 1.43	288,761	56.4	103	69	12.4	7.9	20.6	20.6
Sweden	24,267	58.0 ± 7.6	7.61 ± 1.69	184,706	57.8	106	88	4.1	4.8	21.2	19.3
Denmark	55,007	56.7 ± 4.4	6.69 ± 1.07	368,229	52.2	231	181	18.3	8.5	22.7	26.5
Total	413,044	51.9 ± 10.0	6.38 ± 1.78	2,635,075	69.1	1,094	599	13.9	8.0	20.4	15.6

*Excluding all study subjects who had unknown or missing occupational physical activity data.

None of the different types of physical activity considered here, occupational, household, or recreational activity, independently accounted for the inverse association of total physical activity with colon cancer risk in multivariate models where each type of physical activity was mutually adjusted by the others. However, the inverse association seemed somewhat stronger with recreational activity than with occupational and household activity. The multivariate risk estimate for the highest quartile of recreational activity (≥42.8 MET-h/wk) was 0.88 (95% CI, 0.74-1.05) when compared with the lowest quartile (<12 MET-h/wk).

No association between rectal cancer and total physical activity or any specific type of activity was found (Table 3). Active compared with inactive study participants had a relative risk of 1.02 (95% CI, 0.73-1.44) for total physical activity and comparable null results were found for occupational, household, and recreational activity.

We next examined the association by tumor subsite within the colon (Table 4). The risk reductions seemed to be restricted to right-sided colon cancers. Participants who were in the moderately active or active category of physical activity had an up to 36% decreased relative risk of right-sided colon cancer compared with inactive subjects, with a statistically significant linear trend across categories (P_{trend} = 0.004). A 26% relative risk reduction was seen in the highest compared with lowest quartile of household activity with a marginal statistically significant trend (P_{trend} = 0.05) across quartiles. Occupational activity was also related to lower risk

Table 2. Demographic and lifestyle characteristics at time of enrollment among participants with incident colon and rectal cancer and individuals without cancer in EPIC

Characteristic*	Incident colon cancer cases (n = 1,094)		Incident rectal cancer cases (n = 599)		Individuals without incident colon or rectal cancer (n = 411,351)	
	Males (n = 417)	Females (n = 677)	Males (n = 293)	Females (n = 306)	Males (n = 127,050)	Females (n = 284,301)
Age (mean ± SD), y	57.8 ± 8.4	57.6 ± 8.8	57.4 ± 10.8	56.3 ± 10.0	52.6 ± 10.1	51.1 ± 10.2
BMI (mean ± SD), kg/m^2	27.5 ± 5.3	26.6 ± 5.5	26.7 ± 6.3	26.1 ± 6.4	26.4 ± 4.5	25.7 ± 4.5
Waist hip ratio (mean ± SD)	0.95 ± 0.08	0.82 ± 0.09	0.96 ± 0.12	0.82 ± 0.11	0.94 ± 0.07	0.80 ± 0.11
Dietary intake (mean ± SE)						
Energy intake, kcal	2,404.8 ± 31.5	2,018.8 ± 20.5	2,470.3 ± 37.6	1,985.5 ± 29.2	2,486.6 ± 1.9	2,005.6 ± 1.0
Total red meat, g	65.1 ± 2.4	46.8 ± 1.2	70.0 ± 2.6	49.1 ± 2.0	62.2 ± 0.1	42.1 ± 0.07
Total fish/shellfish, g	41.6 ± 1.8	33.2 ± 1.1	43.1 ± 2.0	31.3 ± 1.6	39.5 ± 0.1	32.8 ± 0.05
Fruits and vegetables, g	385.6 ± 12.8	478.1 ± 10.0	364.4 ± 13.4	439.6 ± 13.3	443.7 ± 0.9	505.8 ± 0.5
Fiber, g	21.6 ± 0.4	21.4 ± 0.3	21.2 ± 0.4	20.5 ± 0.4	23.6 ± 0.03	22.0 ± 0.01
Alcohol intake, g	24.0 ± 1.3	10.1 ± 0.6	29.3 ± 1.6	9.0 ± 0.7	22.4 ± 0.07	8.8 ± 0.02
Smoking status, %						
Never smoker	24.5	58.1	23.2	53.9	30.6	58.2
Ex-smoker	48.2	23.3	45.4	21.2	38.1	22.3
Current smoker	26.6	16.7	31.1	24.5	30.4	17.9
Education, %						
None	4.6	3.3	4.44	1.6	4.2	4.7
Primary school completed	34.8	26.7	32.4	32.7	27.0	21.5
Technical/professional school	22.8	19.5	23.2	27.8	24.3	19.9
Secondary school	13.0	26.0	10.6	17.3	14.7	25.4
University degree	21.6	19.1	26.3	14.1	27.1	23.8
Occupational activity, %						
Nonworker	38.1	52.4	36.9	51.3	23.0	39.1
Sedentary	29.7	18.5	30.7	17.0	36.5	24.8
Standing	15.6	20.2	17.8	18.6	20.2	26.4
Manual/heavy manual	15.1	6.9	13.7	10.1	19.1	7.1
Household activity (mean ± SD), MET-h/wk	34.4 ± 43.5	67.3 ± 46.0	36.4 ± 52.7	63.2 ± 49.1	30.0 ± 39.9	67.0 ± 40.6
Recreational activity (mean, SD), MET-h/wk	28.9 ± 29.5	26.1 ± 31.3	31.2 ± 39.5	32.0 ± 36.8	30.7 ± 27.3	28.8 ± 27.8

*All mean values were adjusted for age and center.

Table 3. Physical activity and risk of colon and rectal cancers, by type of activity for total study population

Type of activity	Colon cancer, total study population				Rectal cancer, total study population				
	No. cases	No. person-years	Age- and center-stratified hazard ratio (95% CI)*	Multivariate hazard ratio (95% CI)†	Quartile definitions/ cut points	No. cases	No. person-years	Age- and center-stratified hazard ratio (95% CI)*	Multivariate hazard ratio (95% CI)‡
Total physical activity									
Inactive	162	443,155	1.0	1.0	Inactive	91	442,864	1.0	1.0
Moderately inactive	397	942,463	0.91 (0.75-1.10)	0.92 (0.76-1.12)	Moderately inactive	192	941,684	1.01 (0.78-1.31)	1.02 (0.78-1.32)
Moderately active	436	943,626	0.84 (0.69-1.01)	0.86 (0.70-1.04)	Moderately active	246	944,956	1.00 (0.78-1.29)	1.02 (0.79-1.32)
Active	80	239,427	0.76 (0.58-1.00)	0.78 (0.59-1.03)	Active	58	239,318	1.01 (0.72-1.40)	1.02 (0.73-1.44)
P_{trend}			0.02	0.04	P_{trend}			0.98	0.91
Occupational activity									
Sedentary	249	727,785	1.0	1.0	Sedentary	142	727,363	1.0	1.0
Standing	202	689,087	0.96 (0.80-1.17)	0.98 (0.81-1.19)	Standing	109	688,712	1.11 (0.86-1.43)	1.11 (0.85-1.43)
Manual/heavy manual	110	274,166	0.89 (0.71-1.12)	0.91 (0.72-1.15)	Manual/heavy manual	71	274,015	0.97 (0.72-1.29)	0.96 (0.71-1.30)
Nonworker	514	879,634	0.90 (0.75-1.08)	0.91 (0.75-1.09)	Nonworker	265	878,734	1.15 (0.90-1.47)	1.16 (0.90-1.49)
P_{trend}§			0.29	0.38	P_{trend}§			0.97	0.82
Household activity (MET-h/wk)									
<19.5	289	673,316	1.0	1.0	<19.5	150	672,766	1.0	1.0
≥19.5-<39.6	281	682,023	0.94 (0.80-1.12)	0.95 (0.80-1.12)	≥19.5-<39.6	157	681,576	1.02 (0.81-1.28)	1.02 (0.81-1.28)
≥39.6-<73.9	272	659,317	0.90 (0.76-1.07)	0.90 (0.76-1.07)	≥39.6-<73.9	167	658,912	1.09 (0.87-1.38)	1.10 (0.87-1.39)
≥73.9	252	618,226	0.92 (0.76-1.13)	0.93 (0.76-1.13)	≥73.9	125	617,747	0.97 (0.74-1.26)	0.98 (0.75-1.29)
P_{trend}			0.34	0.35	P_{trend}			0.97	0.88
Recreational activity (MET-h/wk)									
<12.0	317	675,216	1.0	1.0	<12.8	139	488,157	1.0	1.0
≥12.0-<24.8	255	665,716	0.83 (0.70-0.98)	0.85 (0.71-1.00)	≥12.8-<24.0	144	490,903	1.14 (0.90-1.44)	1.15 (0.90-1.46)
≥24.8-<42.8	258	658,547	0.81 (0.68-0.96)	0.83 (0.70-0.98)	≥24.0-<42.0	158	477,489	1.20 (0.94-1.51)	1.22 (0.96-1.54)
≥42.8	264	633,403	0.85 (0.71-1.01)	0.88 (0.74-1.05)	≥45.8	158	426,419	1.18 (0.92-1.50)	1.21 (0.94-1.54)
P_{trend}			0.05	0.13	P_{trend}			0.18	0.12

*Base models are stratified by age and center and mutually adjusted for each type of physical activity (occupational, recreational, and household).
†Multivariate models are stratified by age and center and adjusted for energy (kilocalories per day in quartiles), education (none, primary school, technical/professional school), smoking (never, former, current, unknown), height (centimeters in tertiles), weight (kilograms in tertiles), and fiber (grams per day in quartiles).
‡Multivariate models are stratified by age and center and adjusted for energy (kilocalories per day in quartiles), education (none, primary school, technical/professional school), smoking (never, former, current, unknown), height (centimeters in tertiles), weight (kilograms in tertiles), fiber (grams per day in quartiles), and fish intake (grams per day in quartiles).
§Test for trend in occupational activity excluded all study participants categorized as nonworkers, missing, or unknown.

although no clear trends were observed by increasing intensity level in occupational activity. Recreational activity was not statistically significantly related to lower risk of right-sided colon cancer.

We examined the consistency of the results in the subcohorts participating in EPIC. There was no heterogeneity of the association of physical activity with colon cancer across the subcohorts participating in the EPIC study ($P_{heterogeneity}$ = 0.92).

When examining effect modifications by BMI, no statistically significant interaction was observed ($P_{interaction}$ = 0.67; Table 5). Some apparent heterogeneity in the association of physical activity with colon cancer across categories of BMI was observed in the participants in the ''active'' category of physical activity. This heterogeneity is probably explained by random variation due to low number of colon cancer patients with BMI >30 kg/m^2 in this category of physical activity.

Because the other major component of energy balance, besides physical activity, is energy intake, we also investigated effect modification of physical activity and colon cancer by energy intake. There was no statistically significant interaction ($P_{interaction}$ = 0.24; Table 5). In analyses stratified by tertiles of energy intake, the inverse association of physical activity with risk of colon cancer was statistically significant (P_{trend} = 0.003) across the categories of total activity for participants with energy intake in the middle tertile (≥1,827-<2,351 kcal/d) for whom a multivariate-adjusted relative risk of 0.59 (95% CI, 0.36-0.97) was found when comparing active with inactive subjects. A more moderate inverse association was observed for individuals in the lowest energy tertile. Among the highest energy intake tertile, there was no association of physical activity across any category of activity.

Finally, additional effect modification of BMI and energy intake by tumor subsite was investigated (Table 6). The interactions for both BMI and energy intake for right-sided colon cancers were statistically significant ($P_{interaction}$ = 0.03 and 0.003, respectively). We found a very strong risk reduction among moderately active and active normal weight participants (BMI <25) with a right-sided colon cancer (0.38; 95% CI, 0.21-0.68) as well as for overweight participants (BMI≥25-<30) for whom the risk was 0.43 (95% CI, 0.24-0.77) when compared with the inactive, obese study subjects. Participants with the lowest daily caloric intake (<1,827 kcal/d) who were most physically active had a 31% nonstatistically significant decreased risk as compared with the inactive, highest energy intake tertile of participants. There were no clear associations for any combination of BMI and energy intake and physical activity for left-sided colon cancers.

Discussion

In this large European prospective study of more than 400,000 participants, we found an inverse association between physical activity and risk of colon cancer, particularly for right-sided tumors. None of the different types of

physical activity considered (occupational, household, and recreational) independently explained the inverse association, although the association was most apparent for recreational activity for all tumors whereas household activity showed the strongest inverse association for right-sided tumors. A particularly strong inverse association for physical activity was observed among lean and active participants and strong dose-response relations were found in those with lower energy intake. Physical activity was not related to rectal cancer in our study.

The strengths and limitations of this study need to be addressed before discussing the results. First, this large European prospective study of more than 400,000 participants provides a heterogeneity of exposures that is unparalleled in other prospective studies conducted to date. Furthermore, the availability of exposure data on a wide range of other risk factors for colon and rectal cancers, as well as of data on tumor location, has provided a detailed and comprehensive assessment of the role of physical activity in the etiology of colon and rectal cancers. Moreover, this is the only international cohort study with such a large number of cases for which the data could be stratified by BMI and energy intake separately for tumor subsites.

The main limitation of the study was in the physical activity assessment method. Although all types of activity were assessed in this study at the time of recruitment, there was no information on the duration and frequency of occupational activity that precluded estimating a sum of all types of activity in MET-hours per week. An assessment of the relative validity and reproducibility of the EPIC physical activity questions was also undertaken (71) and the short version of the questionnaire, used in EPIC and analyzed here, was found to be satisfactory for the ranking of subjects. Short-term reproducibility (i.e., 5 months) for the questionnaire was quite high, ranging from 0.58 to 0.89, whereas longer-term reproducibility (i.e., 11 months) was between 0.47 and 0.83 for the different measures of physical activity (71). Correlations for the relative validity ranged from 0.28 to 0.81 for comparisons between the questionnaire and activity diaries, which are not real gold standards of activity (71). Hence, the assessment of physical activity used in the EPIC study had some limitations but these were not sufficiently serious to preclude the analyses of physical activity and cancer outcomes.

At least 58 studies have been conducted on colon, rectal, or colorectal cancer and physical activity (2-59) including 22 prospective studies of incident cancer (3, 5, 8-12, 18, 19, 29, 30, 33, 37, 39, 41, 42, 46, 51, 53, 54, 57, 58) and three prospective mortality studies (23, 36, 38). A wide range of methods for defining physical activity has been used in these studies including the type, dose, and time period for assessment. When comparing these results with previous studies, the magnitude of the risk reduction found in the EPIC cohort is comparable to those found in most of these studies and, in some subgroups, equaling the largest risk reductions observed. Overall risk reductions of at least 40% in men, or men and women, have been found in nearly half of these studies (27 of 58 studies; refs. 3, 6, 9, 12, 17, 19-24, 27, 31, 32, 34, 35, 37, 44, 45, 47-50, 52, 56, 58, 59) and most associations do not seem to be confounded by other risk factors for colon cancer. Ten studies observed no effect of physical activity on colon or colorectal cancer (2, 5, 8, 25, 29, 39, 41, 46, 53) and no studies found an increased risk of colon cancer with increased activity levels. Evidence for a

Table 4. Physical activity and risk of right and left colon cancer, total study population

Type of activity	Right colon cancer				Left colon cancer				
	No. cases	No. person-years	Age- and center-stratified hazard ratio (95% CI)*	Multivariate † hazard ratio (95% CI)	Quartile definitions/ cut points	No. cases	No. person-years	Age- and center-stratified hazard ratio (95% CI)*	Multivariate † hazard ratio (95% CI)
Total activity									
Inactive	76	442,792	1.0	1.0	Inactive	60	442,726	1.0	1.0
Moderately inactive	157	941,581	0.77 (0.58-1.03)	0.79 (0.59-1.06)	Moderately inactive	161	941,593	1.11 (0.82-1.51)	1.10 (0.81-1.50)
Moderately active	161	944,680	0.61 (0.46-0.82)	0.64 (0.47-0.86)	Moderately active	220	955,076	1.17 (0.86-1.57)	1.15 (0.84-1.56)
Active	32	239,238	0.61 (0.40-0.94)	0.65 (0.43-1.00)	Active	40	239,255	0.98 (0.65-1.47)	0.96 (0.64-1.45)
P_{trend}			0.001	0.004	P_{trend}			0.74	0.83
Occupational activity									
Sedentary	111	727,225	1.0	1.0	Sedentary	102	727,203	1.0	1.0
Standing	67	688,569	0.77 (0.56-1.05)	0.79 (0.58-1.09)	Standing	95	688,670	1.24 (0.93-1.65)	1.22 (0.91-1.64)
Manual/heavy manual	47	273,925	0.85 (0.60-1.21)	0.90 (0.63-1.29)	Manual/heavy manual	54	273,938	0.99 (0.71-1.38)	0.95 (0.67-1.34)
Nonworker	201	878,573	0.77 (0.58-1.03)	0.81 (0.60-1.08)	Nonworker	230	878,612	1.06 (0.80-1.40)	1.01 (0.76-1.35)
P_{trend}			0.18	0.29	P_{trend}			0.89	0.91
Household activity (MET-h/wk)									
<19.5	112	672,660	1.0	1.0	<19.5	120	672,658	1.0	1.0
≥19.5-<39.6	117	681,388	0.97 (0.75-1.27)	0.97 (0.75-1.27)	≥19.5-<39.6	119	681,436	0.97 (0.75-1.26)	0.97 (0.75-1.26)
≥39.6-<73.9	110	658,749	0.85 (0.64-1.12)	0.84 (0.64-1.12)	≥39.6-<73.9	131	658,772	1.06 (0.82-1.38)	1.06 (0.82-1.38)
≥73.9	90	617,641	0.74 (0.54-1.01)	0.74 (0.54-1.02)	≥73.9	121	617,726	1.03 (0.77-1.37)	1.01 (0.75-1.36)
P_{trend}			0.04	0.05	P_{trend}			0.72	0.78
Recreational activity (MET-h/wk)									
<12.8	116	674,487	1.0	1.0	<12.8	144	674,544	1.0	1.0
≥12.8-<24.0	105	665,148	0.96 (0.73-1.26)	0.96 (0.74-1.26)	≥12.8-<24.0	109	665,176	0.79 (0.62-1.02)	0.80 (0.63-1.04)
≥24.0-<42.0	96	657,915	0.83 (0.62-1.09)	0.84 (0.63-1.11)	≥24.0-<42.0	117	657,989	0.82 (0.63-1.05)	0.84 (0.65-1.08)
≥45.8	112	632,888	0.98 (0.74-1.29)	1.01 (0.76-1.33)	≥45.8	121	632,883	0.83 (0.64-1.07)	0.86 (0.66-1.12)
P_{trend}			0.65	0.80	P_{trend}			0.19	0.31

*Base models are stratified by age and center and mutually adjusted for each type of physical activity (occupational, recreational, and household).
†Multivariate models are stratified by age and center and adjusted for energy (kilocalories per day in quartiles), education (none, primary school, technical/professional school), smoking (never, former, current, unknown), height (centimeters in tertiles), weight (kilograms in tertiles), and fiber (grams per day in quartiles).

Table 5. Physical activity and risk of colon cancer by BMI and energy intake, total study population

Type of activity	BMI <25				BMI ≥25-<30				BMI ≥30			
	No. cases	No. person-years	Age- and center-stratified hazard ratios (95% CI)*	Multivariate† hazard ratios (95% CI)	No. cases	No. person-years	Age- and center-stratified hazard ratios (95% CI)*	Multivariate† hazard ratios (95% CI)	No. cases	No. person-years	Age- and center-stratified hazard ratios (95% CI)*	Multivariate† hazard ratios (95% CI)
Inactive	73	246,511	1.0	1.0	63	148,517	1.0	1.0	26	48,127	1.0	1.0
Moderately inactive	179	554,150	0.84 (0.63-1.11)	0.86 (0.64-1.15)	159	285,335	0.97 (0.71-1.31)	0.95 (0.69-1.29)	59	102,978	0.78 (0.51-1.33)	0.82 (0.50-1.33)
Moderately active	179	427,124	0.85 (0.63-1.13)	0.88 (0.66-1.19)	177	359,955	0.79 (0.58-1.07)	0.78 (0.57-1.07)	80	158,546	0.78 (0.51-1.29)	0.83 (0.51-1.34)
Active	24	111,597	0.60 (0.38-0.96)	0.63 (0.39-1.01)	37	93,589	0.81 (0.54-1.23)	0.81 (0.53-1.24)	19	34,241	1.01 (0.55-1.84)	1.03 (0.56-1.90)
P_{trend}			0.08	0.14			0.07	0.08			0.85	0.98
	<1,827 kcal/d				≥1,827-<2,351 kcal/d				>2,351 kcal/d			
Inactive	46	148,484	1.0	1.0	65	150,738	1.0	1.0	51	143,933	1.0	1.0
Moderately inactive	130	311,913	0.89 (0.63-1.27)	0.95 (0.66-1.36)	151	327,204	0.86 (0.63-1.17)	0.85 (0.62-1.16)	116	303,346	0.91 (0.65-1.27)	0.93 (0.66-1.31)
Moderately active	136	318,877	0.75 (0.52-1.07)	0.81 (0.56-1.18)	136	312,059	0.66 (0.48-0.91)	0.66 (0.48-0.92)	164	314,689	1.09 (0.79-1.51)	1.13 (0.81-1.57)
Active	19	61,979	0.74 (0.42-1.27)	0.81 (0.47-1.41)	22	72,610	0.58 (0.36-0.96)	0.59 (0.36-0.97)	39	104,837	0.96 (0.63-1.46)	1.01 (0.66-1.55)
P_{trend}			0.07	0.19			0.002	0.003			0.61	0.44

*Base models are stratified by age and center and mutually adjusted for each type of physical activity (occupational, recreational, and household).
†Multivariate models are stratified by age and center and adjusted for energy (kilocalories per day in quartiles), education (none, primary school, technical/professional school), smoking (never, former, current, unknown), and fiber (grams per day in quartiles).

''dose-response effect'' (i.e., statistically significant linear trend with increasing levels of total activity and decreasing risks) was found for men, or men and women, in 20 of the 26 studies that examined the trend (3, 6, 7, 9, 12, 13, 17, 20, 22, 27, 31, 32, 35, 37, 42-45, 48-51, 54, 55, 58, 59). Our results for rectal cancer are in concordance with previous studies results because only 6 of 30 studies of rectal cancer (2, 7, 9, 12, 13, 15, 17, 18, 20, 24, 25, 27, 29, 31-33, 35-37, 39, 41, 42, 45, 48, 49, 51, 52, 54, 56, 64) in men, or men and women, have found a statistically significant risk reduction or inverse trend among the most physically active study participants. Indeed, there seems to be increasing convincing evidence for no association between rectal cancer and physical activity.

This study found no difference in colon cancer risk according to gender, which is consistent with the literature. In reviewing previously reported risk ratios and 95% CIs for colorectal and colon cancer incidence and mortality, 23 studies of occupational activity (2, 7, 13-19, 22-24, 31-33, 35, 38, 42, 51, 52, 55, 56, 59) and 23 studies of nonoccupational activity (5, 8, 9, 12, 19, 22, 26, 27, 29, 30, 32, 34, 37, 41, 42, 49, 51, 53-56, 58, 59) generally revealed no obvious differences between males and females.

Table 6. Interaction of BMI and energy intake with physical activity, by right and left colon cancers

Total activity	Right colon						Left colon					
	BMI <25		BMI ≥25-<30		BMI ≥30		BMI <25		BMI ≥25-<30		BMI≥30	
	No. cases	Multivariate* risk	No. cases	Multivariate* risk	No. cases	Multivariate* risk	No. cases	Multivariate* risk	No. cases	Multivariate* risk	No. cases	Multivariate* risk
Inactive	34	0.64 (0.34-1.20)	28	0.61 (0.32-1.16)	14	1.0	26	0.98 (0.42-2.30)	32	1.37 (0.60-3.13)	7	1.0
Moderately inactive	73	0.50 (0.28-0.90)	63	0.54 (0.30-0.98)	23	0.57 (0.29-1.12)	64	1.02 (0.46-2.25)	70	1.41 (0.64-3.07)	31	1.47 (0.64-3.39)
Moderately active and active	73	0.38 (0.21-0.68)	82	0.43 (0.24-0.77)	39	0.56 (0.30-1.05)	102	1.25 (0.58-2.70)	104	1.18 (0.56-2.54)	55	1.57 (0.70-3.45)
$P_{interaction}$				0.03						0.39		
	<1,827 kcal/d		≥1,827-<2,351 kcal/d		>2,351 kcal/d		<1,827 kcal/d		≥1,827-<2,351 kcal/d		>2,351 kcal/d	
Inactive	26	1.54 (0.83-2.90)	31	1.72 (0.96-3.08)	19	1.0	12	0.64 (0.30-1.33)	30	1.32 (0.74-2.35)	23	1.0
Moderately inactive	55	1.07 (0.61-1.91)	64	1.30 (0.76-2.23)	40	0.94 (0.54-1.63)	54	0.96 (0.55-1.68)	65	1.27 (0.76-2.13)	46	0.99 (0.59-1.67)
Moderately active and active	51	0.69 (0.39-1.24)	62	0.86 (0.50-1.48)	81	1.15 (0.69-1.92)	81	1.01 (0.59-1.72)	82	1.08 (0.65-1.78)	98	1.18 (0.73-1.92)
$P_{interaction}$				0.003						0.45		

*Multivariate risk models: BMI interaction adjusted for energy intake, fiber, alcohol, smoking, and education; energy interaction also adjusted for height, weight, alcohol, smoking, fiber, and education.

We also compared risks across three types of activity: occupational, household, and recreational. Neither occupational nor nonoccupational activity was clearly more effective in reducing risk. A review of risk estimates from incidence and mortality studies of colorectal and colon cancer [35 studies in men (2, 7, 9, 12-19, 22-24, 26, 27, 29-33, 35, 37, 38, 41, 42, 49, 51-56, 58, 59) and in 22 women (2, 5, 8, 9, 14, 16, 19, 22, 26, 27, 31, 33, 34, 37, 41, 49, 51, 54-56, 58, 59)] similarly suggested no sign of differential protective effects from occupational or nonoccupational activity.

No statistically significant interaction between BMI and physical activity was observed for right and left tumors combined. Of 58 colon and colorectal studies in the literature, only 11 (3, 20, 22, 27, 29, 31, 44, 48, 51, 59) stratified by BMI. These past studies collectively provide no convincing evidence of any statistically significant interaction between BMI, physical activity, and colon cancer in men or women. In contrast, the present study did find statistically significant effect modification by BMI for right-sided tumors. Slattery et al. (44) similarly examined this interaction according to multiple tumor subsites and reported the BMI interaction term as having statistically significantly improved model fit for right-sided (but not left-sided) tumors. Gerhardsson de Verdier et al. (20) also presented evidence of an interaction with BMI but only described left-sided tumors in this regard.

Like BMI, results stratified by energy intake showed no convincing evidence of effect modification for right- and left-sided tumors combined. Very few groups have previously reported on the same two-way stratification (20, 31, 44, 48) and the results have been inconsistent. After stratifying by tumor subsite, Slattery et al. (44) found that an interaction term improved model fit marginally for left-sided (but not right-sided) tumors in men and older individuals. Results of Gerhardsson de Verdier et al. (20) similarly implied effect modification for left-sided tumors. No other groups described interactions between physical activity, energy intake, and right-sided tumors, a statistically significant finding in the present study.

In the EPIC study, we were able to examine the risks by tumor subsite as has previously been done in 9 cohort studies (8, 9, 12, 18, 42, 51, 58, 62, 63) and 21 case-control studies (2, 6, 14-17, 20, 22, 24, 27, 31, 32, 34, 44, 45, 52, 55, 56, 59, 60, 64). Some of those that examined both subsites have found risk decreases that were stronger and often statistically significant for right-sided tumors (6, 9, 15, 18, 24, 31, 51, 52, 59, 60) or left-sided tumors (2, 12, 16, 17, 20, 58, 62-64). Others (8, 14, 22, 27, 34, 42, 44, 45, 55, 56) have found no clear difference between subsites. Although it seems that the associations are not consistently stronger for right- or left-sided tumors, differing methods could account for this. Of 29 studies that compared tumor subsites, only 15 compared two subsite categories (9, 12, 20, 22, 24, 27, 31, 44, 45, 51, 55, 56, 58, 62, 63) using six definitions for right- and left-sided tumors precluding any direct comparisons with our study results. Levi et al. (31) was the only group to dichotomize tumor subsites as in the EPIC study and similarly found a stronger association with right-sided tumors. Gerhardsson de Verdier et al. (18) also found stronger effects in right-sided tumors (cecum and ascending colon, and transverse colon and flexures) than in left (descending, sigmoid colon) and was, to our knowledge, the only other large prospective study to examine tumor subsites in the colon.

The exact biological mechanisms for the differential associations of physical activity with tumor subsites are not known. Previously hypothesized mechanisms for colon cancer include gastrointestinal transit time, immune function, prostaglandin levels, insulin-related pathways, gastrointestinal-pancreatic hormones, serum cholesterol, and bile acids (76, 77), only some of which may differ between the left or right colon. Physical activity, for example, accelerates movement of stool through the colon (78, 79), possibly providing less time for fecal carcinogens to contact colonic mucosa (80). Only the right colon is innervated by the vagus nerve, which induces peristalsis in response to physical activity. Hence, physical activity may affect motility more intensely in the right colon than in the left (81). The effect could be accentuated if foods that correlated with lower BMI (82, 83) and lower energy intake (84) are also those that traverse the colon more rapidly, such as fiber (80). Although plausible, the epidemiologic evidence for the association between gastrointestinal transit time and colon cancer risk has thus far been inconsistent (76).

In conclusion, this large prospective study conducted in a heterogeneous population of Europeans has found 20% to 25% risk reductions for colon cancer among the physically active population, which were particularly evident for right-sided colon tumors where reductions of 35% were observed. The inverse association of physical activity with right-sided colon cancer was very strong among the normal weight (BMI <25) population and among those with low energy intake (<2,351 kcal/d). Hence, there is a clear benefit of physical activity for right-sided colon cancer risk reduction, which is greatest when normal weight or low energy intake is also maintained. It is of public health importance to note that the benefits of physical activity for colon cancer risk were also observed among the overweight population (BMI >25-<30), suggesting that physical activity has a positive influence on colon cancer risk reduction for a large percentage of the at-risk population. The benefits are stronger among those who also maintain a lower BMI and a lower energy intake. The level of physical activity required for the risk reductions observed in this study translates into 1 hour per day of vigorous physical activity (MET = 6) or 2 h per day of moderate intensity physical activity (MET = 3). This activity could be in any combination of occupational, household, or recreational activity. Because these levels of activity are achievable by most of the at-risk population, the potential for colon cancer risk reduction with increased physical activity is worthy of consideration for cancer prevention programs.

Appendix Table 1. Creation of total physical activity index as the cross-classification of occupational and combined recreational and household activity

Occupational activity	Recreational and household activity (MET-h/wk in sex-specific quartiles)			
	Low	Medium	High	Very high
	Males, ≤34.00; females, ≤51.11	Males, >34.00-≤56.76; females, >51.11-≤82.43	Males, >56.76-≤87.06; females, >82.43-≤123.02	Males, >87.06; females, >123.02
Sedentary	Inactive	Inactive	Moderately inactive	Moderately active
Standing	Moderately inactive	Moderately inactive	Moderately active	Active
Manual	Moderately active	Moderately active	Active	Active
Heavy manual	Moderately active	Moderately active	Active	Active
Nonworker	Moderately inactive	Moderately inactive	Moderately active	Moderately active

References

1. Friedenreich CM, Orenstein MR. Physical activity and cancer prevention: etiologic evidence and biological mechanisms. J Nutr 2002;132:3456–64S.
2. Arbman G, Axelson O, Fredriksson M, et al. Do occupational factors influence the risk of colon and rectal cancer in different ways? Cancer 1993; 72:2543–9.
3. Ballard-Barbash R, Schatzkin A, Albanes D, et al. Physical activity and risk of large bowel cancer in the Framingham Study. Cancer Res 1990;50:3610–3.
4. Benito E, Obrador A, Stiggelbout A, et al. A population-based case-control study of colorectal cancer in Majorca. I. Dietary factors. Int J Cancer 1990;45: 69–76.
5. Bostick RM, Potter JD, Kushi LH, et al. Sugar, meat, and fat intake, and non-dietary risk factors for colon cancer incidence in Iowa women (United States). Cancer Causes Control 1994;5:38–52.
6. Boutron-Ruault MC, Senesse P, Meance S, et al. Energy intake, body mass index, physical activity, and the colorectal adenoma-carcinoma sequence. Nutr Cancer 2001;39:50–7.
7. Brownson RC, Chang JC, Davis JR, Smith CA. Physical activity on the job and cancer in Missouri. Am J Public Health 1991;81:639–42.
8. Calton BA, Lacey JV, Jr., Schatzkin A, et al. Physical activity and the risk of colon cancer among women: a prospective cohort study (United States). Int J Cancer 2006;119:385–91.
9. Chao A, Connell CJ, Jacobs EJ, et al. Amount, type, and timing of recreational physical activity in relation to colon and rectal cancer in older adults: the Cancer Prevention Study II Nutrition Cohort. Cancer Epidemiol Biomarkers Prev 2004;13:2187–95.
10. Chow WH, Dosemeci M, Zheng W, et al. Physical activity and occupational risk of colon cancer in Shanghai, China. Int J Epidemiol 1993;22:23–9.
11. Chow WH, Malker HS, Hsing AW, et al. Occupational risks for colon cancer in Sweden. J Occup Med 1994;36:647–51.
12. Colbert LH, Hartman TJ, Malila N, et al. Physical activity in relation to cancer of the colon and rectum in a cohort of male smokers. Cancer Epidemiol Biomarkers Prev 2001;10:265–8.
13. Dosemeci M, Hayes RB, Vetter R, et al. Occupational physical activity, socioeconomic status, and risks of 15 cancer sites in Turkey. Cancer Causes Control 1993;4:313–21.
14. Fernandez E, Gallus S, La Vecchia C, et al. Family history and environmental risk factors for colon cancer. Cancer Epidemiol Biomarkers Prev 2004;13: 658–61.
15. Fraser G, Pearce N. Occupational physical activity and risk of cancer of the colon and rectum in New Zealand males. Cancer Causes Control 1993;4: 45–50.
16. Fredriksson M, Bengtsson NO, Hardell L, Axelson O. Colon cancer, physical activity, and occupational exposures. A case-control study. Cancer 1989;63: 1838–42.
17. Garabrant DH, Peters JM, Mack TM, Bernstein L. Job activity and colon cancer risk. Am J Epidemiol 1984;119:1005–14.
18. Gerhardsson de Verdier M, Norell SE, Kiviranta H, et al. Sedentary jobs and colon cancer. Am J Epidemiol 1986;123:775–80.
19. Gerhardsson de Verdier M, Floderus B, Norell SE. Physical activity and colon cancer risk. Int J Epidemiol 1988;17:743–6.
20. Gerhardsson de Verdier M, Steineck G, Hagman U, et al. Physical activity and colon cancer: a case-referent study in Stockholm. Int J Cancer 1990;46: 985–9.
21. Hauret KG, Bostick RM, Matthews CE, et al. Physical activity and reduced risk of incident sporadic colorectal adenomas: observational support for mechanisms involving energy balance and inflammation modulation. Am J Epidemiol 2004;159:983–92.
22. Hou L, Ji BT, Blair A, et al. Commuting physical activity and risk of colon cancer in Shanghai, China. Am J Epidemiol 2004;160:860–7.
23. Hsing AW, McLaughlin JK, Chow WH, et al. Risk factors for colorectal cancer in a prospective study among U.S. white men. Int J Cancer 1998;77: 549–53.
24. Kato I, Tominaga S, Ikari A. A case-control study of male colorectal cancer in Aichi Prefecture, Japan: with special reference to occupational activity level, drinking habits and family history. Jpn J Cancer Res 1990;81:115–21.
25. Kune GA, Kune S, Watson LF. Body weight and physical activity as predictors of colorectal cancer risk. Nutr Cancer 1990;13:9–17.
26. Lam TH, Ho SY, Hedley AJ, et al. Leisure time physical activity and mortality in Hong Kong: case-control study of all adult deaths in 1998. Ann Epidemiol 2004;14:391–8.
27. Le Marchand L, Wilkens LR, Kolonel LN, et al. Associations of sedentary lifestyle, obesity, smoking, alcohol use, and diabetes with the risk of colorectal cancer. Cancer Res 1997;57:4787–94.
28. Larsen IK, Grotmol T, Almendingen K, Hoff G. Lifestyle as a predictor for colonic neoplasia in asymptomatic individuals. BMC Gastroenterol 2006;6:5.
29. Lee IM, Paffenbarger RS. Physical activity and its relation to cancer risk: a prospective study of college alumni. Med Sci Sports Exerc 1994;26:831–7.
30. Lee IM, Manson JE, Ajani U, et al. Physical activity and risk of colon cancer: the Physicians' Health Study (United States). Cancer Causes Control 1997;8: 568–74.
31. Levi F, Pasche C, Lucchini F, et al. Occupational and leisure-time physical activity and the risk of colorectal cancer. Eur J Cancer Prev 1999;8:487–93.
32. Longnecker MP, Gerhardsson de Verdier M, Frumkin H, Carpenter C. A case-control study of physical activity in relation to risk of cancer of the right colon and rectum in men. Int J Epidemiol 1995;24:42–50.
33. Lynge E, Thygesen L. Use of surveillance systems for occupational cancer: data from the Danish National system. Int J Epidemiol 1988;17:493–500.
34. Marcus PM, Newcomb PA, Storer BE. Early adulthood physical activity and colon cancer risk among Wisconsin women. Cancer Epidemiol Biomarkers Prev 1994;3:641–4.
35. Markowitz S, Morabia A, Garibaldi K, Wynder E. Effect of occupational and recreational activity on the risk of colorectal cancer among males: a case-control study. Int J Epidemiol 1992;21:1057–62.
36. Marti B, Minder CE. [Physical occupational activity and colonic carcinoma mortality in Swiss men 1979-1982]. Soz Praventivmed 1989;34:30–7.
37. Lund Nilsen TI, Vatten LJ. Prospective study of colorectal cancer risk and physical activity, diabetes, blood glucose and BMI: exploring the hyper-insulinaemia hypothesis. Br J Cancer 2001;84:417–22.
38. Paffenbarger RS, Hyde RT, Wing AL. Physical activity and incidence of cancer in diverse populations: a preliminary report. Am J Clin Nutr 1987;45: 312–7.
39. Pukkala E, Kaprio J, Koskenvuo M, et al. Cancer incidence among Finnish world class male athletes. Int J Sports Med 2000;21:216–20.
40. Ravasco P, Monteiro-Grillo I, Marques VP, Camilo ME. Nutritional risks and colorectal cancer in a Portuguese population. Nutr Hosp 2005;20:165–72.
41. Schnohr P, Gronbaek M, Petersen L, et al. Physical activity in leisure-time and risk of cancer: 14-year follow-up of 28,000 Danish men and women. Scand J Public Health 2005;33:244–9.
42. Severson RK, Nomura AM, Grove JS, Stemmermann GN. A prospective analysis of physical activity and cancer. Am J Epidemiol 1989;130:522–9.
43. Slattery ML, Schumacher MC, Smith KR, et al. Physical activity, diet, and risk of colon cancer in Utah. Am J Epidemiol 1988;128:989–99.
44. Slattery ML, Potter J, Caan B, et al. Energy balance and colon cancer-beyond physical activity. Cancer Res 1997;57:75–80.
45. Slattery ML, Edwards S, Curtin K, et al. Physical activity and colorectal cancer. Am J Epidemiol 2003;158:214–24.
46. Steenland K, Nowlin S, Palu S. Cancer incidence in the National Health and Nutrition Survey I. Follow-up data: diabetes, cholesterol, pulse and physical activity. Cancer Epidemiol Biomarkers Prev 1995;4:807–11.
47. Steindorf K, Tobiasz-Adamczyk B, Popiela T, et al. Combined risk assessment of physical activity and dietary habits on the development of colorectal cancer. A hospital-based case-control study in Poland. Eur J Cancer Prev 2000;9:309–16.
48. Steindorf K, Jedrychowski W, Schmidt M, et al. Case-control study of lifetime occupational and recreational physical activity and risks of colon and rectal cancer. Eur J Cancer Prev 2005;14:363–71.
49. Tang R, Wang JY, Lo SK, Hsieh LL. Physical activity, water intake and risk of colorectal cancer in Taiwan: a hospital-based case-control study. Int J Cancer 1999;82:484–9.
50. Thun MJ, Calle EE, Namboodiri MM, et al. Risk factors for fatal colon cancer in a large prospective study. J Natl Cancer Inst 1992;84:1491–500.
51. Thune I, Lund E. Physical activity and risk of colorectal cancer in men and women. Br J Cancer 1996;73:1134–40.
52. Vena JE, Graham S, Zielezny M, et al. Lifetime occupational exercise and colon cancer. Am J Epidemiol 1985;122:357–65.
53. Wannamethee SG, Shaper AG, Walker M. Physical activity and risk of cancer in middle-aged men. Br J Cancer 2001;85:1311–6.
54. Wei EK, Giovannucci E, Wu K, et al. Comparison of risk factors for colon and rectal cancer. Int J Cancer 2004;108:433–42.
55. White E, Jacobs EJ, Daling JR. Physical activity in relation to colon cancer in middle-aged men and women. Am J Epidemiol 1996;144:42–50.
56. Whittemore AS, Wu-Williams AH, Lee M, et al. Diet, physical activity, and colorectal cancer among Chinese in North America and China. J Natl Cancer Inst 1990;82:915–26.
57. Will JC, Galuska DA, Vinicor F, Calle EE. Colorectal cancer: another complication of diabetes mellitus? Am J Epidemiol 1998;147:816–25.
58. Wu AH, Paganini-Hill A, Ross RK, Henderson BE. Alcohol, physical activity and other risk factors for colorectal cancer: a prospective study. Br J Cancer 1987;55:687–94.
59. Zhang Y, Cantor KP, Dosemeci M, et al. Occupational and leisure-time physical activity and risk of colon cancer by subsite. J Occup Environ Med 2006;48:236–43.
60. Brownson RC, Zahm SH, Chang JC, Blair A. Occupational risk of colon cancer. An analysis by anatomic subsite. Am J Epidemiol 1989;130:675–87.
61. Slattery ML, Caan BJ, Benson J, Murtaugh M. Energy balance and rectal cancer: an evaluation of energy intake, energy expenditure, and body mass index. Nutr Cancer 2003;46:166–71.
62. Giovannucci E, Ascherio A, Rimm EB, et al. Physical activity, obesity, and risk for colon cancer and adenoma in men. Ann Intern Med 1995;122:327–34.
63. Martinez ME, Giovannucci E, Spiegelman D, et al.; Nurses' Health Study Research Group. Leisure-time physical activity, body size, and colon cancer in women. J Natl Cancer Inst 1997;89:948–55.
64. Tavani A, Braga C, La Vecchia C, et al. Physical activity and risk of cancers of the colon and rectum: an Italian case-control study. Br J Cancer 1999;79: 1912–6.
65. Bufill JA. Colorectal cancer: evidence for distinct genetic categories based on proximal or distal tumor location. Ann Intern Med 1990;113:779–88.
66. Dubrow R, Bernstein J, Holford TR. Age-period-cohort modelling of large-bowel-cancer incidence by anatomic sub-site and sex in Connecticut. Int J Cancer 1993;53:907–13.
67. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113–24.

68. Ferrari P, Slimani N, Ciampi A, et al. Evaluation of under- and overreporting of energy intake in the 24-hour diet recalls in the European Prospective Investigation into Cancer and Nutrition (EPIC). Public Health Nutr 2002;5: 1329–45.

69. Haftenberger M, Schuit AJ, Tormo MJ, et al. Physical activity of subjects aged 50-64 years involved in the European Prospective Investigation into Cancer and Nutrition (EPIC). Public Health Nutr 2002;5:1163–76.

70. Pols MA, Peeters PH, Bueno-de-Mesquita HB, et al. Validity and repeatability of a modified Baecke questionnaire on physical activity. Int J Epidemiol 1995;24:381–8.

71. Pols MA, Peeters PH, Ocke MC, et al. Estimation of reproducibility and relative validity of the questions included in the EPIC Physical Activity Questionnaire. Int J Epidemiol 1997;26:S181–9.

72. Ainsworth BE, Haskell WL, Whitt MC, et al. Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc 2000;32:S498–504.

73. Wareham NJ, Jakes RW, Rennie KL, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr 2003;6:407–13.

74. James WP, Schofield EC. Human energy requirements: a manual for planners and nutritionists. Oxford: Oxford University Press; 1990.

75. SAS Institute. SAS statistical software [v8.2]. Cary (NC): SAS Institute; 2001.

76. Quadrilatero J, Hoffman-Goetz L. Physical activity and colon cancer. A systematic review of potential mechanisms. J Sports Med Phys Fitness 2003; 43:121–38.

77. McTiernan A, Ulrich C, Slate S, Potter J. Physical activity and cancer etiology: associations and mechanisms. Cancer Causes Control 1998;9:487–509.

78. Cordain L, Latin RW, Behnke JJ. The effects of an aerobic running program on bowel transit time. J Sports Med Phys Fitness 1986;26:101–4.

79. Koffler KH, Menkes A, Redmond RA, et al. Strength training accelerates gastrointestinal transit in middle-aged and older men. Med Sci Sports Exerc 1992;24:415–9.

80. Burkitt DP, Walker AR, Painter NS. Effect of dietary fibre on stools and the transit-times, and its role in the causation of disease. Lancet 1972;2:1408–12.

81. Bartram HP, Wynder EL. Physical activity and colon cancer risk? Physiological considerations. Am J Gastroenterol 1989;84:109–12.

82. Howarth NC, Huang TT, Roberts SB, McCrory MA. Dietary fiber and fat are associated with excess weight in young and middle-aged US adults. J Am Diet Assoc 2005;105:1365–72.

83. Lairon D, Arnault N, Bertrais S, et al. Dietary fiber intake and risk factors for cardiovascular disease in French adults. Am J Clin Nutr 2005;82:1185–94.

84. Howarth NC, Saltzman E, Roberts SB. Dietary fiber and weight regulation. Nutr Rev 2001;59:129–39.