



Effets de régimes hyperlipidique et cafeteria sur le développement de l'obésité et ses désordres associés chez la souris

Charles Desmarchelier

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**Effects of high fat and cafeteria diets on obesity development and
associated metabolic disturbances in mice**

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Abstract

Introduction: Obesity results from a prolonged imbalance between energy intake and energy expenditure, as depending on basal metabolic rate, heat production, thermogenic effects of the diet and physical activity. Diet-induced obesity (DIO) in rodents can be achieved by different regimens and approaches. Diets providing a high fat intake have been established as a “gold standard” to generate obese rodent models and have proven to initiate pathologies similar to those encountered in humans. However, this dietary treatment is far from being standardized and its relevance has been criticised on the basis of findings in humans that total energy intake rather than fat *per se* determines body fat accumulation in humans. Hence, cafeteria diets have been introduced by providing a choice of several palatable food items of variable composition, appearance and texture in addition to a non-purified diet. Those approaches have been shown to induce obesity by a hyperphagia.

Objective: This thesis aimed at comparing the effects of a high fat vs. a cafeteria diet on food intake, weight gain and determinants of energy homeostasis and metabolism in obese mice. **Results:** Our key findings demonstrate that both a high fat and a cafeteria diet were almost equally efficient in driving an obese phenotype but did not necessarily elicit the same metabolic changes. The cafeteria diet as characterised by a higher carbohydrate (mainly sucrose) and lower fat content seemed to be more deleterious for liver steatohepatosis and provoked more pronounced changes in the gut microbiota. Despite a lower cholesterol content than in the high fat diet, mice fed the cafeteria diet presented levels of circulating cholesterol as high as animals on a high fat diet. Changes in gene expression in liver and intestine suggested an increased *de novo* synthesis of cholesterol and altered transport and those effects were more pronounced in animals receiving a high fat diet. Most strikingly, the

pronounced effects of the two high calorie diets causing obesity when compared to animals on control diet remaining lean vanished when diets with identical composition were supplied in powder form and not as standard pellets. Here, even the control diet with a high starch but very low fat content caused a substantial weight gain with only minor differences to the two other high-calorie diets. **Conclusion:** The results presented here raise the question of whether high fat diets used for induction of obesity are the proper models to simulate human obesity and its pathologies. Cafeteria diets are equally effective and are closer to human diets.

Résumé

Introduction : L'obésité est causée par un déséquilibre prolongé entre les apports énergétiques et l'activité physique, dépendant du métabolisme de base, de la production de chaleur et des effets thermogéniques du régime et de l'activité physique. Chez les rongeurs, l'obésité induite par le régime peut être obtenue par différents régimes et approches. A cet égard, les régimes hyperlipidiques sont considérés comme les régimes de référence pour générer des modèles de l'obésité chez le rongeur et engendrent des pathologies similaires à celles rencontrées chez l'homme. Cependant, ce régime alimentaire est loin d'être standardisé et a été critiqué sur le fait que la prise énergétique totale et non uniquement les lipides régissait l'accumulation de graisse corporelle chez l'homme. Ainsi, les régimes cafétéria ont été introduits : en offrant en plus d'un régime non purifié un choix de plusieurs aliments appétants, de composition, d'apparence et de texture différentes, ils permettent le développement de l'obésité en déclenchant l'hyperphagie. **Objectif :** L'objet de ces travaux a été de comparer chez des souris obèses les effets d'un régime hyperlipidique à ceux d'un régime cafétéria sur la prise de nourriture, la prise de poids et les déterminants du métabolisme et de l'homéostasie énergétique.

Résultats : Nos résultats démontrent qu'un régime hyperlipidique et un régime cafeteria permettent tous deux d'obtenir un phénotype obèse mais sans causer nécessairement les mêmes changements métaboliques. Le régime cafétéria, caractérisé par un contenu en glucides (principalement le sucrose) plus élevé et un contenu en lipides plus faible, semble avoir des conséquences plus néfastes pour le foie et provoque des changements plus prononcés au niveau du microbiote intestinal. Malgré un contenu en cholestérol plus faible que dans le régime hyperlipidique, les souris nourries au régime cafétéria présentaient une

cholestérolémie similaire. Les niveaux d'expression des gènes impliqués dans le métabolisme du cholestérol dans l'intestin grêle et le foie suggèrent une augmentation de la synthèse de cholestérol *de novo* et une modification de son transport, ces effets étant plus marqués chez les souris nourries au régime hyperlipidique. **Conclusion** : Ces résultats remettent en question le statut des régimes hyperlipidiques pour déclencher l'obésité et pour générer ses pathologies associées. Les régimes cafétéria sont aussi efficaces à cet égard et sont plus proches des régimes consommés chez l'homme.

Keywords

High fat diet, cafeteria diet, food texture, food intake, adipose tissue, small intestine, intrahepatic triacylglyceride, cholesterol metabolism, gut microbiota, microarrays, Fourier transform infrared spectroscopy, obesity

Mots clés

Régime hyperlipidique, régime cafeteria, texture alimentaire, prise alimentaire, tissu adipeux, intestin grêle, triacylglyceride intrahépatique, métabolisme du cholestérol, microbiote intestinal, puce à ADN, Spectroscopie infrarouge à transformée de Fourier, obésité

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Abbreviations

11- β -HSD-1: 11 β -hydroxysteroid dehydrogenase type 1

Abca1: ATP-binding cassette, sub-family A, 1

Abcg5: ATP-binding cassette, sub-family G, 5

Abcg8: ATP-binding cassette, sub-family G, 8

ACC: acetyl CoA carboxylase

Acc1: acetyl-CoA carboxylase 1

Actb: β -actin

ALT: alanine aminotransferase

AMP: adenosine monophosphate

AMPK: AMP-activated protein kinase

Apo: apolipoprotein

ARH(1): heterogeneous autoregressive order one

AST: aspartate aminotransferase

ATGL: adipose triacylglycerols lipase

ATP: adenosine triphosphate

BAT: brown adipose tissue

BMI: body mass index

Bp: base pairs

Cal: calorie

CD14: cluster of differentiation 14

CD36: cluster of differentiation 36

ChREBP: carbohydrate response element binding protein

CLA: conjugated linoleic acid

CM: chylomicron

CoA: coenzyme A

CPT1: carnitine palmitoyl transferase-1

Cyp27a1: cytochrome P450, family 27, subfamily a, polypeptide 1

Cyp51: cytochrome P450, family 51

DHA: docosahexaenoic acid

Dhcr7: 7-dehydrocholesterol reductase

DIO: diet-induced obesity

EAT: epididymal adipose tissue

EDTA: ethylenediaminetetraacetic acid

EPA: eicosapentaenoic acid

Fas: fatty acid synthase

FATP-4: fatty acid transport protein 4

Fiaf: fasting-induced adipose factor

FISH: fluorescent in situ hybridization

FT-IR: Fourier transform infrared

GAPDH: glyceraldehyde 3-phosphate dehydrogenase

GE: gross energy

GF: germ-free

GIP: gastric inhibitory polypeptide

GLP-1: glucagon-like peptide-1

Gpr41: G protein-coupled receptor 41

Gpr43: G protein-coupled receptor 43

HDL: high-density lipoprotein

Hmgcr: 3-hydroxy-3-methylglutaryl-Coenzyme A reductase

Hmgcs2: 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2

Hprt: hypoxanthine phosphoribosyltransferase

Idh1: isocitrate dehydrogenase 1 (NADP+), soluble

I-FABP: intestinal fatty acid binding protein

IHTG: intrahepatic triacylglyceride

IRS-1: insulin receptor substrate 1

IRS-2: insulin receptor substrate 2

LDL: low-density lipoprotein

LDLr: LDL receptor

L-FABP: liver fatty acid binding protein

LPL: lipoprotein lipase

LPS: lipopolysaccharide

LXR α : liver X receptor α

MAT: mesenteric adipose tissue

MCP-1: monocyte chemotactic protein-1

ME: metabolizable energy

Me1: malic enzyme 1, NADP(+)-dependent, cytosolic

MTP: microsomal triacylglyceride transfer protein

mRNA: messenger ribonucleic acid

MUFA: mono-unsaturated fatty acid

Mvd: mevalonate decarboxylase

N: nitrogen

NADP: nicotinamide adenine dinucleotide phosphate

NAFLD: non-alcoholic fatty liver disease

Npc1l1: Niemann-Pick C1-like protein 1

Nsdhl: NAD(P) dependent steroid dehydrogenase-like

PBS: phosphate buffered saline

PCR: polymerase chain reaction

Pgc-1 α : peroxisomal proliferator activated receptor coactivator 1 α

Pmk: phosphomevalonate kinase

PUFA: poly-unsaturated fatty acid

PYY: peptide YY

qPCR: real-time quantitative polymerase chain reaction

RT: room temperature

Scarb1: scavenger receptor class B, member 1

Scd1: stearyl-Coenzyme A desaturase 1

SCFA: short-chain fatty acids

SDS: sodium dodecyl sulfate

SFA: saturated fatty acid

Slc25a1: solute carrier family 25 (mitochondrial carrier, citrate transporter), member 1

SR-B1: scavenger receptor class B1

SREBP-1: sterol response element binding protein 1

SREBP-2: sterol response element binding protein 2

TG: triacylglycerols

TLR-4: toll-like receptor 4

Tm7sf2: transmembrane 7 superfamily member 2

UCP: uncoupling protein

UN: unstructured

V/v: ratio volume to volume

VLDL: very low-density lipoprotein

W/v: ratio weight to volume

W/w: ratio weight to weight

WAT: white adipose tissue

WHO: world health organization

Publications and scientific communications

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General introduction

Obesity incidence has increased rapidly over the last two decades, reaching an epidemic state. An incidence of above 20% has been observed in most Western countries (WHO 2007), particularly in the US and UK, with an alarmingly high incidence among children (Rocchini 2002). The estimated number of overweight children globally in 2005 was at least 20 million (WHO 2005). Obesity is defined as a body mass index (BMI) of 30 kg/m^2 or more and overweight is defined as a BMI of 25 kg/m^2 or more by the World Health Organization (WHO) (WHO 2005). However, BMI is not necessarily the best parameter in defining obesity, and in particular not for predicting obesity-associated metabolic problems.

Obesity is known to increase the risk of developing a variety of disorders including type 2 diabetes, coronary heart disease, osteoarthritis, as well as certain types of cancer and psychological problems (Pi-Sunyer 1993; Must et al. 1999; Visscher and Seidell 2001; Calle and Kaaks 2004). It is also highly associated with the metabolic syndrome, which includes conditions such as impaired glucose tolerance, insulin resistance, dyslipidemia and hypertension (Grundy 2004; Grundy *et al.* 2004). This condition is a worldwide problem, and is showing a worrying increase in developing countries as well as developed countries (Prentice 2006).

The dramatic rise in obesity and the metabolic syndrome are a consequence of several lifestyle factors in modern societies. Factors such as nutrition, physical activity, smoking, alcohol and stress are well known lifestyle components associated with the development of obesity-associated diseases (Ueno *et al.* 1997). Modern Western lifestyle involve a reduced need for physical activity, due to the sedentary

nature of many jobs with technological developments and the increasing use of computers for everyday tasks as well as readily available, high calorie, ready-made diets.

Thus, there is currently a great need for effective therapies. Numerous medical and behavioural interventions have been tried to treat obese patients but only a few were successful. Pharmacological compounds often had to be withdrawn, due to severe undesired side effects (Farrigan and Pang 2002). Bariatric surgery is considered the most successful treatment in highly obese patients but the significant risk of complications does not allow its wide-range use (Sjostrom et al. 2004). Therefore, there is a huge challenge for the scientific community to search for more effective and better tolerable treatment against obesity.

Part 1: Literature review

I.I. Diet-induced obesity in animal models

I.I.1. Animal models of obesity

To understand the genetic and environmental basis of obesity, animal models have proven useful by allowing manipulations technically or ethically not feasible in humans (Speakman et al. 2008). In these models, obesity can be induced by genetic mutations (See **Table 1.1** for spontaneous single gene loss-of-function mutations), pharmacologically, by injecting gold thioglucose for example (Brecher and Waxler 1949) or by a variety of dietary manoeuvres.

Table 1.1. Single gene mutations associated with an obesity phenotype

Candidate gene	Syndrome
NTRK2	neurotrophic Tyrosine Kinase Receptor Type 2
GPR24	G protein-coupled receptor 24
PCSK1	Proprotein convertase subtilisin/kexin type 1
POMC	Proopiomelanocortin
LEP	leptin (obesity homolog, mouse)
CRHR2	corticotropin releasing hormone receptor 2
MC4R	melanocortin 4 receptor
CRHR1	corticotrophin releasing hormone receptor 1
LEPR	leptin receptor
SIM1	single-minded homolog 1 (Drosophila)
MC3R	melanocortin 3 receptor

Adapted from http://obesitygene.pbrc.edu/static/mendelian_table.htm

These models of obesity have allowed insights into some critical pathways but their overall relevance is nonetheless questionable since common obesity cannot be attributed to a single gene or single pathway. Thus, the polygenic nature of obesity calls for a more realistic approach to generate animal models of obesity. In this respect, diet-induced obesity allows to mimic situations more closely related to what can be observed in humans.

I.1.2. High fat diets in diet-induced obesity

I.1.2.1. The effect of high fat diets on the development of obesity

As early as 1951, Fenton and Carr observed that, when providing diets with increasing fat content to rodents, some strains showed marked weight gains, while others had a much less pronounced response. They reported elevated food utilization with diets high in fat and showed by carcass analysis that the strain responding well to high fat feeding accumulated most of the excess weight as fat (Fenton and Carr 1951). In 1955, Mickelsen *et al.* showed for the first time in rats that obesity could be achieved by feeding a diet high in fat and implied that this could be due to an excess consumption of calories (Mickelsen et al. 1955). High fat diets are now fairly well accepted to model the disorders of human obesity in rodents (Buettner et al. 2007) and have since then been extensively used to induce obesity in animal models. A PubMed search in December 2009 with the keywords “high fat diet” and “obesity” retrieved more than 1500 results, mainly animal studies. To understand the mechanisms behind the excess storage of energy usually associated with feeding high fat diets, some parameters of the energy balance need to be considered.

I.I.2.2. High fat diets and hyperphagia

The most obvious, and possibly easiest, parameter to look at is the energy intake, which is simply calculated by measuring the food consumption and multiplying it by the energy density of the diet. High fat feeding has usually been associated with hyperphagia, meaning the group given the high fat diet tended to consume more calories than the control group. This effect has been observed in mice (Mercer and Trayhurn 1987; West et al. 1995; Gallou-Kabani et al. 2007), rats (Ramirez and Friedman 1990; Shafat et al. 2009) and humans (Lissner et al. 1987). Therefore, it seems that subjects fed a high fat diet are unable to regulate their food intake to meet their needs and develop obesity as a consequence.

Interestingly, the hyperphagia associated with high fat diet does not seem to be due to fat itself but rather to the energy density of the diets. Fat is characterised by a high energy density (in kcal per g of macronutrient: fat, 9; carbohydrate, 4; protein, 4) and thus, high fat diets are often high in energy density. Ramirez and Friedman fed rats either a low fat or a high fat diet but presenting the same energy density. Rats fed the high fat diet then presented decreased body weight and energy intake compared to the mice fed the low fat diet (Ramirez and Friedman 1990). This result has been confirmed by others in rats (Paulino et al. 2008). Therefore, as underlined by Warwick and Schiffman, who reviewed 40 studies comparing the effects of high fat to high carbohydrate diets, when the caloric density of the diets was similar (density of the high fat diet less than 25 % greater than high carbohydrate diet), only 5 out of 10 studies observed greater weight gain in high fat fed animals whereas when the high fat diet had an energy density at least 25 % greater than the high carbohydrate diet, then 28 out of 30 studies observed a greater weight gain in the high fat fed animals (Warwick and Schiffman 1992). These findings have been confirmed in humans as well (Stubbs et al. 1995; Prentice 1998; Rolls 2000). Thus, the hyperphagia

associated with high fat diet feeding is abolished when the diets provided are matched with respect to caloric density. To summarize, the hyperphagia associated with high fat diet seems to be due to the high energy densities of high fat diets and not because of the fat content of the diet *per se*. Of note, contradictory results have been reported in rats when using liquid diets (Warwick 2003).

I.I.2.3. Diet-induced thermogenesis

Diet-induced thermogenesis (DIT) has been shown to have a significant effect on the regulation of energy balance (Himms-Hagen 1985) and mainly takes place in the brown adipose tissue in rodents (Rothwell and Stock 1979; Cannon and Nedergaard 2004). Mercer and Trayhurn showed that mice fed a high fat diet rich in corn oil, meaning a high content of PUFA, exhibited increased energy expenditure, as revealed by an enhanced total thermogenic activity of the BAT, compared to mice fed a low fat diet (Mercer and Trayhurn 1987). Interestingly, the mice fed a high fat diet rich in beef tallow, meaning a high content of SFA, did not show any evidence for an increased DIT which could partly explain why they displayed greater body weight compared to the 2 other groups. Differences in DIT could also partly account for the differences in energy assimilation efficiencies since mice fed the corn oil diet retained only 18 % of the excess energy intake in the carcass whereas mice fed the beef tallow diet retained 77 %, despite similar total energy intakes. A decrease in DIT induced by a diet rich in SFA as compared to diets rich in MUFA or PUFA has been confirmed in rats as well (Takeuchi et al. 1995). Altogether, these results point at differences in DIT as a function of the fat amount, or possibly the total energy intake since DIT has been associated with overfeeding, and the fatty acid composition of the diet which influences the obesity state as well (Corbett et al. 1986). BAT has long been thought to be of negligible importance in humans, based on its mass and

activity, and its role in the energy balance has been neglected until recently where positron emission tomography demonstrated that adult humans had significant depots of metabolically active BAT (Cypess et al. 2009; Saito et al. 2009; van Marken Lichtenbelt et al. 2009; Virtanen et al. 2009). Therefore, there is currently a renewal of interest for the role of the BAT in obesity in humans.

The term high fat diet actually encompasses a fairly wide spectrum of diets. In a 1992 review, Warwick analysed 40 studies comparing the effects of high fat and high carbohydrate diets (Warwick and Schiffman 1992) and reported differences in fat content of the diets used ranging from 28 to 84 % of total energy. The diets were also characterised by different fat sources (corn oil, lard, tallow and others, hence providing quite different fatty acid profiles). Therefore, an analysis of feeding trials with animals on high fat diets has to take into account not only the energy density and the fat content but also the role of the fatty acids provided on the development of obesity.

I.I.2.4. The effect of the dietary fat intake on the development of obesity

The fat content of the diet has been shown to be a major determinant of body weight in mice fed *ad libitum* (West et al. 1995). In this study, the authors fed two strains of mice, AKR/J and SWR/J, with increasing levels of fat (15, 30, 45 kcal %) and observed that dietary fat content was strongly associated with body weight gain and a very marked increase in the weight of adipose tissue depots. However, these effects did not become obvious in the SWR/J mice, addressing the importance of genetic predisposition on the development of obesity. Boozer *et al.* fed rats increasing amounts of dietary fat (12, 24, 36, and 48 energy %) in quantities matched energetically (Boozer et al. 1995). They did not observe a significant increase in total

body weight (although the diet by time interaction was significantly different) but the absolute weights of the white adipose tissue correlated with the amount of dietary fat. The authors concluded that dietary fat promoted adiposity, independently of the energy intake. Pair-feeding studies are the gold-standard to determine the relative contributions of hyperphagia versus metabolic effects of dietary fat in inducing obesity. Although some studies reported that animals fed an isocaloric high fat diet had greater body weight gain than animals fed a control or low fat diet (Wade 1982; Oscai et al. 1987), other data led to the conclusion that there was no difference (Woods et al. 2003).

I.I.2.5. Ketogenic diets

If there is a positive relationship between increasing levels of dietary fat and the development of obesity, it actually only holds true within a defined range of fat content as shown by experiments using ketogenic diets. Ketogenic diets typically consist of at least 80 % of calories from fat with minimal requirements for protein and marginal levels of carbohydrates. In mice fed *ad libitum*, a ketogenic diet (95 kcal %) has been shown to promote weight loss compared to mice fed a control or a high fat diet (45 kcal %) although they ingested similar levels of energy. This effect was due to increased energy expenditure. Interestingly, the ketogenic diet not only prevented mice to develop obesity but was also able to reverse obesity in mice previously fed a high fat diet (Kennedy et al. 2007). Such ketogenic diets have proven to be more efficient for weight loss compared to low fat diets in obese humans, even if only 68 % of the energy was coming from fat (Yancy et al. 2004).

I.I.2.6. The effect of dietary fat composition on the development of obesity

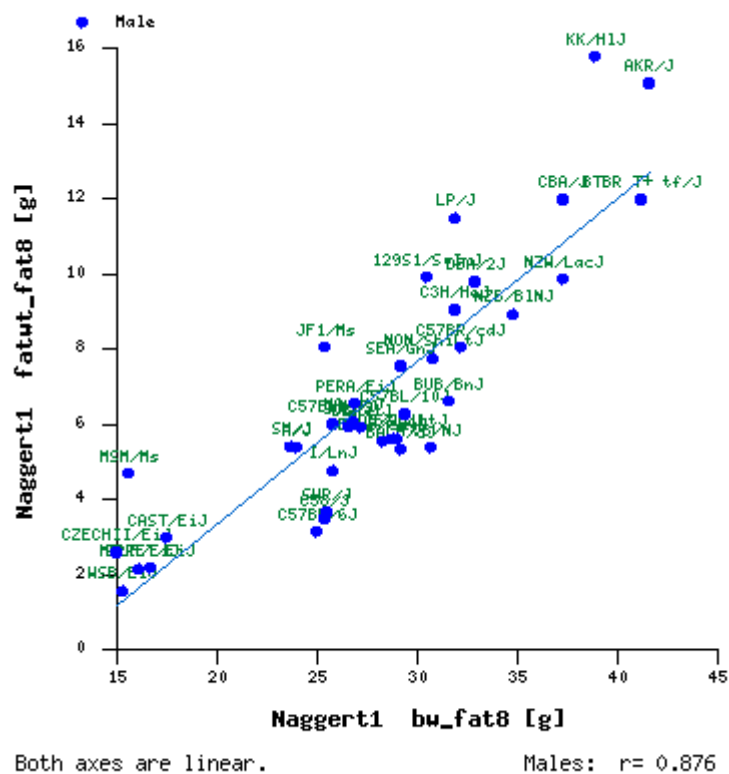
Diets high in fat not only differ with respect to total fat content but also in their fat sources and thereby their fatty acid profiles. For example, beef tallow, butterfat or pork lard are rich in SFAs, coconut oil is rich in medium-chain SFAs, olive oil is rich in MUFAs, corn oil is rich in omega-6 PUFAs and fish oil is rich in omega-3 PUFAs. Therefore, it is difficult to determine the effects of fat quality (SFA, MUFA, PUFA) on the development of obesity, as the fat sources used always consist of a mixture of different fatty acids. Moussavi *et al.* reviewed the possible association between types of fatty acids in diets and weight change (Moussavi *et al.* 2008). In animal studies, diets rich in SFA (beef tallow and lard) seem to initiate a greater weight gain, as reported for mice (Buettner *et al.* 2006) and for rats (Mercer and Trayhurn 1987; Takeuchi *et al.* 1995). Fish oil, rich in PUFA, notably the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have mainly been shown to promote weight loss, except in one study (Awad *et al.* 1990). Only a few epidemiological studies have been carried out in humans and the results are still contradictory with regard to the quality of the dietary fatty acid patterns provided in the diet and the effects on obesity development (Moussavi *et al.* 2008).

I.I.2.7. Strain differences

As already addressed, the association of dietary fat intake and obesity in mice is strain-dependent (Fenton and Carr 1951). West *et al.* showed that the murine AKR/J strain is obesity-prone whereas the murine SWR/J strain is obesity-resistant, although when enough fat was provided in the diet, their body fat eventually increased (West *et al.* 1995). Interestingly, in a following study, the AKR/J mice were shown to have preference for fat while the SWR/J mice preferred other diets depending on the way food was offered (Smith *et al.* 1999). The strain-specific effect

of high fat diets on the development of obesity has been observed in rats as well (Schemmel et al. 1970; Svenson et al. 2007). Svenson *et al.*, from the Jackson Laboratory, carried out a comprehensive study in 2007 where they fed 43 inbred mouse strains for 8 weeks with a high fat diet, revealing large differences with regards to body weight or fat mass gain as shown in **Figure 1.1**.

Figure 1.1. Body fat mass as a function of body weight in 43 strains of inbred mouse fed a high fat diet for 8 weeks (Svenson et al. 2007).



I.I.3. Cafeteria diet in diet-induced obesity

I.I.3.1. Is a high fat diet the most appropriate model to simulate a Western diet in human obesity?

High fat diet feeding in rodents induces changes in body weight and food intake similar to those encountered in humans. Nevertheless, there is still a fierce debate

ongoing over whether dietary fat *per se* determines body fat accumulation or not. Willett has pointed out, based on large epidemiological studies, that obesity has increased in the last decades in the US although fat intake has decreased. Moreover, some studies did not reveal any relation between dietary fat intake and body fatness, and furthermore, a reduction in fat intake had little effect, if any, on the reduction in body weight (Willett 1998b, 1998a). To date, this debate is still open (Bray and Popkin 1998; Willett 2002). However, a Western diet not only provides a higher dietary fat intake but may be best characterized by a hyperphagia, providing also high intakes of carbohydrates and proteins.

I.I.3.2. The cafeteria diet: a high fat / high sugar diet inducing hyperphagia

It appears more sensible to induce obesity not only by increasing the amount of dietary fat but also by inducing hyperphagia. Cafeteria diets have been introduced in this respect: animals are offered a choice of several palatable food items of varied composition, appearance and texture in addition to their nonpurified diet (Sclafani and Springer 1976). These diets have been shown to induce obesity in a very efficient manner, driven by hyperphagia, in rats and mice (Sclafani and Springer 1976; Rothwell and Stock 1988). For example, Rothwell and Stock reported an 80 % increase in energy intake in rats fed a cafeteria diet compared to animals on control diets, although the weight gain was only 27 % greater than that of the control animals. (Rothwell and Stock 1979) This paradox was explained by an increase in DIT in the cafeteria-fed animals, which could thus partly compensate for the excess energy ingested (Rothwell et al. 1985).

I.I.3.3. The effect of flavour on hyperphagia

Since cafeteria diet items are characterised by variations in flavour, texture or macronutrient composition, it is difficult to determine what factor(s) induce hyperphagia. The question of whether flavour variations induce hyperphagia remains controversial. Treit *et al.* fed rats 2 hours per day with powdered chow flavoured with one of 4 options, either changed every 30 min (variety day) or not (control day). The rats given different flavours showed an approximate increase of 25 % in food intake (Treit *et al.* 1983). Rolls *et al.* also showed that this effect was more pronounced when rats were given the palatable items simultaneously than in succession (Rolls *et al.* 1983). Nevertheless, Naim *et al.* did not report any effect of flavour variety on body weight gain or energy intake when they fed rats for 23 days three control diets with different added flavours as compared to a control diet (Naim *et al.* 1985). Thus, it is not yet clear if flavour variety has an effect on energy intake or body weight gain.

I.I.3.4. The limits of the cafeteria diet

In a 1987 article in the Journal of Nutrition, Moore critically assessed the use of cafeteria diets for studies on thermogenesis (Moore 1987). As cafeteria foods are low in vitamins and minerals and the animals tended not to consume enough of the nutritionally adequate nonpurified diet, the animals could face deficiencies. Moreover, the animals usually did not eat the same items and therefore the composition of the diet could greatly differ from one animal to another, which could affect the outcomes of the study since the diet factor was not fully controlled. In a subsequent issue of the Journal of Nutrition, Rothwell and Stock, the most prolific users of the cafeteria diet, convincingly dismissed Moore's criticisms, reporting a 20 % energy intake from the nonpurified diet and similar coefficients of variation for the different macronutrients in both control and cafeteria fed animals. Nonetheless, they acknowledged that "the

major drawbacks of the cafeteria diet are the variations in nutrient composition and the poor control over this factor” (Rothwell and Stock 1988). Controlling this factor is actually possible, albeit painstaking and tedious (Shafat et al. 2009).

I.II. Physiological effects of high fat diets

I.II.1. The adipose tissue

Feeding a high fat diet induces a weight gain and most of this excess weight is based on accumulated fat. However, this fat accumulation has a variety of physiological effects, since not only does the adipose tissue expand but this organ also secretes a large number of endocrine and paracrine factors.

I.II.1.1. Remodelling of the adipose tissue

High fat feeding elicits an increase in adipocyte size (hypertrophy) and number (hyperplasia) (Faust et al. 1978; Berke and Kaplan 1983; Corbett et al. 1986). This hyperplasia is not affected by food restriction, contrary to the adipocyte size, which might indicate permanent deleterious effects of high fat diets on body weight (Rolls et al. 1980). The consequences of this remodelling are also linked to the capacity of the adipose tissue for the secretion of adipokines and cytokines (Huber et al. 2006).

I.II.1.2. Insulin resistance

Lavau *et al.* fed rats for one week either a low fat or a lard-based high fat diet. They reported significantly decreased rates of glucose transport into the adipocytes of rats fed the high fat diet compared to rats fed the low fat diet but the effect of insulin on glucose transport was similar in both groups (Lavau et al. 1979). A decreased

glucose uptake in high fat fed rats, but far less marked and only restricted to the epididymal fat pad when stimulated by insulin, has been reported by Storlien *et al.* (Storlien *et al.* 1986). Maegawa *et al.* also presented data on a decreased glucose uptake but only upon insulin stimulation in high fat fed rats (Maegawa *et al.* 1986). Wilkes *et al.* did not observe any effect of a high fat diet with a balanced fatty acid profile but a decrease of the insulin-stimulated glucose uptake at high insulin concentrations was seen with a high fat diet rich in PUFA (Wilkes *et al.* 1998). The adipose tissue is generally considered to develop a mild insulin resistance upon high fat feeding but very little is actually known on the impairments in the underlying insulin signalling mechanism in the adipocyte (Anai *et al.* 1999; Park *et al.* 2005).

I.II.1.3. Lipoprotein lipase activity

Lipoprotein lipase (LPL) is the enzyme catalyzing the release of free fatty acids and triacylglycerol from circulating triacylglyceride-rich lipoproteins to adipose tissue and muscle. LPL activity has been shown to be enhanced in high fat fed mice in inguinal and mesenteric fat pads but lipase activity did not show any association with insulin levels (Surwit *et al.* 1995). Rossmeisl *et al.* observed an increase in LPL activity in the epididymal fat depots of mice fed a high fat diet for 12 weeks. LPL activity showed a 2-fold increase per unit of weight of tissue and a 4-fold increase if the entire depot was considered (Rossmeisl *et al.* 2005). This increase in lipoprotein lipase activity could promote the storage of excess lipids in adipose tissue.

I.II.1.4. *De novo* lipogenesis

Lavau *et al.* also determined the fate of glucose and observed that glucose incorporation into CO₂ and fatty acids was decreased in rats fed a high fat diet. Interestingly, when the adipocytes were stimulated with insulin, the difference was

dramatically increased, including for glucose incorporation into lipids, and the authors therefore concluded that “high fat feeding markedly decreases the adipocyte’s responsiveness to insulin”. They subsequently measured lipogenic enzyme activities which were all massively reduced. Hence, lipogenesis is already reduced in mice fed a high fat diet for one week (Lavau et al. 1979). This effect on adipocyte lipogenesis and lipogenic enzymes was confirmed in rats fed during 3 or 7 weeks from weaning onwards with a high fat diet (Berke and Kaplan 1983) and was also partly confirmed on transcription level in mice based on microarray analysis (Moraes et al. 2003).

I.II.1.5. Adipokines

The main function of adipose tissue has long been thought to be solely its energy storage capacity. However, during the past years, considerable advances have been made in defining its functions as an endocrine organ (Zhang et al. 1994; Kershaw and Flier 2004). Leptin was the first adipokine discovered (Zhang et al. 1994) and was shown to inhibit food intake and stimulate energy expenditure (Havel 2000). In mice fed a high fat diet, leptin levels were elevated and positively correlated with body weight (Ahrén 1999; Bullen et al. 2007). Adiponectin is the only adipokine currently known to be negatively correlated with body mass and its decrease has been associated with the progression of metabolic syndrome (Hauner 2005). The effects of high fat diet feeding on adiponectin secretion are still controversial since adiponectin plasma levels have been reported to be either unaffected upon high fat diet feeding or elevated (Barnea et al. 2006; Bullen et al. 2007; Lee et al. 2009). Resistin, at least in rodents, has been shown to counteract insulin activity (Steppan et al. 2001) and its secretion has been shown to be increased in mice fed a high fat diet (Steppan et al. 2001; Rajala et al. 2004).

I.II.1.6. Inflammation

Analysis of gene expression levels using microarray in adipose tissue of mice fed a high fat diet identified numerous genes significantly up-regulated that belong to inflammatory pathways (Moraes et al. 2003). Indeed, it was shown with immunohistochemical methods that mice fed a high fat diet showed an increased infiltration of macrophages into their adipose tissues, forming aggregates named Crown-like structures (CLS), and their number was significantly correlated to the adipocyte size (Weisberg et al. 2003; Xu et al. 2003). This infiltration has been associated with increased levels of secretion of the monocyte chemotactic protein-1 (MCP-1), a chemoattractant specific for monocytes and macrophages (Takahashi et al. 2003; Chen et al. 2005). Interestingly, this macrophage infiltration seems to be prevented by a diet containing fish oil, rich in omega-3 PUFA (Todoric et al. 2006).

Figure 1.2. Summary of the effects of high fat diets on the adipose tissue.



I.II.2. Liver

I.II.2.1. Hepatic steatosis

It is well established that diet-induced obesity in animals is associated with the development of hepatic steatosis (also known as NAFLD, non-alcoholic fatty liver

disease), characterized by large vacuoles of triacylglycerides accumulating in hepatocytes (Clarke et al. 1977; Yaqoob et al. 1995). In humans, an increase in intrahepatic triacylglycerides (IHTG) has been associated with hepatic and peripheral insulin resistance (Hwang et al. 2007; Korenblat et al. 2008) and is considered a major determinant of the metabolic syndrome (Marchesini et al. 2003). Recently, Fabbrini *et al.* demonstrated that IHTG content, but not visceral adipose tissue size, was a marker of obesity-related metabolic alterations in humans (Fabbrini et al. 2009). To date, the mechanisms underlying ectopic fat distribution are not known.

I.II.2.2. Hepatic insulin resistance

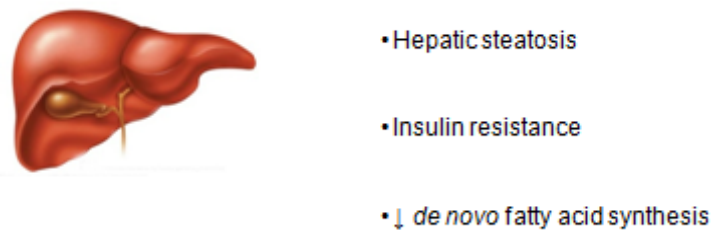
The association of NAFLD and hepatic insulin resistance has been shown in diet-induced obese animals. Using the hyperinsulinemic-euglycemic clamp technique, it was demonstrated that the insulin-stimulated suppression of hepatic glucose production was drastically impaired in rats fed a high fat diet (Storlien et al. 1986; Anai et al. 1999; Li et al. 2006). The mechanisms for insulin resistance in the liver upon high fat feeding seem to be different from those encountered in the muscle and adipose tissue since neither the insulin receptor substrate 1 and 2 (IRS-1 and -2) protein levels, nor their phosphorylation status, are altered. However, phosphoinositide-3-kinase activity, acting downstream of IRS-1 and -2 to translate insulin receptor activation into metabolic responses, is increased (Anai et al. 1999).

I.II.2.3. Hepatic *de novo* lipogenesis

As shown by Lavau *et al.* in adipocytes, glucose incorporation into fatty acids was also decreased in the liver of rats given a high fat diet when compared to rats given a control diet. This difference was even more marked when the incorporation was stimulated with insulin, suggesting a generalized decreased capacity for *de novo*

hepatic lipogenesis (Storlien et al. 1986). Clarke *et al.* showed that this reduction of *de novo* hepatic lipogenesis was more pronounced in rats receiving a PUFA-rich rather than a SFA-rich diet (Clarke et al. 1977).

Figure 1.3. Summary of the effects of high fat diets on the liver.



I.II.3. Intestine

The role of the intestine in the genesis of obesity has been quite underestimated, although it is responsible for fat absorption into the circulation. Very little is known on the effects of feeding high fat diets on the intestine and it only recently received increased interest, notably through the use of microarrays, which allow access to the transcriptome.

I.II.3.1. Fat absorption

In an early study, Singh *et al.*, showed that when feeding rats for 4 weeks a lard-based high fat diet, fecal excretion of radiolabelled lipids was significantly decreased. Moreover, radioactive uptake of oleic acid in everted gut sacs from both jejunum and ileum and its rate of reesterification were found to be greater in the rats fed the high fat diet. This was confirmed by an enhanced activity for the jejunal monoglyceride acyltransferase, a reesterifying enzyme which allows the synthesis of diglycerides in the enterocytes from monoglycerides, a necessary step in triacylglyceride synthesis for their export in chylomicrons (CM). Altogether, these results pointed to an enhanced lipid absorption capacity in the animals fed the high fat diet (Singh et al.

1972). This was confirmed in mice where it was shown that the fecal lipid content was not affected by a 6-week-long high fat feeding. Thus, whether or not through an adaptation in its absorptive capacity, the small intestine shows a very high efficiency for fat absorption (Petit et al. 2007).

Fat absorption is a function of the total absorptive surface area times the absorptive capacity of each enterocyte. In the work by Petit *et al.*, microarray analysis revealed an upregulation in genes involved in fatty acid uptake (such as the transporters FATP-4 and CD36), intracellular fatty acid processing (the fatty acid binding proteins I-FABP and L-FABP for example) and lipoprotein secretion (ApoA-IV and MTP for example). This demonstrated an adaptive upregulation of genes / proteins in the machinery to allow an enhanced lipid absorption and processing (Petit et al. 2007).

The intestinal absorptive surface area has also been found to be increased in high fat fed mice. Petit *et al.* and de Wit *et al.* both observed an increase in the proliferation rate of the enterocytes which could lead to an increase in villus size and ultimately in the absorptive area (Petit et al. 2007; de Wit et al. 2008). Interestingly, de Wit *et al.* also showed a downregulation of genes involved in apoptosis and an upregulation of genes involved in cell cycle, especially in the mid and distal parts of the small intestine. They also confirmed the increase in villus length and the total number of cells per villus in the distal small intestine. These changes could constitute a mechanism to support the small intestinal capacity in absorbing the bulk of dietary lipids.

I.II.3.2. Lipoprotein secretion

It has been shown that after long term feeding of a high fat diet containing long chain fatty acids, postprandial intestinal lipoprotein secretion is increased (Cartwright and Higgins 1999). Interestingly, this effect of diet-induced changes in postprandial lipoprotein secretion was observed as early as after 7 days of feeding and was characterised in the small intestine with a secretion of a smaller number of CM but of larger size (Hernandez Vallejo et al. 2009). As the lipoprotein lipase shows a higher activity towards larger-sized particles, this could be interpreted as a way to “manage the lipid overloading”. An adaptation of CM assembly in the intestine and secretion was confirmed also on the transcriptome basis by increased levels of apolipoprotein B (apoB) or the microsomal triacylglyceride transfer protein (MTP). Surprisingly, mice fed a high fat diet for 6 weeks showed decreased blood triacylglyceride levels which were shown to be due to an enhanced clearance from the blood, possibly through an elevated apoCII / apoCIII ratio. It underlines the prominent role of the small intestine in postprandial triglyceridemia (Petit et al. 2007).

I.II.3.3. Lipid oxidation

As addressed above, the small intestine reacts to a high fat diet by trying to export more efficiently excess lipids, which can be toxic for the enterocyte (Unger and Orci 2002). De Wit *et al.*, analysed the small intestinal transcriptome of mice fed either a low fat or a high fat diet. They reported a significant number of biological processes linked to lipid metabolism, cell cycle and inflammation / immune response being significantly affected by the dietary treatment. Interestingly, they found several genes associated with fatty acid oxidation to be upregulated in the mice fed the high fat diet, speculating that this might act as a detoxifying process, to prevent free fatty acids from impairing the function of the enterocyte (de Wit et al. 2008). Kondo *et al.* also

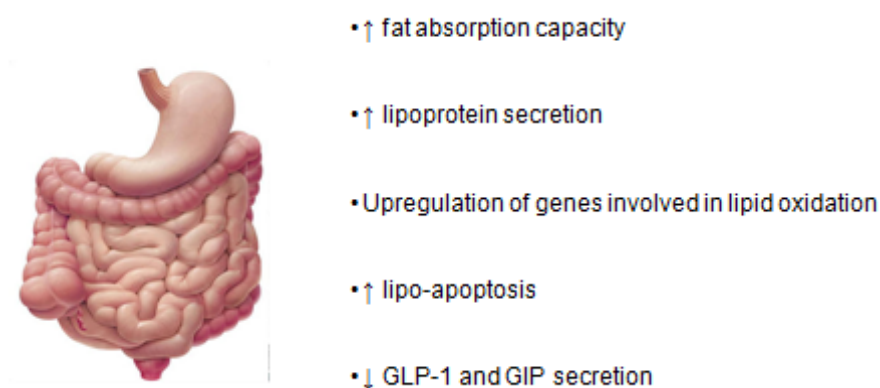
observed an upregulation of genes associated with fatty acid oxidation and interestingly, this effect was more prominent in obesity-resistant A/J mice than in obesity-prone C57Bl/6 mice, therefore suggesting a role of the small intestine in the development of obesity (Kondo et al. 2006). Nonetheless, according to Gniuli *et al.*, this increase in expression of genes linked to fatty acid oxidation and the increase in enterocyte mitotic rate is not sufficient to counteract the lipotoxic effect of the high fat diet since such a diet has been shown to induce apoptosis in rat enterocytes, the longer the diet was being fed, the more prone the enterocytes were to lipid-induced apoptosis (Gniuli et al. 2008). Interestingly, PUFA of marine origin (EPA and DHA) have been found to increase lipid oxidation rates in the small intestine and might therefore protect the small intestine from lipo-apoptosis and reduce the amount of lipids for export via CM to the other organs (van Schothorst et al. 2009).

I.II.3.4. Hormone secretion

When de Wit *et al.* analysed the intestinal transcriptome of mice fed a high fat diet compared to mice fed a low fat diet, they also found several transcripts of secreted proteins being affected by the high fat feeding, suggesting alterations in the communication of the gut with other organs, such as liver, muscle and adipose tissue, via hormones and thereby underpinning the role of the small intestine in metabolic perturbations (de Wit et al. 2008). The gut is not only an absorptive organ but also secretes various hormones involved in energy homeostasis and satiety (Chaudhri et al. 2008). For example, feeding a high fat diet for 30 days has been shown to cause an elevated gastric inhibitory polypeptide (GIP) secretion (an incretin that amplifies insulin secretion) but this response was blunted after 90 days of feeding and was associated with a decreased insulin secretion (Gniuli et al. 2008). Mice subjected to a high fat diet for 8 weeks displayed lower basal plasma glucagon-

like peptide-1 (GLP-1) (another incretin) levels and showed a reduced GLP-1 response following an oral glucose load (Anini and Brubaker 2003). It seems therefore that feeding a high fat diet blunts the response of the small intestine with respect to secreted peptide hormones that are involved in satiety control and in energy homeostasis.

Figure 1.4. Summary of the effects of high fat diets on the small intestine.



I.III. The gut microbiota and obesity

The human gut hosts as many as 100 trillion microbes – collectively called microbiota - mainly located in the colon where densities approach $10^{11} - 10^{12}$ cells/ml. This makes the human gut one of the most densely populated microbial habitats on Earth. There might be thousands of species, dominated by anaerobic microorganisms, which contain an estimated 100 times more genes than the human genome (Ley et al. 2006a). The microbiota is able to perform functions that humans cannot accomplish by converting undigested food components and endogenous substrates, such as plant polysaccharides, phenolic compounds, mucin, cholesterol, biliary acids and steroids. Thereby, it produces essential compounds, including some vitamins,

and releases large quantities of short chain fatty acids, considered to be beneficial for gut health. However, the gut microbiota also produces metabolites that can be harmful. For instance, diets characterized by high saturated fat or low fibre content have been associated with changes in the bacterial metabolism of steroids and bile acids. Resulting metabolites, such as secondary bile acids, have been linked to pathologies, e.g. colon cancer and cholesterol gallstone disease (Blaut and Clavel 2007). The next section will briefly review recent studies linking the gut microbiota to obesity. This field has received great interest in the very recent years, notably through the work of Jeffrey Gordon's group.

I.III.1. Role of the gut microbiota in fat storage

The first study that established a link between the gut microbiota and obesity was published in 2004 by the group of Jeffrey Gordon. In this comprehensive work, Bäckhed *et al.* compared parameters of energy balance in germ-free (GF) (*i.e.* raised in the absence of microorganisms), conventionally raised and conventionalized C57Bl/6 mice fed a standard rodent chow. Conventionalized mice were obtained by spreading resuspended cecal contents of conventionally raised mice on the fur of GF animals. 2 weeks after the colonization of the GF mice, conventionalized and conventionally raised animals showed a 42 % increase in total body fat compared to the GF animals. Surprisingly, the GF mice presented a 40 % higher food intake and a 27 % lower metabolic rate (as measured by O₂ consumption) compared to the mice bearing a gut microbiota. As muscle and liver high-energy phosphate stores were not affected by the microbiota, the authors concluded that the presence of microorganisms induced futile cycles, therefore allowing a dissipation of energy (Bäckhed *et al.* 2004). Moreover, GF mice appeared to be protected against diet-induced obesity since 8 weeks of high fat feeding failed to induce a significant weight

gain in GF animals when compared to GF mice fed a low fat diet. Conventionalized mice did not present any protection against diet-induced obesity (Bäckhed et al. 2007). Whether fed a low fat or a high fat diet, GF mice displayed an increased locomotor activity compared to conventionalized mice, seemingly not due to the difference in adiposity, which could contribute to the energy balance of these animals (Bäckhed et al. 2007). Some of the proposed mechanisms which could be underlying these effects on energy balance will be discussed next.

I.III.2. Gut microbiota and short-chain fatty acids absorption

Polysaccharide fermentation by the gut microbiota produces SCFA, e.g. acetate, propionate or butyrate. These SCFA appear to serve as ligands for the G protein-coupled receptor 41 and 43 (Gpr41 and Gpr43 respectively) which are located in enteroendocrine cells of the gut. To study the contribution of Gpr41 to energy balance, Jeffrey Gordon's group compared wild-type and Gpr41 knock-out mice cocolonized or not with 2 organisms promoting SCFA production from dietary polysaccharides. Knocking out Gpr41 abolished the effect of gut microbiota on fat storage (as described in the previous section I.III.1.): GF mice, whether expressing Gpr41 or not, had the same body weights, fat pad weights and adiposity as cocolonized mice not expressing Gpr41. Cocolonized mice expressing Gpr41 presented significantly higher values for these phenotypic changes. Moreover, deletion of Gpr41 reduced leptin secretion more than expected if only the associated decrease in adiposity was considered, therefore suggesting a distinct role of Gpr41 in microbiota-mediated leptin production. Moreover, an increased fecal energy output, an increased uptake of monosaccharide from the gut and an increased intestinal transit time were also observed. The latter was discussed as a consequence of a blunted peptide YY (PYY) production which, as an anorexigenic gut peptide secreted

by enteroendocrine cells from the gut, might inhibit gastric motility and may be responsible for an increased fecal loss of SCFA in Gpr41-deficient mice. Taken together, these results suggest a prominent role of colonic SCFA in control of satiety, motility and obesity which seems to be mediated by Gpr41 (Samuel et al. 2008).

I.III.3. Gut microbiota and lipoprotein lipase (LPL) activity

In the same study, Bäckhed *et al.* observed a 122 % increase in LPL activity in the epididymal fat pads of the conventionalized animals (Bäckhed et al. 2004). Moreover, when given an olive oil gavage after overnight fasting, GF mice presented a much more delayed serum triacylglyceride clearance compared to conventionalized mice, a difference which can be explained by a reduced LPL activity (Bäckhed et al. 2007). The authors hypothesized that this increase was caused by the suppression of *Fiaf*. *Fiaf*, standing for “fasting-induced adipose factor”, also known as angiopoietin-like protein-4, represents a circulating inhibitor of LPL. Its suppression would induce a higher LPL activity thereby promoting the release of free fatty acids from circulating triacylglyceride-rich lipoproteins to the adipose tissue, which could explain the increased fat storage in conventionalized mice. *Fiaf* expression levels were shown to be strongly downregulated in the small intestine, but not in the liver or white adipose tissue of conventionalized mice. A deletion of *Fiaf* drastically abolished the differences in body fat between germ-free and conventionalized animals, therefore confirming the importance of small intestinal *Fiaf* as an important target of the gut microbiota with respect to peripheral fat storage (Bäckhed et al. 2004). Of note, *Fiaf*-deficient mice present decreased levels of genes encoding for key enzymes of fatty acid oxidation, possibly through the decreased expression levels of the peroxisomal proliferator activated receptor coactivator 1 α (Pgc-1 α) (Bäckhed et al. 2007). Thus, not only does the gut microbiota allow an increased energy harvest from the diet by

processing for example plant polysaccharides but it also modifies the genes of the host, promoting fat deposition in adipocytes.

I.III.4. Gut microbiota and hepatic lipogenesis

In the studies by Bäckhed *et al.*, conventionalized mice presented a 2.3-fold increase in liver triacylglyceride content, probably through the activation of 2 transcription factors, the sterol response element binding protein 1 (SREBP-1) and the carbohydrate response element binding protein (ChREBP), whose mRNA levels were higher in conventionalized mice. These transcription factors predominantly control the expression of lipogenic enzymes in the liver and the genes encoding for the acetyl-CoA carboxylase (Acc1) and the fatty acid synthase (Fas), two key enzymes in hepatic lipogenesis, appeared to be upregulated (Bäckhed *et al.* 2004; Samuel *et al.* 2008). SCFA serve as substrate in the liver for *de novo* lipogenesis (Rolandelli *et al.* 1989). Therefore, the increase in SCFA production and absorption associated to the gut microbiota promotes elevated fat deposition in the liver through the activation of the *de novo* lipogenesis pathway.

I.III.5. Gut microbiota and AMP-activated protein kinase

In 2007, Jeffrey Gordon's group also showed that GF animals displayed 40 % higher levels of phosphorylated AMP kinase (AMPK) in their gastrocnemius muscle compared to conventionalized mice and 50 % higher levels of AMP (Bäckhed *et al.* 2007). Phosphorylation of AMPK stimulates fatty acid oxidation by phosphorylating acetyl CoA carboxylase (ACC), which inhibits the activity of this enzyme, the rate-controlling step in malonyl CoA production. A decrease in malonyl CoA concentration causes a de-inhibition of the carnitine palmitoyl transferase-1 (CPT1), which catalyzes the rate-limiting step for the entry of long-chain fatty acyl-CoA into

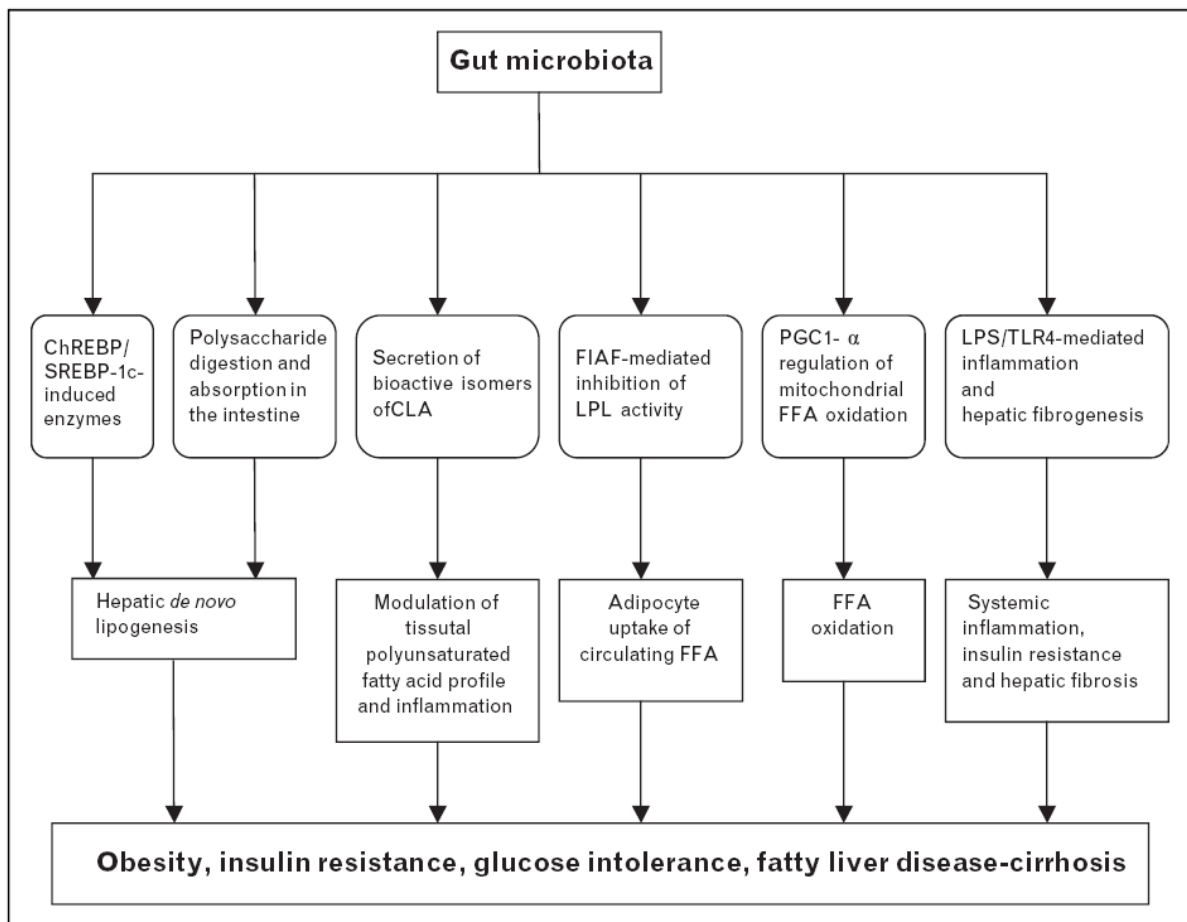
mitochondria for oxidation (Kahn et al. 2005). As observed by Bäckhed *et al.*, levels of phosphorylated acetyl CoA carboxylase (ACC) were increased by 43 % and CPT1 activity was increased by 17%. Taken together, these results suggest that the gut microbiota could induce a reduction in fatty acid oxidation in skeletal muscle through the phosphorylation of AMPK (Bäckhed et al. 2007). A similar increase in the levels of phosphorylated AMPK in the livers of the GF mice was accompanied by reduced glycogen levels and a decreased glycogen synthase activity. As addressed before (see **I.III.4**), mice not bearing a microbiota are protected against hepatic fat deposition (Bäckhed et al. 2004) which could partly be due to increased fatty acid oxidation through increased AMPK activity as also seen in the skeletal muscle (Bäckhed et al. 2007).

I.III.6. Gut microbiota and metabolic endotoxemia

Lipopolysaccharides (LPS) are a membrane component of Gram-negative bacteria and are released from dead bacteria. Cani *et al.* showed that high fat feeding in mice resulted in an increase in plasma LPS levels. Mice injected with a chronic low rate of LPS presented similar phenotypic characteristics to the mice fed the high fat diet, including elevated body weight, fasting hyperglycaemia and inflammation, despite a normal energy intake. Mice deficient for CD14, the LPS receptor, exhibited a delayed response to high fat feeding. Thus the effects of high fat feeding on metabolic disorders such as obesity, insulin resistance and inflammation could be at least partly mediated by LPS, produced by the gut microbiota (Cani et al. 2007). Moreover, it was shown that high fat feeding or genetic obesity (by the use of leptin-deficient mice) increased intestinal permeability and thus LPS translocation into circulation. Most interestingly, some of the disorders induced by high fat feeding or obesity in the mice

were improved by antibiotics treatment confirming that changing the gut microbiota is a sensible approach to treat obesity or its associated disorders (Cani et al. 2008).

Figure 1.5. Summary of the effects of gut microbiota on host metabolic and inflammatory processes (Musso et al. 2009).



I.III.7. Composition of the gut microbiota in obese states

I.III.7.1. In genetically obese mice

Of the 70 known divisions of bacteria, the *Firmicutes* and the *Bacteroidetes* are the two most prominent classes accounting respectively for 60 – 80 % and 20 – 40 % of the sequences found. These proportions are similar between humans and mice (Backhed et al. 2005; Ley et al. 2005). Interestingly, genetically obese mice, homozygous for a mutation in the leptin gene (ob/ob), and their lean ob/+ or +/+

littermates do not present the same microbiota at the division (superkingdom) level: Ley *et al.* observed that the obese mice were characterized by a shift in the proportion of *Firmicutes* and *Bacteroidetes* present in their ceca with a 50 % reduction of *Bacteroidetes* compensated by an increase in *Firmicutes*. Moreover, this shift was independent of the difference in body weight or chow consumption between the different genotypes. This shift was division-wide since it did not affect a specific class of bacteria. The authors proposed 2 explanations for this shift: it could serve either as an adiposity promoting factor or on the contrary as a defence mechanism from the host, for example by limiting the energy harvest from polysaccharides (Ley *et al.* 2005). To answer this question, Turnbaugh *et al.* performed a microbiota transplantation: GF mice were colonized with a microbiota either coming from genetically obese (ob/ob) or lean (+/+) mice. After 2 weeks of colonization, the mice still presented the same *Firmicutes* to *Bacteroidetes* ratio as the donors, implying that the microbiota, and therefore its induced phenotype, are transmissible. The most interesting finding of this study was that after 2 weeks, the mice colonized with an ob/ob microbiota displayed a significantly greater increase in body fat than the mice colonized with a +/+ microbiota. Since there were no differences between the groups with respect to chow consumption, the authors concluded that the microbiota from obese donors exhibited an increased capacity for energy harvest from the diet (Turnbaugh *et al.* 2006).

I.III.7.2. In diet-induced obese mice

As mentioned earlier in this literature review (see I.I.1.), leptin-deficient mice may not really be an appropriate model of human obesity. Therefore, in another study, Turnbaugh *et al.* investigated the changes in the gut microbiota associated with diet-induced obesity. Mice fed a low fat or a high fat diet, conventionally raised or

conventionalized, did not present the same microbiota: they observed the same shift towards an elevated *Firmicutes* to *Bacteroidetes* ratio. However, this shift was not division-wide since the diversity of the *Firmicutes* in the high fat fed mice was profoundly altered by the blooming of a single class of the *Firmicutes*, the *Mollicutes*. They carried out a microbiota transplantation to GF mice from mice fed the low fat or the high fat diet and after 2 weeks, the mice colonized with a DIO-associated microbiota displayed a significantly greater increase in body fat than the mice colonized with a low fat diet fed donor flora. Here again, there were no differences between the groups with respect to chow consumption. When the microbiota of mice fed Western diets reduced in carbohydrates or fat, which promoted weight stabilization, were analysed, a decrease in the proportion of *Mollicutes* with a concomitant increase in the proportion of *Bacteroidetes* was observed. This effect was more pronounced in the mice fed the Western diet reduced in carbohydrates. It was therefore concluded that “diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome”. Moreover, microbiota transplantation from these animals to GF mice failed to induce obesity. Altogether, these results confirm the observations made in genetically obese mice (shift in bacterial populations and an increased energy harvest from the diet) but through different mechanisms since there was a massive bloom for one specific class of the *Firmicutes*, the *Mollicutes* (Turnbaugh et al. 2008). Interestingly, high fat diet itself can trigger changes in gut microbiota. Hildebrandt *et al.* took advantage of RELM β knockout mice which fail to develop obesity upon high fat feeding. They could thus study diet specific changes in the gut microbiota. However, they presented results different from those of Turnbaugh *et al.* since they did not observe a bloom of *Mollicutes* but rather of *Clostridia* and *Proteobacteria* (Hildebrandt et al. 2009).

I.III.7.3. In humans

The same dominance of *Firmicutes* over *Bacteroidetes* could be observed in humans. Moreover, this proportion could also be altered upon dietary treatment: the composition of the gut microbiota in 12 obese people given either a fat-restricted or a carbohydrate-restricted diet was analysed during 1 year and it was found that the proportion of *Bacteroidetes* increased from 2 to more than 20 % of total bacteria, correlating well with the percentage of body weight lost and indicating here again that manipulation of the gut microbiota could be a sensible approach to treat obesity (Ley et al. 2006b). Duncan *et al.* carried out a similar study but did not observe different *Firmicutes* to *Bacteroidetes* ratios between obese and non obese subjects or an increase in *Bacteroidetes* upon weight loss (Duncan et al. 2008). Kalliomäki *et al.* studied the composition of the gut microbiota in children at 6 and 12 months of age, and later followed-up the cohort to know if they either developed overweight or not at the age of 7 years. Interestingly, low numbers of *Bifidobacteria* and high numbers of *S. aureus* were found in children who later in life developed overweight. This led the authors to conclude that differences in the gut microbiota may precede and predict overweight (Kalliomaki et al. 2008).

Part 2: Experimental work conducted

Part 3: Final discussion and conclusion

Diet-induced obesity allows us to generate obese models in a more realistic manner than monogenic or pharmacological manipulations and has therefore been extensively used in the study of obesity-associated disorders. In this respect, high fat diets are regarded as the “gold standard” although they present two major drawbacks: 1) they only poorly mimic Western diets and 2) they are not standardized since, as we have seen in the literature review of this thesis, fat quantity and quality play significant roles in the development of metabolic disorders. Therefore, we decided in this thesis to compare a widely used high fat diet to a cafeteria diet. Cafeteria diets are supposed to elicit an obese phenotype through hyperphagia which is driven by the flavour variety.

In the first study, we aimed at determining the most appropriate form in which the diets should be offered to the mice. We showed that mice fed powder diets developed the same body weights, regardless of the diet composition, whereas when we fed them the same diets in pellet form, mice on the control diet remained lean and mice on the high fat and cafeteria diet clearly presented an obese phenotype. This surprising finding allowed us to separate pathophysiological changes due to body weight from those due to specific dietary effects. Thus, we could conclude that a cafeteria diet seemed more deleterious for the liver than the commonly used high fat diet and reported some diet-specific changes in the adipose tissue. Nevertheless, this study allowed us to critically ask what a proper control diet is. To our knowledge, the fact that when fed pellets, mice fed a control diet need four times as much energy to put on one gram of body weight as compared to mice fed a high fat or a cafeteria diet has never been thoroughly addressed in the literature. Although we did not propose here any mechanism to explain this feature, we suggested some explanation

(energetic cost of chewing and handling, energy loss through the gut microbiota or diet-induced thermogenesis) and these would surely be directions worth looking into for future studies. We are actually carrying out a new experiment in which we are feeding mice either a control diet as pellets or a control diet as powder to look at possible differences in energy expenditure, using indirect calorimetry. Nevertheless, this work raises an essential question for all scientists studying obesity using rodent models: what is a proper control diet? Does maybe a high carbohydrate pellet diet – used as a standard in mice – produce an artificial lean phenotype? However, since we needed a lean control to assess the effects of a high fat and a cafeteria diet on obesity-associated disorders, we decided to feed the mice in the subsequent studies with pellets.

In the second study, we used pellet diets to investigate the effects of a high fat and a cafeteria diet on the small intestine. The small intestine has long been neglected and has only seen a renewal of interest in the past years with the development of microarray, which allows an access to the transcriptome. In this study, we confirmed the effects of diets high in fat on lipid oxidation and transport as seen by others before. Nevertheless, we highlighted a very surprising paradox: the mice fed the diets high in fat, which also contained cholesterol, presented decreased levels of cholesterol in the small intestine, and the liver, as compared to mice fed a cholesterol-free control diet. This fall in cholesterol was counteracted on mRNA level. The next step would be of course to measure expression levels of some targeted proteins, such as the LDL receptor or the 3-hydroxy-3-methylglutaryl-Coenzyme A reductase, or some enzyme activities. Moreover, we have been analyzing phospholipids profiles in the small intestine and liver, measuring the concentrations of

more than a hundred metabolites, belonging to the family of acylcarnitine, phosphatidylcholine and sphingolipid, using liquid chromatography-mass spectrometry, and it appears so far that both organs display fairly similar profiles. It would be of great interest to assess to which extent cholesterol synthesis in the small intestine contributes to the total cholesterol pool in obese states or upon dietary intervention.

In the last study, we characterized the effects of a high fat and a cafeteria diet on the gut microbiota. As seen in the introduction, the gut microbiota greatly contributes to the energy balance and it is therefore of interest to study bacterial populations and how they are affected by dietary treatment or obesity. To do so, we used Fourier transform infrared spectroscopy and could show that not only an obese phenotype but also the quality of the diet affected the gut microbiota. Fourier transform infrared spectroscopy appeared to be a sensible approach to study the gut microbiota. Moreover, the dietary treatment did not only affect bacterial populations but also their chemical composition, pointing at a possible modification of bacterial activities. Hence, it would be highly valuable to dig further in this direction and characterize changes in bacterial functions upon the development of obesity or upon dietary treatment and investigate the consequences on digestive functions of these alterations. Metaproteomics studies might be the next step in this field. We are currently carrying out several experiments in this respect:

- We are looking at FT-IR spectra of mouse intestinal contents after antibiotic treatment to assess to which extent the spectra depend on bacterial composition, independently of changes in the diet.

- We are looking at FT-IR spectra in germ-free mice and mice colonized with one single strain of *Bacteroides thetaiotaomicron*, when mice are fed with the different diets
- We are trying to analyse the cecal proteome in mice fed the different diets to identify microbial functions of particular relevance in the context of diet-induced obesity
- Finally, we have been isolating gut microbes from the cecum of mice fed the different diets using selective media to assess differences in composition of specific cultivable bacteria

The primary aim of this thesis was to compare the effects of a high fat vs. a cafeteria diet on some components / determinants of energy homeostasis, namely the adipose tissue, the liver, the small intestine and the gut microbiota. This was covered in three parts: while a cafeteria diet was shown to induce more severe liver steatosis and damages (study 1), a high fat diet elicited more dramatic changes in the small intestine with regards to cholesterol metabolism (study 2). Finally, dietary treatment was shown to affect the gut microbiota composition, an effect which was more marked with the cafeteria diet (study 3). Thus, it seems that a cafeteria diet elicits disturbances as or even more severe than a high fat diet, although it is characterised by a lesser energy density and fat content. Hence, cafeteria diet constitute a good diet to promote diet-induced obesity. To conclude, this work brings new knowledge in the effects of dietary treatment on obesity-associated disturbances, a crucial step in the elaboration of dietary recommendations for obese patients.

Part 4: References

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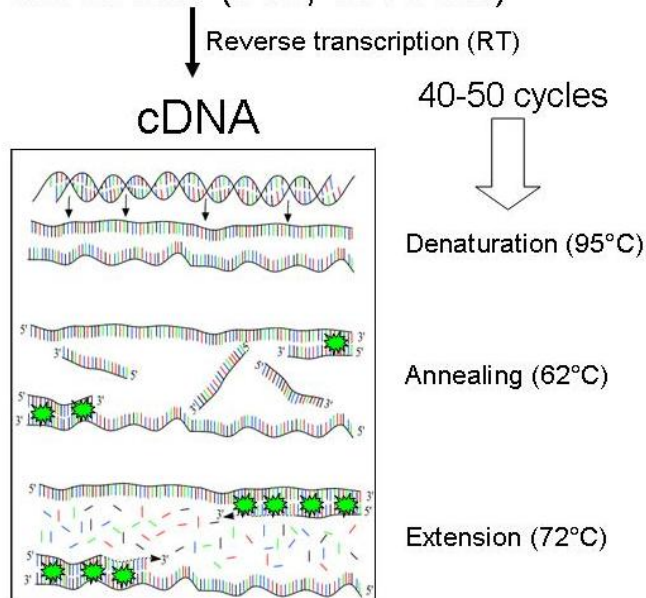
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Part 5: Appendices

Principles of real-time quantitative PCR (qPCR) with the use of SYBR green

Real-time quantitative PCR (qPCR) allows to simultaneously amplify and quantify the products of the polymerase chain reaction. The principle is based on the detection and the quantification of a fluorescent molecule, SYBR green in our case, which fluoresces upon light excitation when bound to double-stranded DNA. The fluorescence is proportional to the amount of double-stranded DNA and is measured after each cycle, thus allowing DNA concentrations to be quantified.

RNA extraction (cell, tissue...)



By using sequence-specific DNA probes consisting of oligonucleotides (named primers), it is possible to know the relative expression of a specific gene. Fluorescence is used to detect the threshold cycle (C_q) which corresponds to the number of cycles needed to obtain a defined number of PCR products. The lower the C_t is, the more copies of the gene were present in the sample at the beginning of the reaction. The fluorescence intensity is then normalized to a housekeeping gene (such as GADPH or β -actin) to account for differences in DNA concentrations at the beginning of the reaction.

Principles of DNA microarrays

DNA microarrays are based on the same principle of RT-PCR but extended to a much bigger number of target genes (more than 15 000 in our case): DNA oligonucleotides (called probes) are spotted onto a chip and bind to cDNA from the sample (called target). The hybridization is detected by fluorescence and the level of fluorescence of each target is then detected, measured and translated into an expression level of the specific gene.



Affymetrix chip ~ 15 000 genes

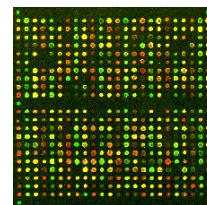


Image scanning and analysis