Biomedical Investigations for the Optimized Diagnosis and Monitoring of Severe Acute Malnutrition : The OptiDiag Study

Trenton Dailey-Chwalibóg

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Biomedische onderzoeken voor geoptimaliseerde diagnose en monitoring van ernstige acute ondervoeding

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Résumé substantiel en français

Introduction

Les recommandations actuelles de l'OMS séparent les enfants atteints de malnutrition aiguë sévère (MAS) non-œdémateux en trois phénotypes anthropométriques : (1) faible périmètre brachiale (PB) seul ; faible indice poids-taille (IPT) seul ; ou faible PB et ITP. Tous sont éligibles à une réhabilitation nutritionnelle selon les lignes directrices de l'OMS.

Les premières comparaisons de PB et l'IPT ont montré que les deux indices sont mal corrélés et identifient des enfants différents;[5,6] depuis, rien n'a changé.[7–13] Une colossale analyse secondaire des données d'enquête de 47 pays a rapporté qu'en moyenne, seulement 16% des enfants atteints de MAS présentent à la fois un faible PB et un faible IPT ; en outre, l'ampleur et la direction de la divergence diagnostique varient considérablement d'un pays à l'autre.[13]

Deux hypothèses concurrentes, parmi d'autres, ont été avancées pour expliquer ce phénomène.

Une première hypothèse suggère que le PB et l'IPT évaluent différemment l'état nutritionnel. Grellety *et al.* émettent l'hypothèse que les deux critères sont complémentaires, chacun sélectionnant différents enfants présentant un risque accru de décès.[13] Grijalva-Eternod *et al.* suggèrent que l'IPT reflète mieux la masse tissulaire, alors que le PB est un indice composite de faible croissance (combinant le retard de croissance, émaciation et faible adiposité).[14]

Une deuxième hypothèse suggère que le PB et l'IPT sont des indices anthropométriques imparfaits—et chacun surestime ou sous-estime la malnutrition aiguë dans des situations spécifiques. Le diagnostic du PB utilise un seul absolu, indépendant de l'âge, de la taille et du sexe.[13] Des travaux suggèrent, pour cette raison, que le PB surestime la malnutrition aiguë chez les jeunes enfants,[15–17] chez les filles,[7,12,18,19] et en cas de retard de croissance.[7,12,20] A l'inverse, le PB pourrait sous-estimer la malnutrition chez les enfants plus âgés. Certaines données suggèrent que les enfants plus âgés sont proportionnellement plus atteint de malnutrition dans les contextes de crise aiguë.[21] L'utilisation du PB seul dans ce contexte entraînerait donc une sous-estimation du nombre de cas lors de crises humanitaires aiguës—un phénomène qui a été confirmé récemment sur le terrain dans des contextes à forte prévalence.[22]

Orientations de recherche

Deux difficultés empêchent le diagnostic et une priorisation correct de la MAS. Premièrement, le diagnostic est basé sur des outils anthropométriques rudimentaires et donc imparfaits (*i.e.*, PB et IPT). Deuxièmement, le diagnostic fait par ces outil diverge fortement : ils ciblent des groupes d'enfants différents pour la réhabilitation nutritionnelle.[11–13,23] La question de quel outil est le meilleur prédicteur de la mortalité fait l'objet d'un débat intense.[1,2,11,13,24–28]

Cependant, pour des raisons de facilité d'utilisation, et sur la base de données suggérant une surmortalité chez les enfants atteints de MAS diagnostiquée par le seul PB, plusieurs agences et gouvernements considèrent ce dernier critère comme nécessaire et suffisant à l'éligibilité pour une prise en charge de la malnutrition aiguë sévère, excluant de fait les enfants présentant uniquement un faible IPT. Ces enfants sont non seulement à risque accrue de décès s'ils ne sont pas traités[1,2,13,27,28] mais ils représentent également près de la moitié de la charge mondiale de malnutrition,[13] soit environ 7 millions d'enfants aujourd'hui.[29]

Ce changement de paradigme semble prématuré : les liens entre phénotype anthropométrique, retentissement fonctionnelle et risque associés n'ont pas encore été élucidés. En effet, plusieurs réanalysé de données anciennes montrent que les enfants atteints de MAS inéligibles à une prise en charge sur la base du seul PB (c'est-à-dire les enfants avec uniquement un faible IPT) présentent un risque de décès similaire à ceux éligibles. De plus, ces analyses suggèrent que les enfants présentant simultanément les deux déficits anthropométriques ont un risque de mortalité encore supérieur.

Cette situation complique le choix de décideurs politique des enfants à traiter, ce qui a conduit l'OMS et la communauté scientifique à formellement appeler à l'investigation de ces questions.[29]

Le but de cette thèse est de participer à la résolution de ces questions, et de produire une caractérisation robuste et objective de l'état de santé et nutritionnel lié au phénotype diagnostic dans la MAS. Nous espérons que cette contribution à un domaine de la recherche très largement ignoré sera rapidement prise en compte par les décideurs, et les éléments d'information apportée incluse dans le rationnel de la prise en charge des enfants atteints de malnutrition.

Avec ces travaux, nous avons pour objective de combler une lacune, très largement ignorée, dans les connaissance du domaine—connaissances dont les décideurs politiques ont urgemment besoin afin de prendre des décisions éclairées sur les enfants à cibler pour le traitement.

Objectifs de recherche

L'objectif de cette thèse est double. Tout d'abord, elle cherche à décrire et comparer la physiopathologie et la sévérité fonctionnelle liées au déficit anthropométrique dans la MAS. Ce faisant, elle demande : existe-t-il des différences de risque de morbi-mortalité parmi (et entre) les trois phénotypes de MAS (*e.g.*, PB seul, PB & IPT, IPT seul) ? Dans le même ordre d'idées, le deuxième objectif de cette thèse est de piloter l'utilisation de marqueurs émergents de l'alimentation, du métabolisme (analyse isotopique stable) et de la composition corporelle (bio-impédancemétrie) dans cette population, en posant la question : ces nouveaux indicateurs peuvent-ils aider à élucider la signification clinique du déficit anthropométrique ?

Plus précisément, cette thèse vise à :

1. Décrire et comparer les caractéristiques cliniques, biochimiques, et anamnestiques de santé et/ou nutritionnel des enfants atteints de MAS à l'admission, parmi et entre les phénotypes (*e.g.*, PB seul, PB & IPT, IPT seul) ;
2. Évaluer l'association entre le $\delta^{13}C$ et $\delta^{15}N$ dans le cheveux, le régime alimentaire et la gravité de la malnutrition aiguë ou chronique dans une population représentative de jeunes enfants (6-59 mois) au Bangladesh ;
3. Évaluer la capacité du $\delta^{15}N$ et du $\delta^{13}C$ dans le cheveux à différencier les enfants atteints de MAS, diagnostiqués avec PB ou IPT ; et,
4. Décrire et comparer la composition corporelle (en utilisant la bio-impédancemétrie) des enfants atteints de MAS à l'admission, parmi et entre les phénotypes.

Méthodes

Une étude de cohorte multicentrique a été réalisé chez des enfants atteints de MAS sans complications ni œdèmes au Bangladesh, au Burkina Faso, et au Libéria. Les patients ont été inclus de façon équilibré parmi les trois phénotypes anthropométriques. Un large panel de paramètres cliniques et biochimiques indicateurs du statut nutritionnel et de l'état de santé a été recueilli à l'admission à – et à des points clés pendant – la réhabilitation nutritionnelle. Nous avons évalué de nouveaux biomarqueurs de la physiopathologie et de la survie en plus des biomarqueurs conventionnels de l'état de santé et de la dénutrition : la leptine sérique, un paramètre prédictif robuste de mortalité chez les enfants atteints de MAS ; l'abondance isotopique naturelle du carbone et de l'azote ($\delta^{13}C$ et $\delta^{15}N$) dans le cheveux, offrant un intéressant historique nutritionnel et métabolique ; la bio-impédancemétrie, une technique mobile et non-invasive pour l'évaluation de la composition corporelle adaptée à une utilisation sur le terrain ; et la mesure de paramètres biochimiques d'inflammation (protéines de la phase aiguë) et de déficience en micronutriments (vitamine A et fer).

Conclusions

Les résultats de cette thèse mettent en évidence la complexité et l'hétérogénéité particulière des profils cliniques, biochimiques et métaboliques chez les enfants atteints de MAS. Comme souligné précédemment, le déficit anthropométrique est un indicateur utile pour identifier rapidement et efficacement la carence nutritionnelle chez les enfants sur le terrain. Cependant, les outils anthropométriques sont limités dans la mesure où ils ne fournissent pas d'informations étiologiques sur les causes complexes et souvent multifactorielles qui sous-tendent la malnutrition aiguë sévère. Alors que nos travaux mettent en évidence des particularités cliniques et biochimiques spécifiques à certains profils anthropométriques, nos résultats révèlent surtout que tous les enfants atteints de MAS présentent des profils caractéristiques de déficits nutritionnels sévères—ces enfants nécessitent sans équivoque une prise en charge. Notre travail est étayé par des preuves récentes sur le risque de mortalité associé au déficit anthropométrique dans la MAS, qui a montré un risque élevé et similaire entre les enfants atteints de MAS avec déficit d'ITP et de PB, et un risque accru chez ceux qui ont les deux déficits anthropométriques.[1,2,27,28]

Nos résultats remettent en question la stratégie opérationnelle et de plaidoyer des programmes basés sur l'utilisation exclusive du PB, car les enfants atteints de MAS qui seraient exclus du traitement (*i.e.*, à faible IPT seul) dans de tels programmes sont à haut risque de morbidité et de mortalité aiguës et à long terme.

Recommandations

Cette thèse est le premier pas sur un long chemin vers une meilleure compréhension de l'état de santé et nutritionnel des différents phénotypes des enfants atteints de MAS. Une priorité de recherche devrait être d'exploiter la capacité technologique pour développer des méthodes de diagnostic innovantes pour identifier les enfants à faible IPT seul dans la communauté. En attendant le développement éventuel de ces technologies, l'IPT et le PB doivent être conservés comme critères de diagnostic indépendants de la MAS, conformément aux recommandations répétées de l'OMS.

Abstract

Current WHO diagnostic recommendations segregate non-edematous children with severe acute malnutrition (SAM) into one of three anthropometric phenotypes, those with: (1) low mid-upper arm circumference (MUAC) only; (2) low weight-for-height *z*-score (WHZ) only; or (3) both low MUAC and low WHZ—all of which are eligible for nutritional rehabilitation according to WHO guidelines.

But, based on both ease of use and reports purporting higher mortality in SAM identified by MUAC, many agencies and some national governments use only MUAC as the sole diagnostic criterion for admission to therapeutic refeeding programs—disqualifying low WHZ only children from access to treatment. This diagnostic paradigm shift is premature because the links between anthropometric phenotype and functional severity have not yet been clearly delineated. In fact, recent secondary analyses of historic databases have shown that children with SAM that are excluded from treatment within the framework of a MUAC-only program (*i.e.*, low WHZ only) have a similar risk of death as those who are included; moreover, children with both anthropometric deficits (*i.e.*, low MUAC and low WHZ) have a higher risk of death.[1,2]

This dissertation aims to describe and compare the pathophysiology and functional severity associated with the anthropometric phenotypes of children with SAM today. Building on existing comparative work on vulnerability in SAM, it asks: how does the vulnerability profile of children with SAM who are excluded from treatment within the framework of a MUAC-only program (*i.e.*, low WHZ only) compare to the profiles of those children who are included (*i.e.*, low MUAC only and/or both low MUAC and low WHZ)?

A multi-centric cohort study was conducted in uncomplicated, non-edematous children with SAM in Bangladesh, Burkina Faso and Liberia. Participants were recruited equally into each of the three anthropometric phenotypes. A wide range of clinical and biochemical indicators of health and nutritional status were collected at admission to, and at key time points throughout, therapeutic refeeding. We assessed emerging biomarkers of pathophysiology and viability in addition to traditional indicators of health status and nutritional deprivation. These included: serum leptin, a robust biochemical predictor of mortality in children with SAM;[3,4] natural isotopic abundances of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) in hair, promising archives of metabolic status; bio-electric impedance, a portable, non-invasive technique for assessing body composition in the field-setting; and combined biochemical assessment of micronutrient deficiencies (vitamin A and iron) and inflammation (acute phase proteins).

Analysis of these indicators demonstrated that all children with SAM (*i.e.*, low WHZ and/or low MUAC) presents with clinical evidence of nutritional deprivation and micronutrient deficiencies, with significant heterogeneities on key criteria. Children with low WHZ only have biochemical and clinical deficits that are more severe than those in children with low MUAC only. These results also indicate

that children with both anthropometric deficits have the highest risk of acute and post-discharge death and morbidity. On this basis, low WHZ must be retained as an independent diagnostic criterion, in line with WHO recommendations. Further research is urgently needed to develop innovative diagnostic solutions to identify low WHZ children in the community.

Abstract in het nederlands

De WGO verdeelt niet-oedemateuze acuut en ernstig ondervoede kinderen (SAM) in drie antropometrische fenotypes: (1) alleen lage midden-bovenarmomtrek (MUAC); (2) alleen lage gewicht-voor-lengte z-score (WHZ); of (3) zowel lage MUAC als lage WHZ. Allen komen in aanmerking voor nutritionele revalidatie volgens de WGO.

Gebaseerd op zowel gebruiksgemak, gebruiken veel organisaties MUAC alleen als diagnostische criterium voor opname rehabilitatie en behandelen zo kinderen niet met een lage WHZ score. Deze paradigmaverschuiving is voorbarig omdat het verband tussen antropometrisch fenotype en functionele ernst nog niet duidelijk is afgebakend.

Dit proefschrift heeft als doel de pathofysiologie van ondervoeding te beschrijven, fenotypes van ondervoeding te onderscheiden en deze te vergelijken met antropometrische categorieën van ernstige ondervoeding.

Een multi-centrisch cohortonderzoek werd uitgevoerd bij ongecompliceerde, niet-oedemateuze kinderen met SAM in Bangladesh, Burkina Faso en Liberia. Een reeks klinische en biochemische indicatoren van gezondheid en voedingsstatus werden verzameld bij opname.

Kinderen met SAM vertonen klinische voedingstekorten en tekorten aan micronutriënten. Kinderen met alleen een lage WHZ, hebben ernstigere biochemische en klinische tekorten vergeleken deze met alleen een lage MUAC. Een lage WHZ moet derhalve behouden blijven als onafhankelijk diagnostisch criterium. Verder onderzoek is nodig om gemakkelijk kinderen met SAM en lage WHZ te identificeren.

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List of abbreviations

AGP: α-1-Acid Glycoprotein
APP: Acute Phase Protein
APR: Acute-Phase Response
ASF: Animal Source Foods
ASR: Absolute Synthesis Rate
AT: Adaptive Thermogenesis
BIA: Bio-electric Impedance Analysis
BIS: Body Iron Stores
BIVA: Bio-electric Impedance Vector Analysis
BMRC: Bangladesh Medical Research Council
CERS: Comité d'éthique pour la recherche en santé
CHW: Community Health Worker
CMAM: Community-Based Management of Acute Malnutrition
CRP: C-Reactive Protein
DGPLM: Direction Générale de la Pharmacie, du Médicament et des Laboratoire
ECF: Extracellular Fluid
EE: Environmental Enteropathy
F-100: F-100 therapeutic milk formula
F-75: F-75 therapeutic milk formula
FDR: False Discovery Rate
FFM: Fat-Free Mass
FFMI: Fat-Free Mass Index
FM: Fat Mass
FMI: Fat Mass Index
FSR: Fractional Synthesis Rate
GMP: Growth Monitoring and Promotion
GSH: Glutathione

H: Height

H^2: Height^2

H^2/Z: Impedance Index

HAZ: Height-for-Age Z-Score

ICC: Intraclass Correlation Coefficient

ICF: Intracellular Fluid

IDA: Iron Deficiency Anemia

IMCI: Integrated Management of Childhood Illness

IPT: Indice Poids-Taille

IQR: Interquartile Range

IV: Intravenous

IYCF: Infant and Young Child Feeding

MANGO: Modelling an Alternative Nutrition Protocol Generalizable for Outpatient

MAS: Malnutrition Aiguë Sévère

MGRS: Multicenter Growth Reference Study

MUAC: Mid-Upper Arm Circumference

NCD: Non-Communicable Disease

NERC: National Research Ethics Committee

ODK: Open Data Kit

ORS: Oral Rehydration Solution

OTP: Outpatient Therapeutic Feeding Program

PA: Phase Angle

PB: Périmètre Brachiale

PLB: Phospholipid Bilayer

R: Resistance

R/F Ratio: Serum Transferrin Receptor/Serum Ferritin

RBP: Retinol Binding Protein

RDT: Rapid Diagnostic Test

REE: Resting Energy Expenditure

RUTF: Ready-to-Use Therapeutic Food

SAM: Severe Acute Malnutrition

SC: Stabilization Center

SD: Standard Deviation

SDS: Standard Deviation Score, 121; Standard Deviation Scores

SIA: Stable Isotope Analysis

SIR: Stable Isotope Ratio

SMART: Standardized Monitoring and Assessment of Relief and Transitions
SSR: Sitting-Standing Ratio
SSRZ: Sitting-Standing Ratio Z-Score
sTfR: Soluble Transferrin Receptor
TBW: Total Body Water
TfR: Transferrin Receptor
UN: United Nations
UOR: Unadjusted Odds Ratio)
VAD: Vitamin A Deficiency
VADD: Vitamin A Deficiency Disorders
VAI: Vitamin A Insufficency
VIF: Variance Inflation Factor
V-PDB: Vienna Pee-Dee Belemnite
WHO: World Health Organization
WHZ: Weight-for-Height Z-score
Xc: Reactance
Z: Impedance

I General Introduction

1. Dissertation outline

The main chapters of this dissertation are as follows:

I. General Introduction

II. Alternative Indicators of Health and Nutrition Status

III. Methods

IV. Article 1: Clinical and biochemical markers of functional severity in severe acute malnutrition (SAM): comparative analysis of anthropometric phenotypes from the OptiDiag study

V. Article 2: Weaning and stunting affect nitrogen and carbon stable isotope natural abundances in the hair of young children

VI. Article 3: Preliminary stable isotope analyses in the hair of young children with SAM at admission and during nutritional rehabilitation

VII. Article 4: Preliminary bio-electric impedance (vector) analyses in young children with SAM at admission to nutritional rehabilitation

VIII. General Discussion

2. Malnutrition

2.1. Background

The first clinical description of malnutrition in the scientific literature dates back to 1926 in French Indochina where Léon Normet described a condition he called "La Boufissure d'Annam," (Annam swelling)[30] published alongside the following photo (**Figure 1**):[31]

Figure 1. Bouffisure d'Annam (Annam swelling)

Left, at hospital admission; middle, after resolution of edema and ascites; right, at recovery.

Source: Normet *et al.*, 1926.

Shortly thereafter, Jamaican physician Cicely Williams described a pediatric "deficiency disease" she observed in the hospital setting in present day Ghana.[32] Three years later Williams *et al.* employ the local Ga word "kwashiorkor" to describe a nutritional disease "the deposed baby gets when the next is born";[33] by 1938 she uses the word malnutrition.[34]

Since then, a more nuanced definition of malnutrition has evolved. Currently WHO defines the term as excesses or imbalances in energy and/or nutrient intakes.[35] Malnutrition covers two broad groups of conditions: (1) undernutrition, which includes stunting[1], wasting[2], and underweight[3] and micronutrient insufficiencies/deficiencies;[35] and (2) overweight (*i.e.*, obesity and diet-related non-communicable diseases (NCDs).[35] Undernutrition diagnosis can be based on anthropometric criteria and/or clinical characteristics (detailed in sections 2.3 *Anthropometric Definitions of Malnutrition* and 2.4 *Clinical Definitions of Malnutrition: Marasmus and Kwashiorkor*).

Today malnutrition contributes to almost half of all childhood deaths in the developing world.[36] The burden of malnutrition remains unacceptably high and progress towards alleviating it unacceptably slow.[37]

2.2. The Definition of Undernutrition: An Evolution

Anthropometry is a useful metric of body size that allows the comparison of an individual child to a growth reference derived from a healthy population.[38] Anthropometric indices have been widely used to determine health and nutritional risk, especially in children.[39] Anthropometry is relatively easy to measure, non-invasive, inexpensive, and does not require specialized technology.[40] The usefulness of any anthropometric index is based on simplicity of measurement, age-independence, and an accurate reflection of the severity and nature of malnutrition; yet, the ultimate value of an index depends on its capacity to identify children at high risk of adverse health consequences.[41]

Throughout history, auxologists have cycled through various anthropometric indices and references used to assess undernutrition. First, growth references have been progressively replaced, updated, and improved over time.[38] Second, the methods used to quantify the degree of deficit measured against these references, to classify severity, have evolved.[42] Because of this, prevalence estimates and severity cut-offs are inconsistent in the literature and make cross-comparisons over time difficult. Arguably, the only common thread to be traced across the history of undernutrition diagnosis is the recognition that some form of anthropometric index is useful to: (1) quantify the severity of malnutrition in the community; and (2) target beneficiaries for nutrition intervention.[42]

The story begins in the 1950s, when Thomson *et al.*, in their review of the diagnosis of malnutrition, concluded that no specific criteria existed.[43,44] Two years later, Mexican pediatrician Federico Gómez proposed three 'degrees' of malnutrition based on an index of weight-for-age,[45] and a reference drawn

1. Low height-for-age Z-score (HAZ)
2. Low weight-for-height Z-score (WHZ) and/or low mid-upper arm circumference (MUAC)
3. Low weight-for-age Z-score (WAZ)

from healthy Mexican children.[46] The Gómez classification became widely used to classify malnutrition both in hospital and community settings[46] and was soon redefined using the "Harvard" reference, and adopted by the Wellcome Trust.[47] Gómez never claimed a physiological or pathological basis for his classification.[42]

In the late 1960s, American pediatric nutritionists and couple Derrick and E. F. Patrice Jetliffe published on the potential use of mid-upper arm circumference (MUAC) as an anthropometric index for malnutrition.[48] In emergency situations (like famine or refugee crises) MUAC would sometimes be used as alternative indicator for nutrition status when weight and height could not be easily assessed; low MUAC, under a single cut-off value, would be substituted as a proxy.[15]

By the early 1970s, several attempts to refine anthropometric indices were presented. Researchers recognized the limits of Goméz's weight-for-age index because caretakers were often unable to estimate the age of their malnourished children, even to the nearest year; an age-independent index of weight-for-height was produced in 1971.[49] That same year, the United Nations (UN) expert committee on nutrition emphasized the need for a common definition for malnutrition.[50] A major change came the following year when British physiologist John Waterlow (and others)[44,51] began to criticize Gómez's weight-for-age index of malnutrition for blending two different conditions: (1) chronic malnutrition resulting in height-for-age deficit (what was called "stunting"); and (2) acute malnutrition resulting in weight-for-height deficit (what was called "wasting").[52] He proposed that "current" (*i.e.*, acute) malnutrition be classified using an index of weight-for-height.[53]

By the early 1980s, WHO published the first manual on the management of severe malnutrition; yet, in it the classification of malnutrition was unclear.[54] Throughout the 1980s anthropometric classifications continued to be criticized for being arbitrary, and having little to no physiological validity.[42,46]

During the 1990s WHO deemed the growth reference used since 1970 inadequate. The Multicenter Growth Reference Study (MGRS) was launched to produce a standard (not just a reference[4]) "for assessing the growth and development of children the world over."[56] WHO also updated the recommendations for the management of severe acute malnutrition and formalized the use of an index of weight-for-height for acute malnutrition.[57]

In 2006 the Child Growth Standards were released and are still in use today. That same year WHO commissioned a review of the methods used to detect severe acute malnutrition in the community; in it MUAC (<110 mm) was proposed to screen for acute malnutrition in the community, while weight-for-height was kept for monitoring during, and discharge from, therapeutic refeeding.[58] The UN

4. It is important to distinguish between reference values and standard.[46]. References differ from standards in that they provide a baseline for population comparison with no implication of normality or inference about the observed differences.[55] Standards, however, are elaborated to show how children should grow in all countries (*i.e.*, the standard derived from a healthy population living under conditions considered to be optimal for growth) and thus embrace a value judgement.[38,55,56]

formalized this recommendation the following year,[59] and increased the MUAC threshold (<115 mm) two years later.[60]

2.3. Anthropometric Definitions of Malnutrition

Based on current classification recommendations, childhood undernutrition comes in several anthropometric phenotypes, including stunting (low height-for-age), wasting (low weight-for-height and/or low mid-upper arm circumference, and underweight (low weight-for-age). Each of these phenotypes can be moderate (between –2 and –3 SD for *z*-scores; between 115-125 mm for MUAC) or severe (<–3 SD for *z*-scores; <115 mm for MUAC). Acute malnutrition is synonymous with wasting (and/or nutritional edema) and is associated with altered body composition; chronic malnutrition is synonymous with stunting, a reduction in linear growth.[61,62] Sometimes both occur simultaneously. Underweight is a composite indicator of both acute and/or chronic malnutrition, although it cannot distinguish between them.[55,63]

2.4. Clinical Definitions of Malnutrition: Marasmus and Kwashiorkor

Anthropometric indices are insufficient to define edematous malnutrition in children. Acute malnutrition presents as three clinical phenotypes: (1) non-edematous, acute malnutrition characterized by severe wasting (marasmus); (2) edematous, acute malnutrition (kwashiorkor), and (3) a combination of the two (marasmic-kwashiorkor).

Marasmus, or extreme emaciation, is a wasting syndrome without specific symptoms.[62] It occurs when adipose and muscle stores become depleted so as to sustain essential visceral function in the body.[64] Kwashiorkor, or edematous malnutrition, is a complex condition characterized by bilateral pitting edema, steatosis and diarrhea.[65–67] Marasmic-kwashiorkor combines edema with extreme wasting. Despite vastly different clinical presentation and outcomes, the treatment protocols used to manage marasmus, kwashiorkor and marasmatic-kwashiorkor are the same.[67]

The Definition of Severe Acute Malnutrition (SAM)

This dissertation will focus primarily on acute malnutrition, and severe acute malnutrition (SAM) in particular. SAM is associated with high mortality (~5-20 times higher than well-nourished children) and a primary focus of community based management of acute malnutrition (CMAM), detailed in section **5.4 Treatment**), which is a principal action of humanitarian aid.[59] WHO currently defines SAM as follows:[68]

- Weight-for-height Z-score (WHZ) ⩽–3; and/or
- MUAC <115 mm; and/or
- Presence of bilateral edema.

2.5. Diagnostic Discrepancy Between MUAC and WHZ

The earliest comparisons of MUAC and WHZ showed that the two indices correlate poorly and identify different children;[5,6] nothing has changed.[7,10–13,60,69] A colossal secondary analysis of survey data from 47 countries reported that, on average, only 16% of children with SAM present with both low MUAC and low WHZ; moreover both the magnitude and direction of diagnostic discrepancy varies dramatically across countries **Figure 2** reports the diagnosis of SAM by both MUAC and WHZ in 47 countries.[13]

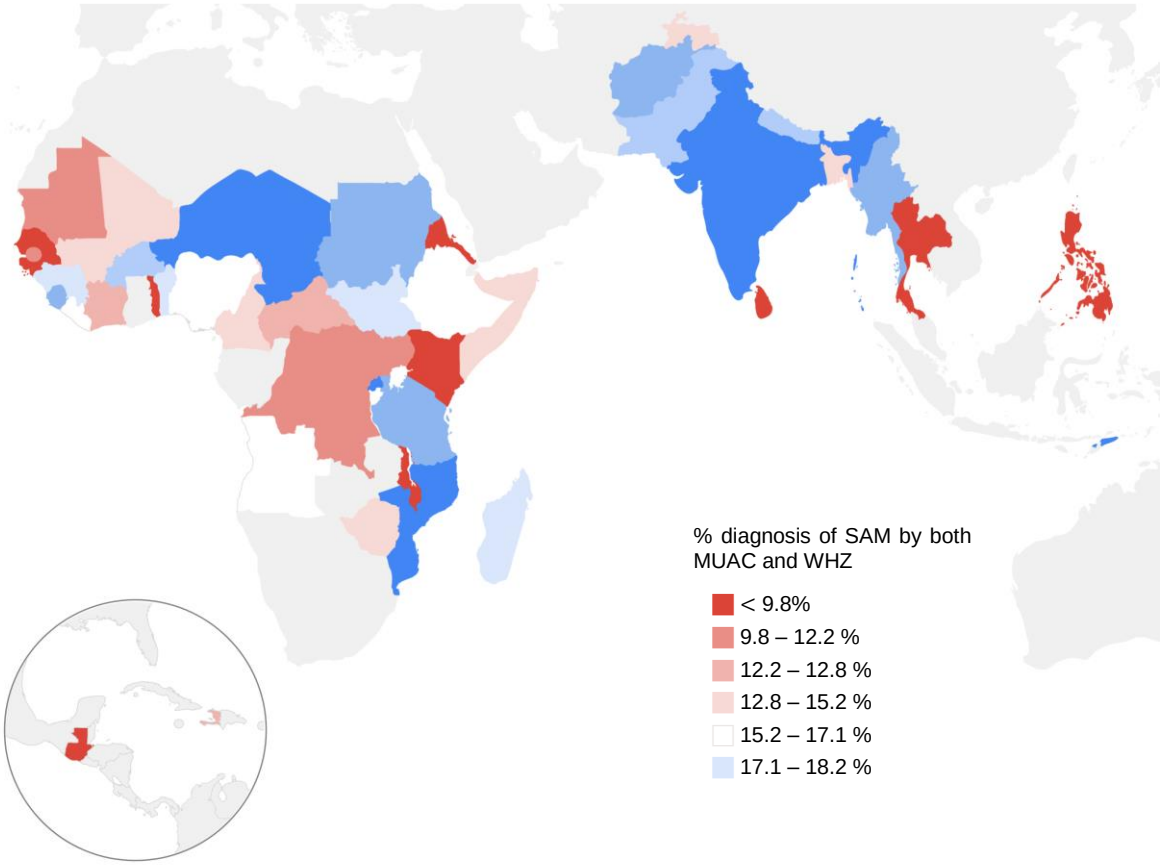

Figure 2. Diagnosis of SAM by both MUAC and WHZ for 47 countries
Adapted from: Grellety and Golden 2016.

Among others, two competing hypotheses have been put forward to explain this phenomenon.

A first hypothesis suggests that MUAC and WHZ index nutritional status differentially. Grellety *et al.* hypothesize that the two criteria are complementary, each selecting different children at increased risk of death.[13] Grijalva-Eternod *et al.* suggest that WHZ best reflects tissue mass whereas MUAC is a composite index of poor growth (combining stunting, wasting and low adiposity).[70]

A second hypothesis is that both MUAC and WHZ are imperfect anthropometric indices with limitations, each of which over- and underestimates acute malnutrition in specific situations. MUAC diagnosis uses a single, absolute cut-off point independent of age, height, and sex.[13] Because of this,

research suggests MUAC overestimates acute malnutrition in young children,[15,16,71] in girls,[7,12,18,72] and in stunting.[7,12,73] For the same reason, MUAC might underestimate malnutrition in older children. Some evidence suggests that older children are proportionally more affected in acute crisis contexts.[74] Use of MUAC-only in this context would therefore result in caseload underestimation during acute humanitarian crises—a phenomenon that was confirmed by recent field experiences in high caseload contexts.[75]

The assumption that MUAC is age and sex independent has been widely criticized and largely refuted. It can be traced back half a century to an observation, drawn from a sample of well-fed Polish children, that from birth to 5 years of age MUAC remains relatively stable, and differs only a few millimeters between boys and girls;[15,71,76] this is untrue.[7,11,12,71,73,77,78] Critics of MUAC suggest its power to predict mortality is biased by these links, particularly the link with age;[41,79–82] since mortality falls with age, its predictive power may be linked to an inherent increased risk of death in young children, and not in low MUAC in and of itself.[26] WHZ may overestimate acute malnutrition in children with low sitting-to-standing height ratio (SSR) observed in pastoralist communities;[83] these children have long legs, and thus lower WHZ because legs weigh less per unit length than the trunk.[26] Another hypothesis suggests that WHZ deficit mischaracterizes vulnerability[25] in Asian children that have a "thin-fat" phenotype (*i.e.*, small abdominal viscera, low muscle mass[84–86] and hidden adiposy[87] which provides them an energy reserve and survival advantage during nutritional deprivation.[87]

In light of this debate WHO and the scientific community have repeatedly called for formal investigation into the pathophysiology and functional severity associated with the anthropometric phenotypes of SAM—but little has been done so far, until now.

3. Determinants and Pathogenesis of Malnutrition

The determinants of SAM (and undernutrition in general) are multifactorial and synergistic; the UNICEF conceptual framework for malnutrition outlines a complex model linking factors across the causal hierarchy (**Figure 3**). These range from distal sociologic and political determinants to two main proximal determinants: (1) inadequate dietary intake; and (2) infectious disease.[88] Distal socioeconomic and political determinants act on proximal ones via various causal pathways, eventually leading to malnutrition (and death). Poverty, poor education, inadequate water, sanitation and hygiene, food insecurity, weak health systems, and environmental insults all contribute to both disease and insufficient energy and nutrient intake.[55]

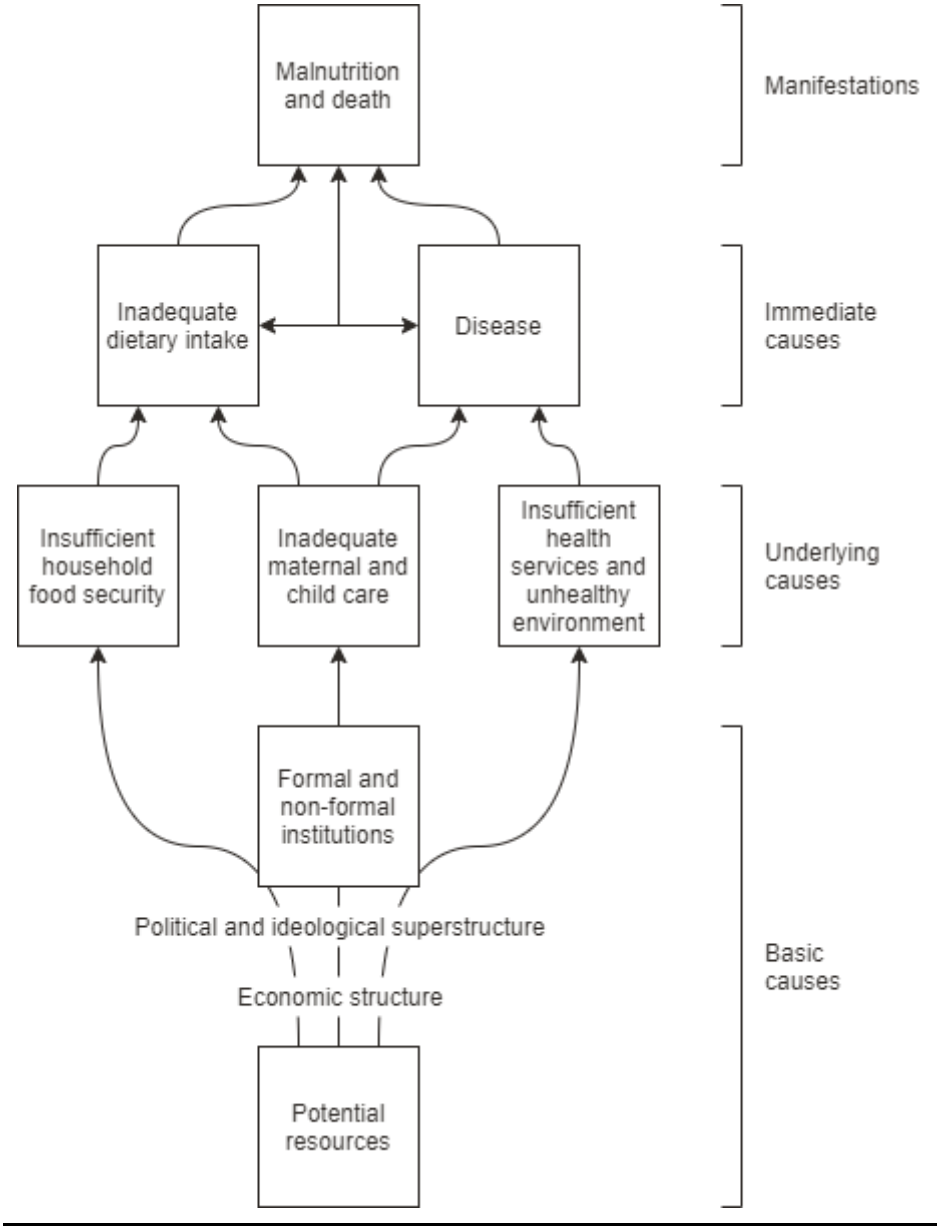

Figure 3. Causes of malnutrition and death
Adapted from: UNICEF 1991.

Recent work on the etiologic relationship between the acute (wasting) and chronic (stunting) forms of malnutrition suggests that stunting is a biological response to previous episodes of wasting.[89] Schoenbucher *et al.* hypothesize that linear growth slowing may represent a deleterious, internal adjustment to more overt episodes of malnutrition, by which limited resources are diverted to tissue accretion and away from growth.[89]

3.1. Malnutrition and Dietary Intake

It is well established that one primary cause of undernutrition is inadequate dietary intake.[61] Dietary intake refers to the energy, macronutrients and micronutrients required for healthy growth and development—a favorable diet is one that exerts the appropriate regulatory anabolic drive[90] to promote growth and provide the necessary substrates, all within an external environment that presents minimal inflammatory insults.[91] When these requirements are not met, linear and ponderal growth fail, leading to undernutrition. Million *et al.* describe the "impoverished," plant-based diet of a child with SAM as restricted to starchy, staple root crops, little meat, and non-starchy vegetables and fruits.[62] Of note is that western vegan children who eat similar diets, but that are complemented with protein and supplemented with limiting nutrients, exhibit near-normal linear growth.[91,92] But, interpreting studies of growth failure conducted in the developing world is challenging because it requires an untangling of the effects of nutritionally poor diets and the associated impoverished environment.[91]

Distinct dietary features have been associated with clinical phenotypes of SAM. The principal dietary characteristic leading to marasmus is low intake of all nutrients and energy in particular; the main dietary feature of kwashiorkor is imbalance, particularly in protein.[64] Contrary to popular belief, the protein:energy ratio requirement for young children is low (~5% energy).[91,93,94] Most cereal-based diets provide adequate protein, with the exception of some very low protein, starchy-root, staple diets (*e.g.*, plantain, cassava, taro, and sweet potato)[91] like those seen in kwashiorkor (along with oxidative stress[95] and infection[96]). Dietary protein intake in young children in the developing world generally exceeds protein requirements.[97] However, when breastmilk is no longer sufficient to meet the nutritional needs of the infant in and of itself, complementary foods must contain the energy (and protein) density to compensate. Indeed, Arsenault *et al.* highlight the importance of proper infant and young child feeding practices (IYCF), specifically during the transition from exclusive breastfeeding to family foods (typically from 6 to 18-24 months of age), when children are very vulnerable and the onset of malnutrition is likely to occur.[98]

Distinct dietary features linked to chronic (as opposed to acute) malnutrition have been reported as well. For example, serum concentrations of most amino acids (16/19) were low in stunted children without SAM,[99] particularly leucine, among other essential amino acids.[100] Moreover, observational studies have linked the consumption of animal source foods (ASF) to improved growth outcomes (adjusted for covariates including nutritional status).[101]

3.2. Malnutrition, Infection, and the Gut

The role of infectious disease in determining nutritional status has been closely examined for much of the 20th century.[55,102] A "vicious-cycle" between acute malnutrition and infection was first described in 1968 by Scrimshaw *et al.* and remains an important conceptual framework that has evolved and complexified over the years.[103–105] Malnutrition increases the risk of infection and infection itself contributes to malnutrition.[106]

In **Figure 4** Mata *et al.*[107] clearly illustrate the deleterious effect that repeated infections can have on growth in children, causing weight loss and limiting the opportunity for catch-up growth between infectious episodes.[108]

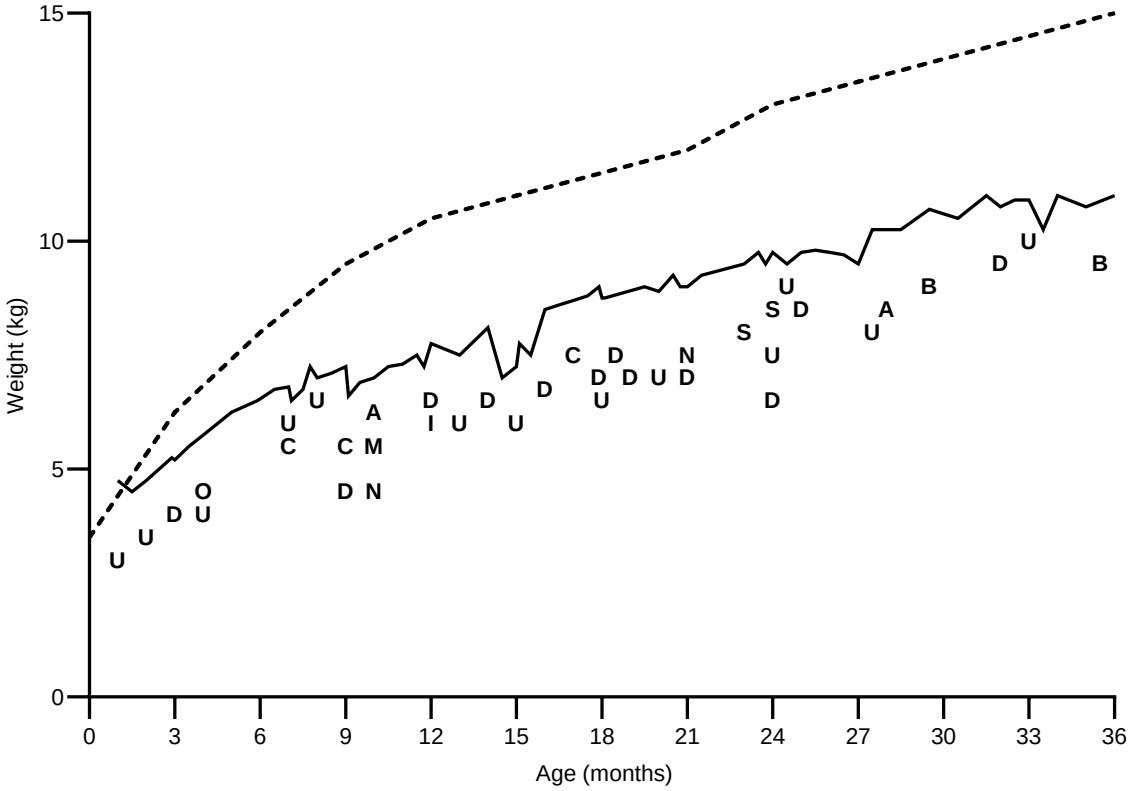

Figure 4. Repeated infection and weight gain in a Guatemalan child

The solid line is the weight of the child; the broken line indicates the median of the Jackson-Kelly standard curve. The letter symbols indicate individual episodes of Infectious disease. Note the frequency with which weight loss occurred after bouts of diarrhea and other infectious diseases.

Abbreviation: A, abscess; B, bronchitis; N, bronchopneumonia; C, conjunctivitis; D, diarrhea, I, impetigo, M, measles; S, stomatitis; O, oral thrush; U, upper respiratory tract infection.

Adapted from: Jackson and Kelly 1945; Mata *et al.*, 1977.

3.2.1. Infection Itself Contributes to Malnutrition

In response to infection, the immune system launches innate and acquired defense functions (*e.g.*, immune cell activation and propagation, DNA, RNA and protein synthesis) that consume anabolic energy.[106] Thus, severe infectious diseases in childhood (*e.g.*, measles, diarrhea, pneumonia, meningitis and malaria) can cause wasting.[36] Episodes of infection potentiate undernutrition by way of anorexia, nutrient diversion to inflammatory responses and tissue repair, nutrient loss, and reduced absorption of nutrients. Inflammatory cascade activation leads to a reduction in appetite and loss of both lean tissue and fat.[105] Infection is linked to net protein loss and amino acid diversion to acute

phase and immune response proteins.[105] Gastrointestinal infection and diarrhea can result in a significant loss of protein, vitamin A, zinc, and other micronutrients and is linked to malabsorption.[104] Chronic infection (*e.g.*, HIV/AIDS and tuberculosis) can cause wasting and anemia,[106] and intestinal parasites can cause anemia and nutrition deprivation.[103,106] Acute mortality in undernutrition has been attributed in large part to an increased risk of death from common infectious diseases like pneumonia, diarrhea, and bacterial sepsis.[105]

The contribution of persistent, low-grade infection to undernutrition has drawn significant interest. Environmental enteropathy (EE) is an acquired condition of the small intestine in which prolonged fecal-oral exposure to enteropathogens leads to a persistent acute phase response and chronic inflammation.[109–111] Environmental enteropathy can be characterized by: villus shortening, crypt hyperplasia, and macro- and micronutrient malabsorption,[109,112] intestinal leakage and permeability[113,114]—leading to the translocation of microbes and systemic immune infection.[111] Together, chronic inflammation and malabsorption are the two key mechanisms by which EE is thought to contribute to undernutrition.[111,115,116]

3.2.2. Malnutrition Increases the Risk of Infection

Moreover poor nutrition increases susceptibility to infectious disease, disrupting immunological function and initiating metabolic responses that further compromise nutritional status,[102] particularly in children[64,117,118] in low-income countries.[106] For example, severe malnutrition in early life affects the development of the thymus, reducing peripheral lymphocyte count, compromising immunity[119] and increasing susceptibility to opportunistic infection;[106] this has been termed nutritionally acquired immunodeficiency syndrome.[120]

3.3. Inflammation

Infection and malnutrition are inextricably linked. Malnutrition is the main cause of immunodeficiency globally whereas parasitic infection leads to malnutrition [121]—and so begins the 'vicious cycle' of nutrition and infection, described previously in section *3.2 Malnutrition, Infection, and the Gut*.

Inflammation refers to a series of complex physiological reactions launched by the host to stem ongoing tissue damage, locate and destroy the infective pathogen(s), and lastly being the repair process [122].

3.3.1. Acute Phase Response (APR) and Acute Phase Proteins (APP)

The early and immediate set of inflammatory reactions is termed the acute phase response (APR).[122] A hallmark of this response is the decreased synthesis of negative acute-phase proteins (APP) (*e.g.*, retinol binding protein, among others) to redirect amino acids for the rapid synthesis of positive acute-phase proteins (*e.g.*, C-reactive protein),[123] which aim to control infection and resolve tissue damage.[124] Acute phase proteins are useful in detecting inflammation in the absence of clinical symptoms and in the monitoring of infection severity.[125]

C-Reactive Protein (CRP)

C-reactive protein (CRP) is a non-specific, positive acute-phase reactant that plays an integral part in the innate immune system. Synthesized in the liver, CRP has a major role in the identification and elimination of foreign pathogens via complement binding for macrophage phagocytosis.[126] It is also thought to clear necrotic tissue and apoptotic cells.[127] CRP concentrations in serum increase within mere hours,[128] plateau within a few days[129] at or around the appearance of clinical symptoms,[125] before finally decreasing to normal a few days after the proinflammatory stimulus is eliminated.[130]

α-1-Acid Glycoprotein (AGP)

Like CRP, α-1-acid glycoprotein (AGP) is also a positive acute-phase reactant synthesized in the liver. It acts as a carrier, although its precise role is yet to be defined.[131] But unlike CRP, AGP is a moderate acute-phase protein that increases more slowly and remains elevated for longer, (as opposed to major APPs that increase rapidly[131]). AGP does not fall as clinical symptoms subside but remains elevated during convalescence.[125,132] The characteristic delay in synthesis and decay between CRP and AGP is used to derive correction factors to adjust micronutrient analyses in the presence of subclinical inflammation (*i.e.*, ferritin and retinol binding protein) detailed in the sections on *Ferritin* and *Biomarker of Vitamin A Status: Retinol Binding Protein (RBP)*.

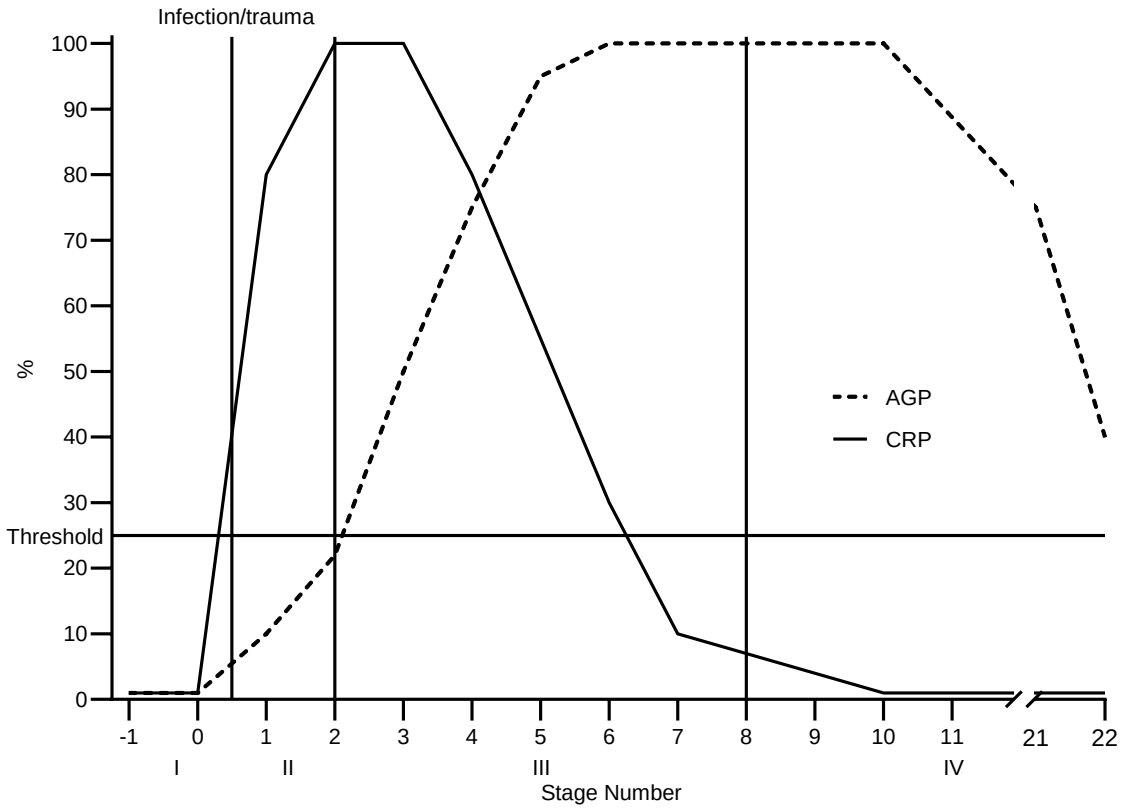

Figure 5. Model of response by C-reactive protein (CRP) and α-1-Acid Glycoprotein (AGP) after an inflammatory stimulus to show methods of categorizing inflammation groups

I, reference; II, incubation; III, early convalescence; IV, late convalescence.

Adapted from: Thurnham *et al.*, 2013.

3.4. Dehydration and diarrhea

Dehydration and diarrhea are also inextricably linked. Severe diarrhea with complications is most common in malnutrition; diarrhea can cause malnutrition, or further aggravate existing malnutrition, due to profuse loss of nutrients from the body, or by diversion of nutrients for tissue repair rather than for growth.[133] Indeed, the two main dangers of diarrhea are death and malnutrition. Death from acute diarrhea is caused by dehydration, *i.e.*, an extreme, acute loss of body water and salt.[133] A healthy bowel absorbs the necessary water and salt into the bloodstream. In the presence of diarrhea, water and salt are diverted from the bloodstream into the stool resulting in larger than normal losses of water and salt from the body, leading to dehydration.[133] Dehydration is treated using an oral rehydration solution (ORS) or intravenous (IV) fluids for severe cases.

Dehydration from diarrhea is common in children with SAM[134] and its management has been controversial.[135] Furthermore, some of the standard clinical features used to assess dehydration are unreliable in children with SAM because reduced skin turgor and sunken eyes are features of malnutrition itself.[134] Dehydration is classified according to WHO recommendations, outlined in **Table 1.**

Table 1. Classification table for dehydration

Classification	Clinical signs
Severe dehydration	Two of the following signs: • Lethargic or unconscious • Sunken eyes • Not able to drink or drinking poorly • Skin pinch goes back very slowly (⩾2 seconds)
Some dehydration	Two of the following signs: • Restless, irritable • Sunken eyes • Drinks eagerly, thirsty • Skin pinch goes back slowly (<2 seconds)
No dehydration	Not enough signs to classify as some or severe dehydration

Dehydration can occur as a complication of gastroenteritis[136,137] as well as severe malaria,[138,139] among other conditions.[7] Berkely *et al.* suggest that severe dehydration could reduce WHZ, but found that dehydration does not have a significant confounding effect (in the hospital setting in Kenya).[7]

3.5. Visible Severe Wasting

Visible severe wasting is defined as the presence of muscle wasting in the gluteal region, loss of subcutaneous fat, and/or the prominence of bony structures, particularly over the thorax.[140] Until 2013, it was a standalone criterion used to diagnose SAM.[59,68] In children, it has been linked to very high short-term mortality.[141] Indeed, an inpatient evaluation of the prognostic performance of visible severe wasting for SAM reported that children with both were at higher risk of death than SAM children without.[140] The WHO classification for visible severe wasting is shown in **Table 2**, an illustration for which is shown in **Figure 6**.

Table 2. Classification of visible severe wasting in severe acute malnutrition

	Front view	Back view
Severe wasting	• Ribs are easily seen • Skin on upper arms look loose • Skin on things look loose	• Ribs and shoulders are easily seen • Flesh is missing from the buttocks
Extreme wasting	• Folds of skin on buttocks and thighs • Looks as if the child is wearing 'baggy pants'	

Source: WHO 2002.

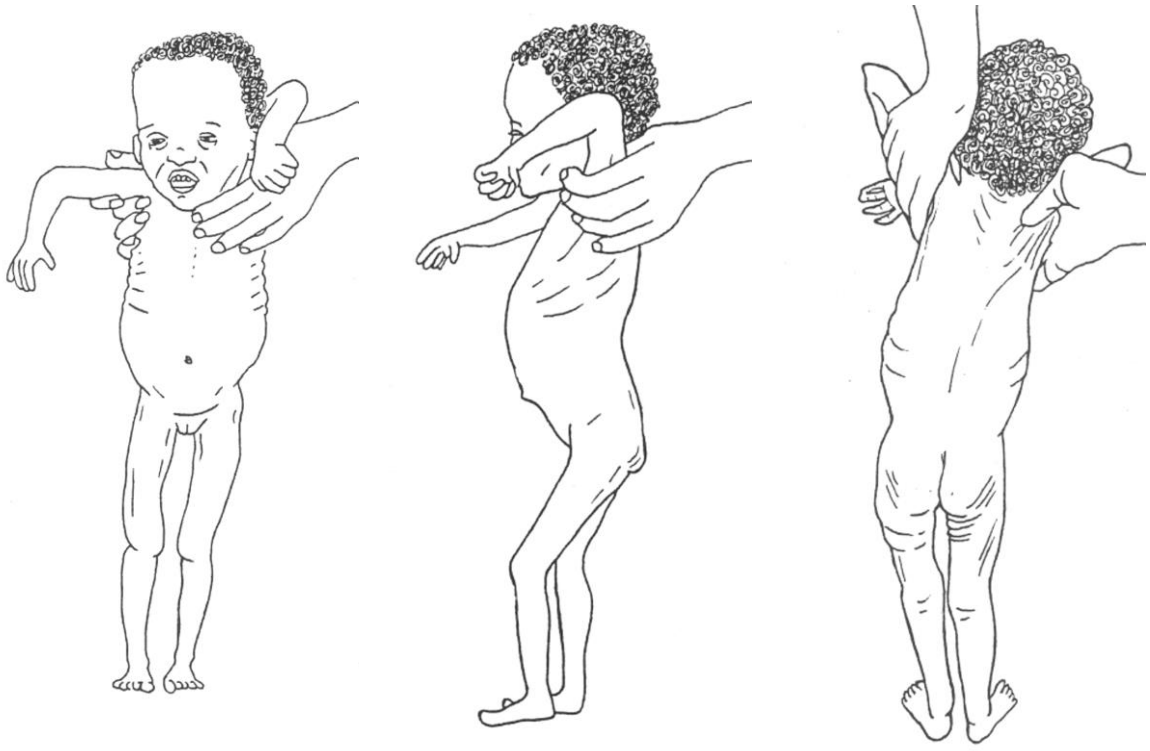

Figure 6. Illustration of visible severe wasting in children

Source: WHO 2002.

3.6. Edema

Even today, the pathogenic factors that, in response to food deprivation, lead to divergent clinical outcomes, in particular the development of kwashiorkor (compared to marasmus), are poorly understood. Yet in recent years, two promising hypotheses have crystallized. First, the rate of protein breakdown in kwashiorkor is slower, suggesting a reduced supply of the amino acids required for both nutrient transportation and the acute phase response to infection.[142] Second, children with edematous malnutrition have lower blood levels of the intracellular antioxidant glutathione (GSH) (**Figure 7**). With less GSH, these children have weakened antioxidant defense mechanisms and are less able to remove the oxidant load caused by infections and noxious stimuli;[143] free radicals cause damage to cellular membranes resulting in the clinical manifestation of kwashiorkor.[142]

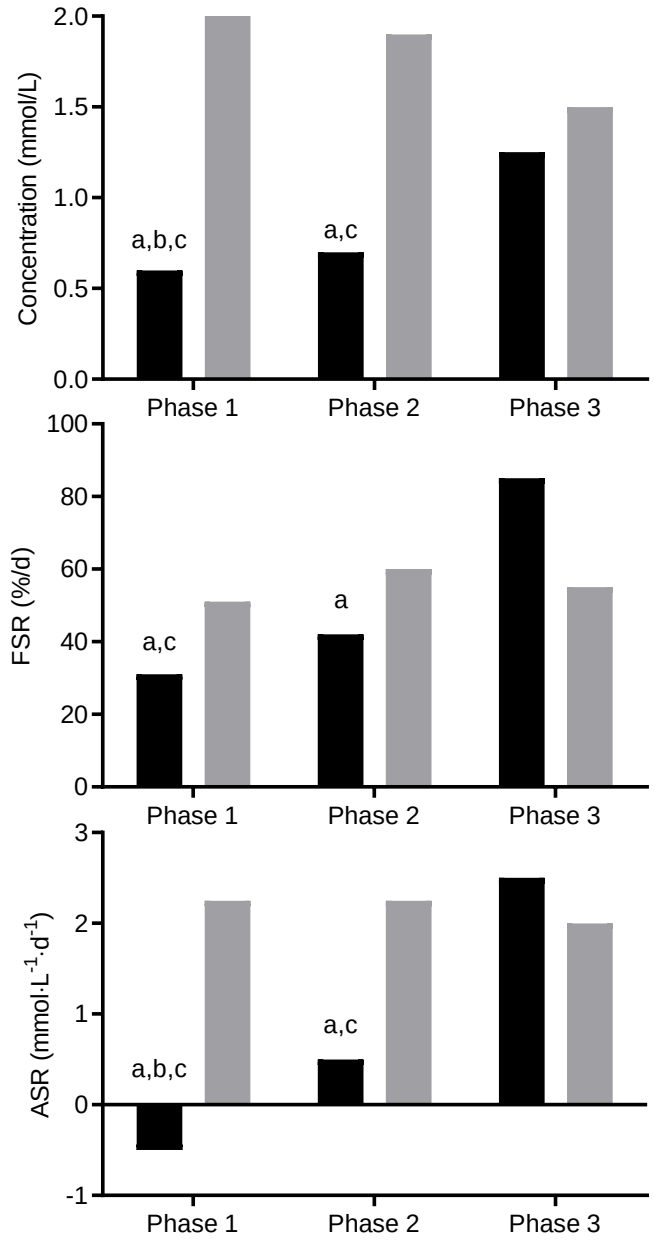

Figure 7. Erythrocyte glutathione concentration, fractional synthesis rate (FSR), and absolute synthesis rate (ASR)

Data are from in children with nonedematous (n=7) and edematous (n=7) SAM during the period of malnourishment and infection (Phase 1), at the time infections were cleared and edema had resolved (Phase 2), and after complete recovery (Phase 3).

Values are means SEM. a, significantly different from Phase 3 value, p<0.05; b, significantly different from Phase 2 value, p<0.05; c, significantly different from the same-phase value of nonedematous group, p<0.05.

■ edematous; ■ non-edematous.

Adapted from: Reid *et al.***,** 2000; Jahoor *et al*., 2012.

4. Micronutrients

4.1. Micronutrient Deficiencies

Micronutrient deficiencies pose a major public health problem affecting millions of children and women (of reproductive age), primarily in low-income countries. In children, they are linked to an increased prevalence of infectious disease and poor cognitive development.[144] Micronutrient deficiencies help sustain the intergenerational cycle of malnutrition[145] by way of hampered reproductive function and reduced fetal development in women,[36] leading to impaired growth and poor school achievement,[144] and so on and so forth…

4.1.1. Vitamin A

Vitamin A is a generic term that refers to a group of fat-soluble compounds including retinol (retinal, retonic acid) and several provitamin A carotenoids (*e.g.*, beta-carotene). Retinol is found in liver, egg yolk and dairy fats.[146]. Carotenoids, which are converted to retinol in the gut, are found in orange fruits (*e.g.*, papaya, mango), green-leafy vegetables and some unrefined oil (*e.g.*, red palm oil).[147] However, carotenoids are less biologically active than retinol—as it has been proposed that 4 to more than 20 µg carotenoids (depending of the food matrix) are required to synthetize 1 µg of retinol.[146]

Vitamin A Deficiency: Epidemiology

Vitamin A deficiency (VAD) is major public health challenge that significantly contributes to the global burden of disease, particularly in impoverished rural communities in Asia and Africa. Various estimates indicate that 190 million preschool age children had low serum retinol,[148] of which 4.4 million present with severe ocular manifestations.[149] Together, Vitamin A deficiency disorders (VADD) (*i.e.*, health disorders attributed to vitamin A deficiency) are estimated to account for 6.5% of deaths in children under five globally.[150]

Vitamin A: Physiology

Vitamin A plays a crucial role in the growth, regulation, and specialization of nearly all cells in the human body. A lack thereof will inevitably influence various bodily functions. Vitamin A, in acid form (*i.e.*, retonic acid) binds to nuclear receptors, regulating gene expression and cellular differentiation.[147] In vitamin A deficiency, mucosal epithelial cells (*e.g.*, in the eyes, respiratory, and urinary tracts) are replaced by keratinized, squamous epithelium—a process called keratinizing metaplasis. The conjunctiva become dry; the ciliated cells in the respiratory tract are lost, increasing respiratory disease;[151] and, the keratinized urinary tract becomes a culture medium for bacterial

growth and development.[147,152] A vicious cycle of micronutrient deficiencies, nutritionally related immunosuppression, and oxidative stress limit immune defenses—particularly to viral infection (*e.g.*, measles).[153]

Vitamin A Deficiency: Causation

Vitamin A and carotenoid liver stores provide an important buffer to mitigate the effect of variations in intake and use, but the availability of vitamin A stores largely depends on nutritional status.[146] Children with marginal vitamin A intake (and finite stores) are more susceptible to liver store depletion from impaired absorption (*i.e.*, gastroenteritis), increased metabolic demand (*i.e.*, fever or measles), or diet changes.[146]

Vitamin A Deficiency: Adverse Effects

Vitamin A deficiency disorders increase the risk of infectious disease[154] and associated mobidity and mortality[155] and cause xerophthalmia—the leading preventable cause of childhood blindness.[156]

Over the past 30 years, data gathered have led to a major shift in understanding regarding vitamin A deficiency as a public health problem. Formerly, the frequency and severity of vitamin A deficiency was determined based solely on ocular manifestations (*i.e.*, xerophthalmia).[147] Today, we know that systemic clinical manifestations preclude ocular ones, including: impaired iron utilization (*i.e.*, exacerbating anemia); reduced resistance to infection (*i.e.*, increasing severity and case-fatality linked to infectious disease); and growth retardation[147] (**Figure 8**).

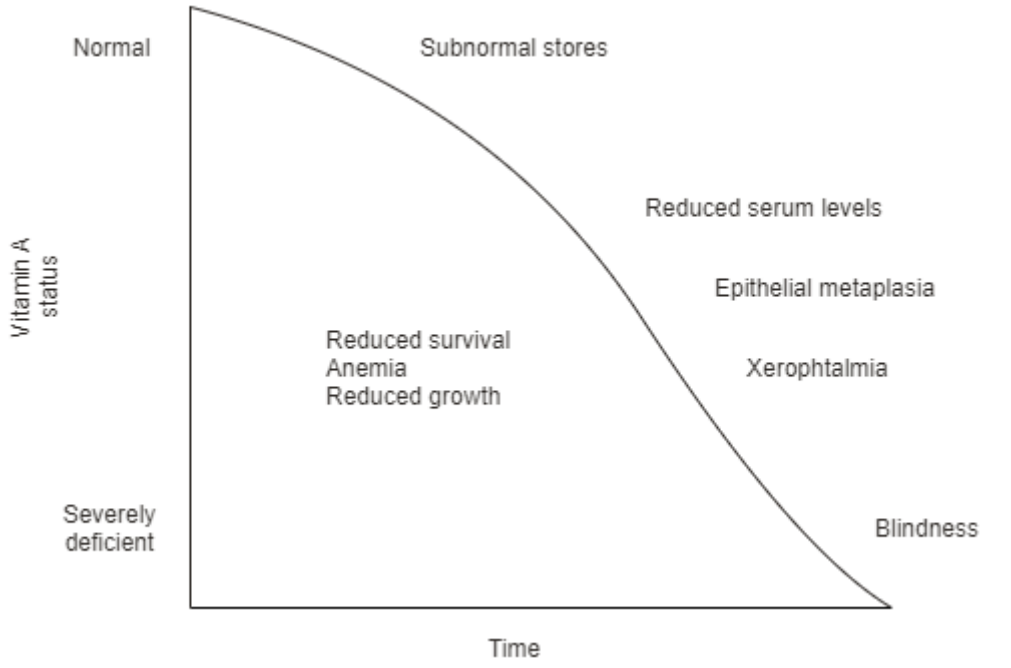

Figure 8. Manifestations of vitamin A deficiency
Adapated from: Sommer *et al.*, 1995.

Ocular manifestations of vitamin A deficiency (and an associated heightened severity of infectious disease) have had devastating effects on children in developing nations.[146] While widespread vitamin A supplementation has significantly stemmed the problem[146] clinical presentations associated with vitamin A deficiency still persist in low-income countries today.[157] Clinical classifications of xerophalmia are reported in **Table 3.**

Table 3. Classification of xerophthalmia

Code	Classification
XN	Night blindness
X1A	Conjunctival xerosis
X1B	Bitot's spots
X2	Corneal xerosis
X3A	Corneal ulceration/keratomatacia <1/3 corneal surface
X3B	Corneal ulceration/keratomatacia ⩾1/3 corneal surface
XS	Corneal scar
XF	Xerophthalmic fundus

Source Sommer *et al.*, 1995.

Biomarker of Vitamin A Status: Retinol Binding Protein (RBP)

Serum retinol and retinol binding protein are the two biochemical indicators currently recommended to determine vitamin A status. Both occur in a 1:1 complex and therefore can be substituted for one another. Compared to retinol, assessment of RBP is easier, less expensive, and requires a very small amount of serum that can be obtained from a finger prick.[158] Because RBP (and retinol) are homeostatically controlled,[158] circulating levels reflect liver vitamin A stores only when they are severely depleted[159] (<0.07 µmol/g liver) or extremely high (>1.05 µmol/g liver).

The serum retinol threshold values for vitamin A deficiency (VAD) and vitamin A insufficiency (VAI) are <0.07 µmol/L[155] and <1.05 µmol/L,[160] respectively. No standard cut-off for RBP is defined therefore investigators often apply the retinol cut-offs to RBP.[161]

RBP is affected by the acute-phase response, leading to potential misinterpretations of vitamin A deficiency in the presence of infection and inflammation.[162] RBP a negative acute phase protein, decreasing in concentration in response to inflammation due to decreased synthesis.[160] During the acute phase response, negative acute phase protein synthesis in the liver (*e.g.*, RBP) is reduced to compensate the increased synthesis of positive acute phase proteins (*e.g.*, C-reactive protein,) to maintain homeostasis in plasma protein concentration.[163] Thus, a correction factor is applied to RBP to accurately quantify vitamin A deficiency in the presence of inflammation.[160]

4.1.2. Iron

Iron is an essential bioelement for most living organisms. It comes in two forms, heme and nonheme (*i.e.*, inorganic).[164] Nonheme dietary iron is found in plants and iron-fortified foods whereas both heme and nonheme iron are found in meat, seafood and poultry.[165]

Iron Deficiency: Epidemiology

Human iron status varies across a continuum from iron deficiency with anemia (IDA) to iron deficiency (ID) without anemia, to normal iron status with iron reserves of various size.[166]

Iron deficiency is one of the leading risk factors for disability and death worldwide.[167] Iron deficiency leads to substantial economic losses by way of cognitive losses in childhood and productivity losses in adults[168]—and Africa and Asia bear 71% of the total global mortality burden.[169] WHO estimates that 39% of children under five in developing countries are anemic,[166] half of which have iron deficiency anemia.[167] A pooled, economic analysis of iron deficiency across ten developing nations estimates that the median productivity loss per year per head is $0.32 or 0.75% of gross domestic product.[168]

Iron Deficiency: Physiology

In the human body, iron is critical to oxygen transport and use, among a number of other diverse cellular functions. Transport proteins (*e.g.*, hemoglobin and myoglobin), heme enzymes and other oxidation-reduction enzymes require iron for in their activity.[170] Iron losses occur as a consequence

of epithelial renewal and blood loss. Iron homeostasis is maintained by way of tight regulation of absorption[170] in the proximal small intestine.[167]

Iron Deficiency: Causation

Nutritional iron deficiency occurs when dietary iron absorption does not meet physiological needs. Bioavailability of dietary iron is low in populations that consume plant-based diets with little meat, as is the case in many developing countries.[171] Approximately 25% of iron found in meat (primarily heme iron) is absorbed into the body. Yet, less 10% of iron in plant products (primarily non-heme iron) is absorbed.[171,172] The dietary iron in plant-based diets commonly consumed in low-income countries is mostly non-heme iron.[171,172]

Because dietary iron intake correlates with overall energy intake,[173] deficiency occurs when iron requirements outweigh energy requirements, like in infants and young children.[167] Rapid growth and development in infancy quickly exhausts the iron stores accumulated during gestation, leading to deficiency if weaning foods cannot compensate for these losses.[167] Common plant-based weaning foods in developing countries are rarely iron fortified, and in these countries more than 50% of children under 4 are anemic.[166]

Iron Deficiency and Iron Deficiency Anemia: Adverse Effects

The high prevalence of iron deficiency in low-income countries has considerable health and economic costs. Iron deficiency anemia in pregnant women increases the risk of preterm labor, low birthweight, infant mortality, and iron deficiency in their babies.[167,174,175] Several studies suggest that iron deficiency has adverse effects on cognitive and motor development[167] that are only partly reversible,[176,177] and decreases performance in school-aged children.[177] However, the data are equivocal because environmental factors limit their interpretation.[167,176–178]

Ferritin

Ferritin is the iron storage molecule of the body, located predominantly in the reticuloendothelial cells and hepatocytes, the two predominantly contributing cell types to the plasma ferritin pool.[179] Plasma ferritin variations mirror iron balance in the body (**Figure 9**).

The serum ferritin threshold value for depleted iron stores is <12.0 µg/L for children less than 5 years of age, and <30.0 µg/L in the presence of infection.[180]

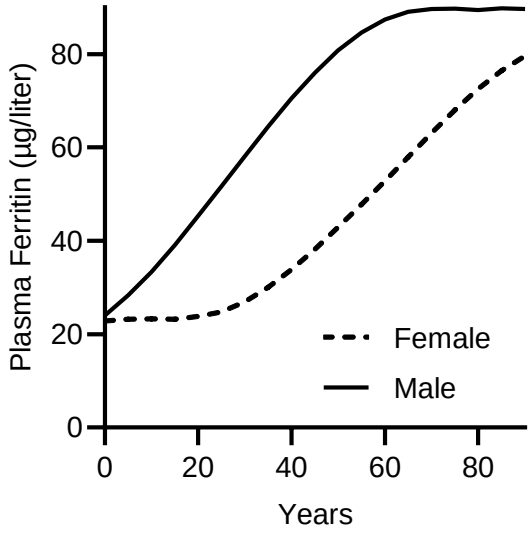

Figure 9. Mean ferritin concentration as a function of age

Adapted from: Finch *et al.*, 1986.

Although WHO recommends serum ferritin as the best indicator of iron deficiency[181] it is also a positive acute-phase protein (APP) that is elevated in the presence of infection or inflammation, and therefore requires adjustment.

In inflammation, plasma iron concentrations decrease and plasma ferritin levels (and ferritin synthesis in reticuloendothelial cells) increase.[182] Investigators hypothesize that the mechanism behind this hypoferraemic-hyperferritinaemic response to inflammation is an integral part of the inflammatory response, whereby iron is deviated into ferritin and not released in the plasma.[182] Indeed, virtually all human pathogens require iron. Because of this, iron distribution regulation is a key component of the innate immune mechanism against invading pathogens; several elements of human iron metabolism block it from use by pathogenic microorganisms.[183] Yet, both the physiological function, and the source (*i.e.*, from damaged cells or actively secreted by regulatory mechanisms), of increased serum ferritin in inflammation are yet to be fully defined.[184] Regardless of the mechanism of action, this poses a major diagnostic challenge in developing countries where the frequency of infection is high.

As is the case with retinol binding protein, a correction factor is applied to ferritin to accurately quantify iron deficiency in the presence of inflammation.[181]

Soluble Transferrin Receptor (sTfR)

Iron transport in plasma is mediated by transferrin, before being donated to cells by way of the transferrin receptor (TfR).[185] The soluble form of TfR (sTfR) in found in serum. TfR density on the surface of proliferating cells is related to iron availability: iron deprivation promotes TfR synthesis

whereas excess iron suppresses it.[185] Because sTfR measured in serum is proportional to the mass of cellular TfR, sTfR is a valuable marker of mild tissue iron deficiency[185] of recent onset.[186]

No international standard threshold value exists to diagnose iron deficiency using sTfR, and WHO recommends the application of cut-off values recommended by the assay manufacturer [187]. However, the test kit reference sTfR threshold value for iron deficiency is typically >8.3 mg/L.[188,189]

Unlike RBP and ferritin, sTfR levels are not increased in inflammation[185] and are thus not corrected for.

Body Iron Stores (BIS)

Elevated sTfR levels characterize functional iron deficiency (*i.e.*, tissue iron deficiency despite adequate iron stores)[185] whereas low ferritin levels reflect depleted iron stores. Due to a reciprocal relationship between sTfR and ferritin, the ratio of sTfR/ferritin (R/F ratio) describes a log-linear relationship[186] of iron availability over a wide range of stores.[185] Body iron stores (BIS) are calculated based on the R/F ratio using Cook's method[190] as follows:

$$\text{Body Iron Stores (mg/kg)} = -[\log(\text{R/F ratio}) - 2.8229] / 0.1207$$

Body iron stores are expressed in either iron surplus in stores *(i.e.*, a positive value) or iron deficit in tissues (*i.e.*, a negative value).[190]

5. Burden, risks, and management

5.1. Global Burden of Malnutrition

The 2018 Global Nutrition Report clearly states that "the problem of malnutrition remains severe [and] the world is not on track to achieve the targets it has set itself."[37] The most recent estimates report that 49.5 million children under five years of age are wasted (7.3% of the total population), 16.6 million severely so.[29] More than half of all wasted children (30.2 million) live in Southern/South-eastern Asia and 14 million in Africa.[29] These estimates draw from incomplete prevalence data based on WHZ only (excluding children with low MUAC-only and/or edema), and the actual prevalence is much higher than this estimated burden.[1]

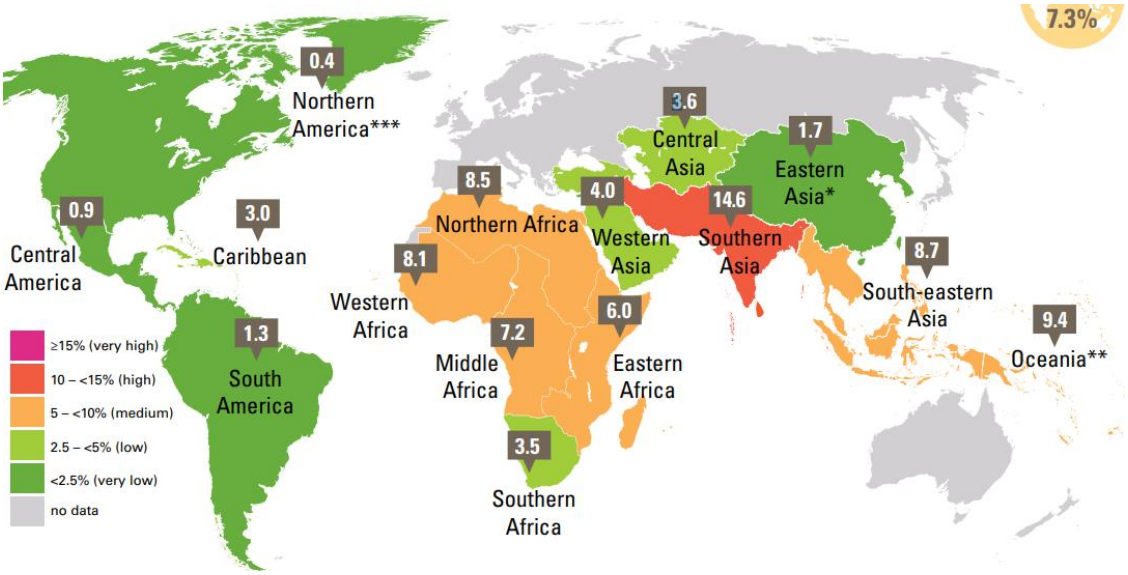

Figure 10. Percentage of wasted children under five by United Nations sub-region 2018
Source: UNICEF *et al.* 2019.

Moreover 149 million (21.9%) children under five years are moderately stunted.[29] Global stunting prevalence has slowly but steadily declined, falling from 32.6% in 2000 to 22.2% in 2017.[37]

5.2. Risks: Mortality and Morbidity

Undernutrition results in substantial increased mortality and morbidity (*e.g.*, anemia, diarrhea, pneumonia, etc.). The 2008 *Lancet Series* on maternal and child undernutrition estimated that SAM was responsible for ~450,000 deaths (4.6% of worldwide deaths in children under five). A second series published in 2013 considered a broader definition of undernutrition (including stunting, wasting, and micronutrient deficiencies)—together estimated to underlie nearly 3.1 million child deaths annually.[191]

5.3. Consequences (Short-, Mid-, and Long-Term Outcomes)

Children admitted to hospital with complicated SAM are discharged upon the resolution of their complications and continue therapeutic feeding in the community.[67] However, the data indicate that mortality is high in post-discharge SAM patients following hospitalization for complications.[67,192–194] Kerac *et al.* present the most comprehensive evaluation of growth and mortality in children with SAM (FuSAM study) and report that, in Malawi, 42% of children with SAM admitted for treatment died within 12 months of follow-up, and 44% of these deaths occurred after discharge from follow-up.[67,193]

The deleterious consequence of SAM persist in the mid- and long-term as well. Lelijveld *et al.* showed that 7 years after discharge (ChroSAM study), formerly SAM children had poorer growth, body composition, and physical function (all of which are indicators of future cardiovascular and metabolic disease) compared to their siblings and community controls.[194] Moreover, a recent retrospective analysis suggests that stunting is an adaptive (and deleterious) biological response to repeated episodes of wasting by which the effects of acute malnutrition are reabsorbed at the cost of linear growth slowing.[89] Undernutrition in childhood has been linked to high glucose concentrations, high blood pressure, harmful lipid profiles, impaired cognitive development, metabolic disorders, suboptimal economic productivity, and maybe even mental illness in adulthood.[36,195]

5.4. Treatment

Treatment of childhood undernutrition occupies a unique position between clinical medicine and public health intervention.[196] The gold standard service delivery model for SAM is community-based management of acute malnutrition (CMAM). This model provides a framework for an integrated public-health response.[196] Treatment guidelines distinguish two groups: (1) children with uncomplicated SAM who are managed in the community and; (2) SAM children with medical complications who are hospitalized and undergo initial stabilization (during which acute medical conditions are managed[197]), followed by nutritional rehabilitation.[67] During the stabilization phase, children with complicated SAM are cautiously refed using the milk-based starter formula F-75 (75 kcal/100 mL and 0.9 g protein/100 mL), due to their fragile physiological state and reduced homeostatic capacity. However, during the rehabilitation phase, a vigorous refeeding approach using F-100 therapeutic milk (100 kcal/100 mL and 2.9 g protein/100 mL) is used to ensure rapid weight gain (>10 g gain/kg/day) for catch-up growth.[197]

Children with uncomplicated SAM receive take-home rations (200 kcal/kg/day, **Table 4**) of ready-to-use therapeutic food (RUTF), a course of broad-spectrum antibiotics, vitamin A, folic acid, anthelminthics, and antimalarials (if appropriate).[196]

Table 4. Nutritional composition for key therapeutic products used in the management of severe acute malnutrition*

	F-75 (100 mL)	F-100 (100 mL)	RUTF (92 g)
Energy	77 kcal	100 kcal	500 kcal
Proteins	5 %	11 %	128 g
Lipids	29.9 %	51 %	30.3 g
Carbohydrates	65 %	38 %	45 g
Calcium	77 mg	76 mg	302 mg
Phosphorus	77 mg	76 mg	343 mg
Potassium	142 mg	228 mg	1,171 mg
Magnesium	9.5 mg	21 mg	80 mg
Zinc	2.5 mg	2.4 mg	11.8 mg
Copper	0.27 mg	0.3 mg	1.5 mg
Iron	< 0.06 mg	< 0.07 mg	10.3 mg
Iodine	17.9 µg	20 µg	98 µg
Selenium	5.1 µg	5.7 µg	28 µg
Sodium	< 17 mg	< 56 mg	165 mg
Vitamin A	0.14 – 0.3 mg	0.15 – 0.3 mg	0.79 mg
Vitamin D	2.6 – 4.9 µg	2.9 – 5.7 µg	14 µg
Vitamin E	3.4 – 4.9 mg	3.8 – 5.7 mg	18.4 mg
Vitamin C	> 8.5 mg	9.5 mg	46 mg
Vitamin B1	> 85 µg	95 µg	0.46 mg
Vitamin B2	> 0.3 mg	0.3 mg	1.5 mg
Vitamin B6	> 0.1 mg	0.1 mg	0.55 mg
Vitamin B12	> 0.3 µg	0.3 µg	1.5 µg
Vitamin K	2.6 – 8.2 µg	2.8 – 9.5 µg	14.4 µg
Biotin	> 10 µg	11 µg	56 µg
Folic acid	> 34 µg	38 µg	184 µg
Pantothenic acid	> 0.51 mg	> 0.57 mg	2.8 mg
Niacin	> 0.85 mg	> 0.95 mg	4.8 mg

*The values given in this table are based on data on the intrinsic nutrient content of the raw materials and their variability, as well as the variability of the process.

Source: Nutriset.

The global scale-up of outpatient CMAM care for the treatment of uncomplicated SAM was a major paradigm shift that drastically reduced inpatient caseloads and case-fatality rates and increased coverage.[196] The efficacy of this approach is backed by observational and programmatic data.[198,199]

II Alternative Indicators of Health and Nutrition Status

6. Leptin

Leptin (from the Greek λεπτός *leptos*, meaning "thin") is an adipokine[200] that regulates energy homeostasis.[201] Discovered in 1994 via genetic analysis of obese mice,[202] leptin renewed interest in the hormonal regulation of energy balance in hopes that it could be used to treat obesity.[201] To the disappointment of many, an RCT conducted shortly thereafter revealed elevated endogenous leptin in obese individuals, suggesting leptin resistance with increasing adiposity.[203] Yet, it is clear today that leptin plays a vital role at the other end of the spectrum—as a signal of energy deficiency.[201]

6.1. Physiology

Leptin is predominantly secreted by white adipose tissue and expressed by mammary glands, the stomach, the placenta,[204] and by immune cells.[205] In fact, the role of leptin helped redefine the understanding of adipose tissue from a site of inert energy storage to an important endocrine organ.[201] Circulating leptin is highly correlated with adipose tissue reserves[206] and acutely sensitive to energy restriction.[207] It relays information about energy availability to the hypothalamus, where it regulates appetite and energy expenditure[201] and reproductive function. Leptin plays an important role in the pubertal transition,[208] and low leptin levels predict amenorrhea[209]—linking the metabolic status with the reproductive axis by way of this important putative signal.[208] The leptin receptor is also expressed in innate and adaptive immune cells where it modulates immune development and function.[205]

6.2. Energy homeostasis and neuroendocrine function

During starvation leptin levels fall and activate behavioral, hormonal, and metabolic adaptations to energy restriction.[210] Metabolic adaptations include a decrease in resting energy expenditure (REE) and adaptive thermogenesis (AT).[211] These adaptations are thought to be protective, as they divert energy from non-essential processes to promote survival, but become counterproductive over time.[201] Conversely, weight gain increases circulating leptin. Intensive investigation of leptin's hypothalamic action revealed a complex neural mechanism that maintains energy homeostasis by way of orexigenic and anorexigenic signaling.[212,213]

6.3. Leptin as a modulator of immune function

Leptin is considered an essential mediator connecting nutrition and immunity.[106] The starvation state is associated with increased susceptibility to infection (see section **3.2 Malnutrition, Infection, and**

the Gut), and leptin is known to influence both the innate and adaptive immune systems. Regarding the former, leptin upregulates macrophage phagocytosis,[214] proflammatory cytokine synthesis [215] and chemotaxis in polymorphonuclear neutrophils.[216] Regarding the latter, leptin has a powerful proliferative effect on lymphocytes[205] and shifts the immune response towards a proinflammatory state.[201]

6.4. Leptin and mortality in severe acute malnutrition (SAM)

Recent studies have used non-targeted metabolomic/proteomic analysis in children with SAM to identify biomarkers that predict in-patient mortality.[3,4,217] Leptin was identified as a major biochemical marker of acute as well as post-hospital discharge mortality in both edematous and non-edematous children with SAM.[3,4] While the mechanisms by which leptin modulates mortality risk remain unclear, it is likely that prolonged stress leads to white adipose store depletion, which compromises the ability of children with SAM to sustain energy production for cardiorespiratory function, and increases the risk of death from cardiopulmonary failure.[217] Hypoleptenimia may also predispose children with SAM to infectious disease by way of impeding innate and/or acquired immune response function.[217] Disruption in any of leptin's critical immunoregulatory properties has a range of effects on disease susceptibility,[218] weakening the response to infectious pathogens[219] and thereby impeding viability.

6.5. Implications

Bartz *et al.* and Njunge *et al.* highlight the importance of leptin signaling for survival in undernutrition.[220] The potential implications of this work are numerous. First, leptin profiling in malnutrition could help elucidate the biological response to energy restriction, and to nutritional rehabilitation. Second, as a marker of adipose tissue reserve, leptin may one day be used to identify children with mild and moderate acute malnutrition, who are at risk for further nutritional deterioration[217] in the absence of intervention. Third, leptin's predictive capacity for mortality could be used to identify children with SAM at the highest risk of death. And finally, further work to clarify the relationships between nutrition, leptin, and immunity could provide critical information to improve the impact of community-based management of SAM.

7. Stable isotope analysis

The atomic number of an element is the number of protons (and thereby electrons, for neutral atoms) in its nucleus. Atomic mass, however, is the sum of protons and neutrons. Isotopes are two variants of the same element which differ only in the number of neutrons, and therefore in atomic mass. The word isotope (from the Greek ἴσος, *isos*, meaning "equal" and τόπος, *topos*, meaning "place") refers to the fact that different isotopes of the same element are found in the same place on the period table, which is organized by atomic number. Most elements have naturally occurring stable (*i.e.*, that do not undergo radioactive decay) isotopes, although one isotope often far surpasses the other in terms of natural abundance[221]—and this is often the lightest one. **Table 5** gives stable isotope abundancies for a selection of elements common in biological molecules.

Table 5. Naturally occurring stable isotopes common in biological molecules

Element	Stable Isotopes	Abundance (%)
Hydrogen	^{1}H	99.985
	^{2}H	0.015
Carbon	^{12}C	98.892
	^{13}C	1.108
Nitrogen	^{14}N	99.635
	^{15}N	0.365
Oxygen	^{16}O	99.759
	^{17}O	0.037
	^{18}O	0.204

Source: O'Brien 2015.

The stable isotope ratio (SIR) measures the ratio of these abundancies in the foods we eat, and in our own bodies, built from the food consumed. Assessing variation in SIRs is easily done with modern instrumentation[222] but of note is that these differences are extremely small, often occurring at the fourth, fifth or even sixth decimal place.[221] Given the subtle nature of these variations, relative differences (as opposed to absolute values) are more precisely assessed.[223] To do so, SIRs are determined in tandem with a reference gas[221] and reported in units of relative abundance (δ) rather than absolute isotope ratios[224,225] and expressed in permil (‰) units. References used to calculate $\delta^{15}N$

and $\delta^{13}C$ are atmospheric N_2 and Vienna Pee-Dee belemnite (B-PDB), respectively. Living organisms tend to be enriched in ^{15}N compared to atmospheric N_2, but are depleted in ^{13}C compared to limestone. Consequently, $\delta^{15}N$ are generally positive while $\delta^{13}C$ are negative.

SIRs are unique in that they are measured at the atomic, rather than molecular, level. Thus they are not affected by even extreme changes in molecular structure (*e.g.*, autoclaving[226,227] freeze-thaw cycles, and short- to long-term storage[221]). Depending on both the growth and turnover rate of the tissue in which SIR is assessed, the data from stable isotope analysis (SIA) are indicative of short-term (*e.g.*, blood) or long-term (*e.g.*, hair) timeframes.[228] Hair keratin, for example, is an excellent isotopic archive because it does not undergo elemental turnover.[221] Aside from blood and hair, SIA has been conducted in a variety of human sample types including feces,[229] fingernails,[230] and urine.[229]

7.1. Isotopic fractionation and trophic step

Due to their different masses, the light and heavy isotopes of carbon and nitrogen undergo fractionation during biochemical reactions. Fractionation refers to the preferential accumulation of one isotope on one side of the reaction, and the simultaneous accumulation of the other isotope on the other side. This process is due to differences in the strength of chemical bonds involving light or heavy isotopes. Less energy is required to form or break a bond with the light isotope, compared to a heavy isotope, leading to slightly higher enzymatic reaction rate for the former. This isotopic effect causes differences in isotopic abundances between living organisms and their environment. Carbon, for example, is captured from atmospheric CO_2 by plants via photosynthesis. Rubisco, involved in CO_2 fixation, has a strong preference for $^{12}CO_2$[231] and plants have a lower $\delta^{13}C$ than atmospheric CO_2.[221] However, plants native to dry settings (*i.e.*, C4 type plants) have evolved physiological adaptations to their environment that reduce water loss,[221] but also limit their ability to discriminate against ^{13}C.[231] Compared to C3 plants, C4 plants have ~12-13‰ higher $\delta^{13}C$ values.[221] Isotopic fractionation also occurs during the oxidation of pyruvate to acetyl-CoA by pyruvate dehydrogenase, leading to the preferential elimination of $^{12}CO_2$. This fractionation makes animals slightly enriched in ^{13}C compared to their diet (+1‰).

As for nitrogen, reactions involved in the transfer of amino groups (transamination, deamination) which lead to nitrogen excretion as urea and ammonia, have an enzymatic preference for the lighter isotope.[232–234] As a result, animals are enriched in relation to their diet by ~3.5‰.[235] The difference in isotopic enrichment between individuals and their diet is called the trophic step (Δ) where:

$$\Delta^{15}N = \delta^{15}N_{individual} - \delta^{15}N_{diet}$$

7.2. Stable isotopes for dietary assessment

Stable isotope ratios are well-established tools for dietary assessment.[221] Recently, Votruba *et al.* reported strong, positive correlations between fish and meat consumption and $\delta^{15}N$ in plasma, red blood cells and hair, in an intervention study in 32 subjects consuming diets of various composition for 12 weeks. Similarly, the EPIC-Norfolk study reported that animal source food (ASF) consumption was positively associated with plasma $\delta^{15}N$, and fish consumption was associated with high $\delta^{13}C$ and $\delta^{15}N$ values.[236]

Differences in ^{13}C enrichment between C3 and C4 plants have proven useful in epidemiological studies. For example, in North America, most of the sugars used by the food industry come from C4 plants (*e.g.*, corn, sugar cane) and $\delta^{13}C$ in blood and plasma have been shown to be reliable biomarkers of sugar-sweetened beverage intake [237–239] there.

To date, the vast majority of research on $\delta^{15}N$ and $\delta^{13}C$ measurement in humans has been conducted in developed countries. There is limited information on SIR values in populations in low-income countries. A notable exception is a study by Correia *et al.* which reports $\delta^{15}N$ and $\delta^{13}C$ values in hair and fingernail clipping of individuals from six African communities, with four different subsistence strategies (agriculture, fishing, hunter-gatherer, and pastoralism). In this study, SIRs varied widely according to subsistence strategies. They showed that the proportion of C4 plants that comprise the diet has an important effect on these variations. However Correia *et al.* did not detect increased $\delta^{13}C$ and $\delta^{15}N$ values associated with freshwater fish consumption in African lakes—likely due to short food chains for these fish, and thereby low $\delta^{15}N$ values.

7.3. Stable isotopes for metabolic assessment

While diet remains the primary determining factor for carbon and nitrogen SIRs in tissue, specific metabolic states have been shown to influence these ratios as well. In particular, tissue $\delta^{15}N$ is influenced by nitrogen balance. Several animal studies have shown that a nutritional stress, and an associated negative nitrogen, in linked to an increase in $\delta^{15}N$ in urine and splanchnic proteins.[240–243] These increases result from the activation of muscle proteolysis, leading to the release of ^{15}N-enriched amino acids in the plasma, combined with an activation of metabolic deamination and transamination pathways, allowing amino acids to fuel energy metabolism.[243] In humans, several studies have compared the evolution of $\delta^{15}N$ along the hair follicle and weight fluctuation, over a period of several weeks. Synthesized at a relatively constant rate,[221] and inert after synthesis, hair keratin can provide an isotopic window into the past, as far back as the length of the sample. Since hair growth rate is on average 1 cm/month, each 2.5 mm segment contains the retrospective archive of the $\delta^{15}N$ value of plasma amino acids (*i.e.*, keratin precursors) over a period of one week, with the most recent recording being in the 2.5 mm segment closest to the scalp. Using this technique, $\delta^{15}N$ in hair has been shown to increase with weight loss in pregnant women with morning sickness in their first trimester[244,245] (**Figure 11**) and in anorexia nervosa.[246,247]

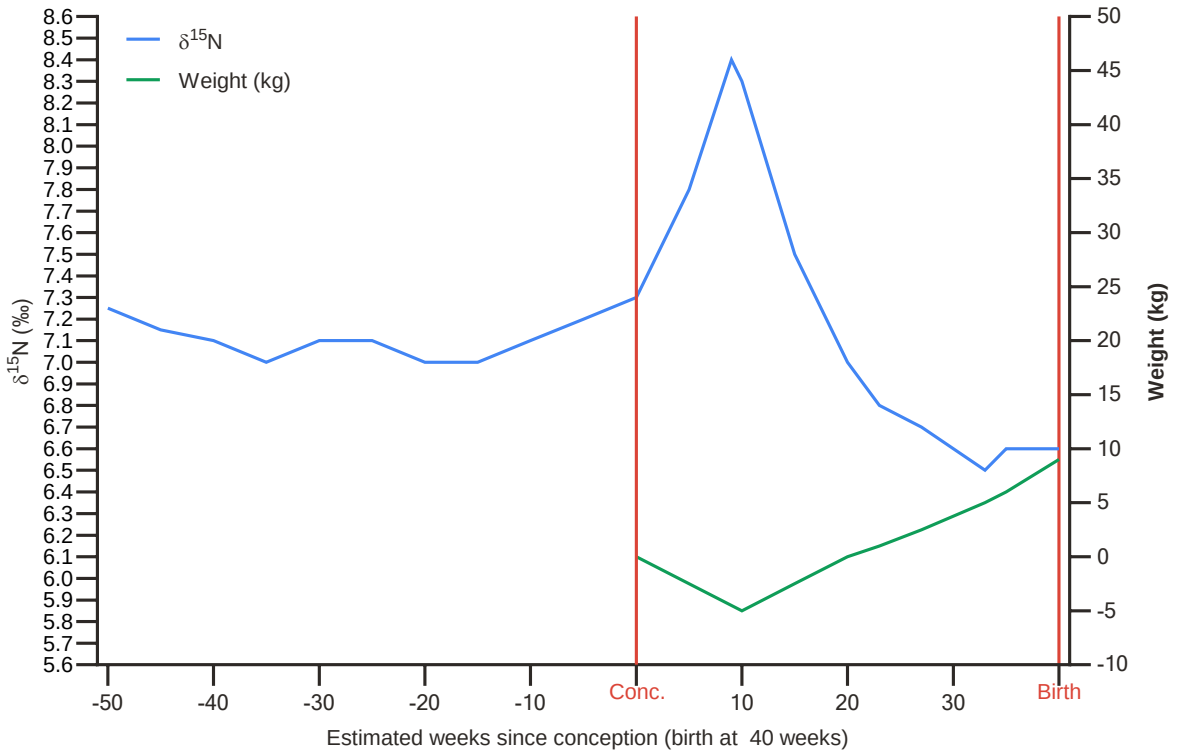

Figure 11. Hair $\delta^{15}N$ variations and weight fluctuation during pregnancy with morning sickness

Hair samples were isotopically analyzed in 1 or 1.5cm sections corresponding to 4 or 6 week intervals of growth, respectively. This subject experienced persistent nausea and vomiting during the first trimester of pregnancy, resulting in a loss of 6.4 kg and a simultaneous increase in $\delta^{15}N$ ~1‰. Her morning sickness subsided after the 10th week of pregnancy, resulting in rapid weight gain (13.9 kg) and a sharp decrease in $\delta^{15}N$ of 1.7‰.

Adapted from: Fuller *et al.,* 2005.

Conversely, weight gain observed during pregnancy (**Figure 12**) or during nutritional rehabilitation of anorexic patients was associated with a gradual decrease in hair $\delta^{15}N$.

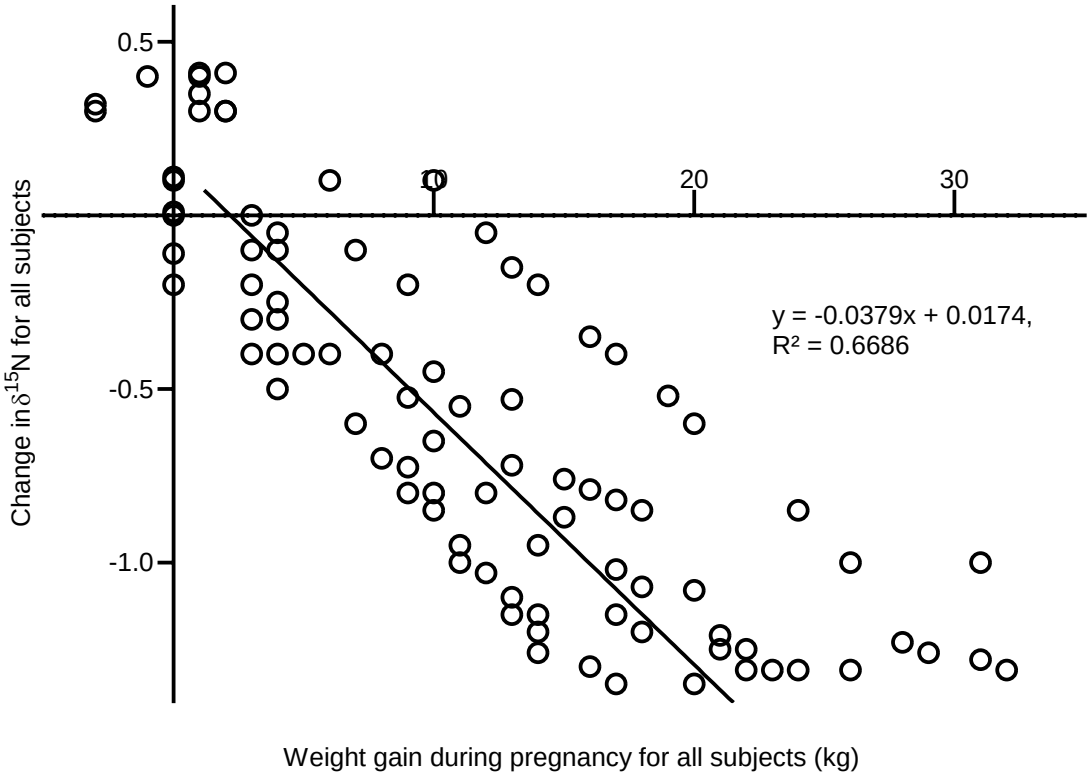

Figure 12. Changes in $\delta^{15}N$ plotted against maternal weight gain during pregnancy

An inverse correlation is observed such that decreasing $\delta^{15}N$ values correspond with increases in weight and thus positive nitrogen balance.

Adapted from: Fuller *et al.*, 2004.

To our knowledge, similar studies in young children do not exist. To what extent $\delta^{15}N$ and $\delta^{13}C$ are affected by specific metabolic (*i.e.*, catabolic) states linked to acute and/or chronic malnutrition are yet to be clearly defined.

7.4. Implications

Stable isotope analysis of carbon and nitrogen in hair have great potential as biomarkers of dietary intake and maybe even metabolic orientation. The impressive integrity of keratin is ideal for field collection in resource poor settings, and offers potential for retrospective analysis.[221] **Article 2** and **Article 3** detail our endeavors to pilot this technique in a representative population of children, and then to apply it to use in severe acute malnutrition.

8. Bio-electric impedance analysis

Introduction

The electrical properties of tissue were first described in 1871.[248] And while these properties are not fully understood,[249] the clinical use of bio-electrical impedance to characterize body composition and disease states, and to monitor nutrition, is widespread and well-established. The fine details of this technique are beyond the scope of this chapter (see reference[250]). However, the following simple model, based on the main components of the tissue should help clarify the physical basis of impedance.

The phospholipid bilayer (PLB) of a cell separates the intra- (ICF) and extracellular fluids (ECF). This membrane is a poor conductor whereas ICF and ECF are good conductors because they contain important ions (K^+, HPO_4^{2-}, SO_4^{2-}, Na^+, and Cl^-, among others) that are critical to cell function. The cell can therefore be modelled as a simple group of electric components shown in **Figure 13** below.[251]

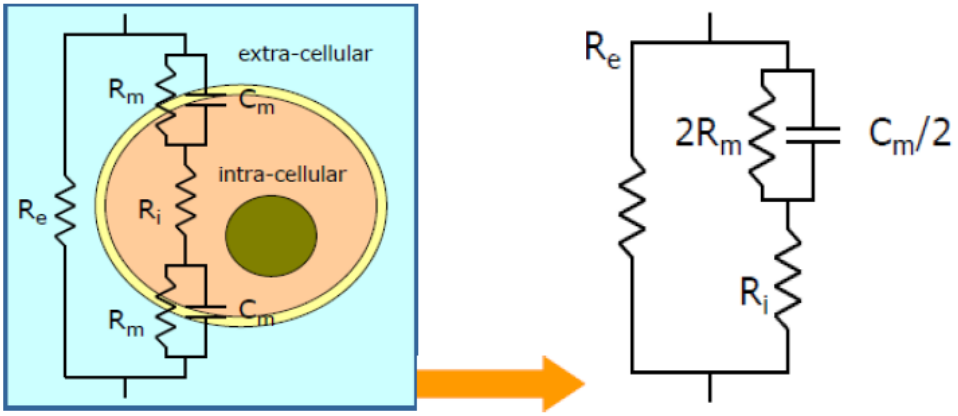

Figure 13. Simple bio-electric impedance equivalence circuit

Current injected into the ECF can flow through the cell across the PLB (Cm) or across the ionic channels (Rm) or can circulate around the cell (Re). Once the current is into the cell it 'travels' through the ICF (Ri) and leaves the cell across the cell membrane (Rm || Cm).

Source: Ivorra *et al*., 2003 and Girma 2014.

Electrical impedance is defined as the opposition, or resistance, that a circuit presents to an alternating current when a voltage is applied.[252] Bio-electric impedance applies this definition to the body. It deals with the passive electrical properties of tissue (*i.e.*, the ability of tissue to oppose, or impede, the flow of electricity).[250] It is measured by the impedance vector (Z) which is derived by two main components: resistance (R) and reactance (Xc). As electrical current flows through the body via ion movement in physiological fluids, resistance is the opposition to this movement by way of fluid viscosity and other effects.[253] Reactance can be seen as the opposite: when current is applied to tissue, it charges cell membranes and other interfaces, that act as capacitors.[253] This framework also includes a "phase shift" (*i.e.*, phase angle (PA)) between voltage and current.[249]

Impedance and phase angle are derived from resistance and reactance as follows:

$$Z = \sqrt{R^2 + Xc^2}$$

$$PA = arctan\left(\frac{Xc}{R}\right) \times \left(\frac{180}{\pi}\right)$$

The relationship between PA, R, Xc, and Z is shown in **Figure 14** below.

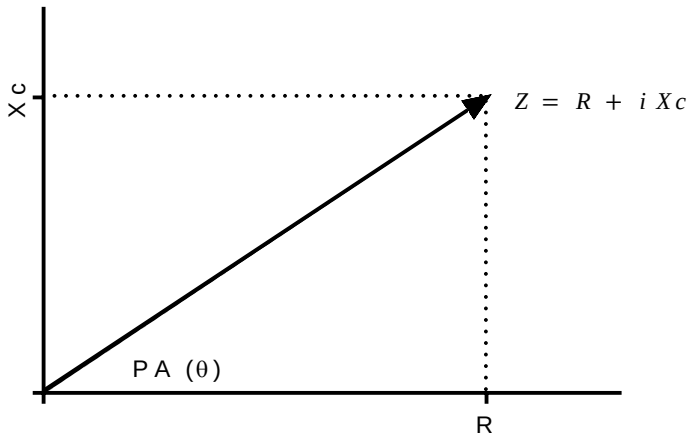

Figure 14. Argand diagram of PA, R, Xc, and Z

The impedance vector (Z) is expressed as a pair of numbers (R, Xc) in a complex plane where R is resistance, Xc is reactance, Θ is phase angle (PA) and i is the imaginary unit that satisfies the equation $i^2 = -1$

Adapted from: Girma 2014.

Use and physiology

At low frequencies (<50 kHz) current flows through the extracellular space around the cell without penetrating cellular components. At high frequencies, membrane capacitance can no longer impede the current and current flows indiscriminately across both the ICF and ECF, shown in **Figure 15** below:

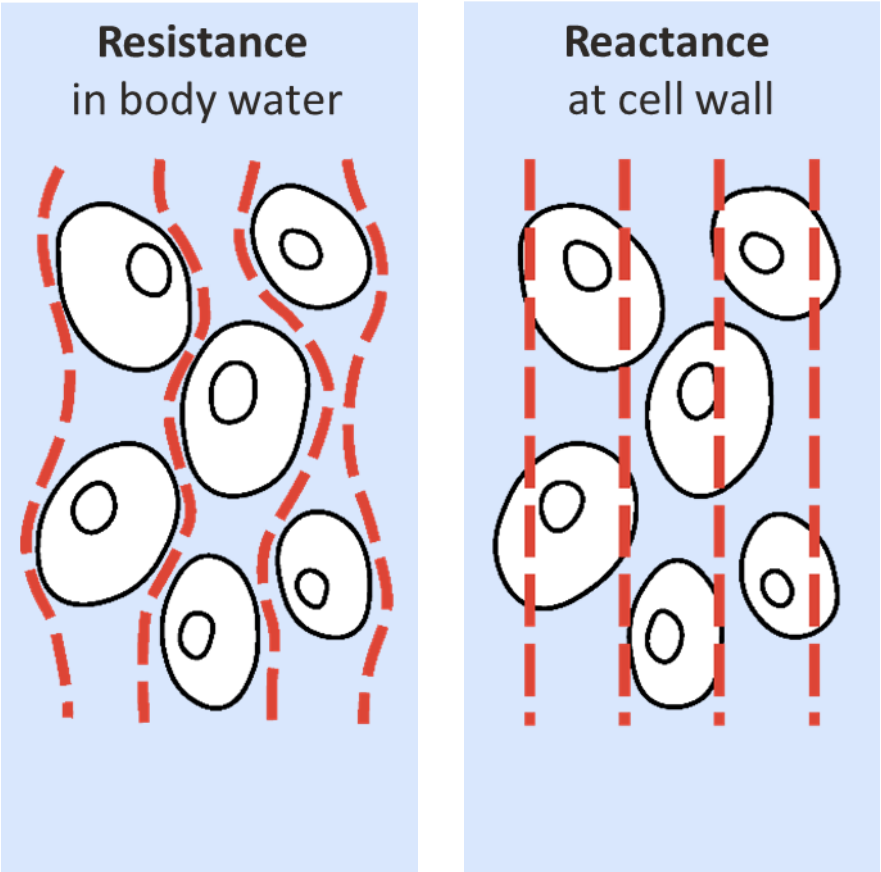

Figure 15. Tissue pathways for low-frequency (left) and high-frequency currents (right)
Adapted from: De Lorenzo *et al.*, 1997

Bioimpedance analyzers use low intensity alternating currents too weak to be felt (1-10 µA) at a frequency of >10 kHz to measure the electrical properties of the body, which are then used to estimate body compartments indirectly.

Bio-electric impedance and severe acute malnutrition

Severe acute malnutrition is a multi-system disorder with similar pathophysiologic causes,[249] despite multifactorial underlying causes.[88] The reductive adaptations in SAM are thought to promote survival, but cause significant atrophy, organ damage, and result in physiological dysfunction.[249,254] Chief among them are fluid and electrolyte disturbances resulting from cardiac, cell membrane, and renal dysfunctions.[255,256] Tissue hydration is further compromised by concomitant diarrheal disease, impaired immunity, and systemic infections.[257]

While disease-associated alterations to bioimpedance have not yet been systematically addressed[249] the literature reports that hydration status, fluid distribution disturbance, and changes in the ratio of ECF and ICF are all significant modifiers.[249,258,259] Furthermore, variations in serum electrolyte balance[260] and blood flow[249] will induce changes in impedance.

Analysis, interpretation, and limits

Bio-electric impedance data can be interpreted quantitatively or qualitatively. Quantitative bio-electric impedance analysis (BIA) reduces the body into a two-compartment model comprised of fat-mass (FM) and fat-free mass (FFM).[261] As described in **Figure 15**, at low frequencies, the current flows primarily through the extracellular space around the cell. However, at high frequencies (>50 kHz), the current (no longer impeded by the cell) passes directly through the extracellular and intracellular space.[253] FFM contains electrolytes and behaves like an electrical conductor. Fat mass contains little water and electrolytes and is thus a poor conductor. If the human body is seen as a simple cylinder with a given length (*i.e.*, body height) and area (*i.e.*, body volume), Ohm's Law can be used to model the relationship between impedance (Z) and body volume (V) as follows:

$$V = \frac{\rho H^2}{Z}$$

where height (H) is assumed to represent conductor length[253] and tissue-specific resistivity (ρ) is a constant (that is inversely proportional to the number of free ions per unit of body volume[262]). Based on this theory, the impedance index (H^2/Z) can be used to estimate TBW. FFM is then estimated from TBW[261,263,264] using a hydration coefficient.[261,265] Once FFM is determined, it is subtracted from the weight of the person to calculate FM. The major limitation of the quantitative BIA approach is that is requires population-, age-, and disease-specific references to accurately estimate TBW (and FFM).[261,266–270]

On the other hand, qualitative bio-electric impedance vector analysis (BIVA) adjusts resistance (R) and reactance (Xc) by height (*i.e.*, R/H and Xc/H), which are then and plotted on a Cartesian plane called the "R/H-Xc/H graph."[271] The data form an ellipse[272] bisected by two axes that are proposed to indicate the level of hydration and cell mass,[271] respectively, shown in **Figure 16** below.

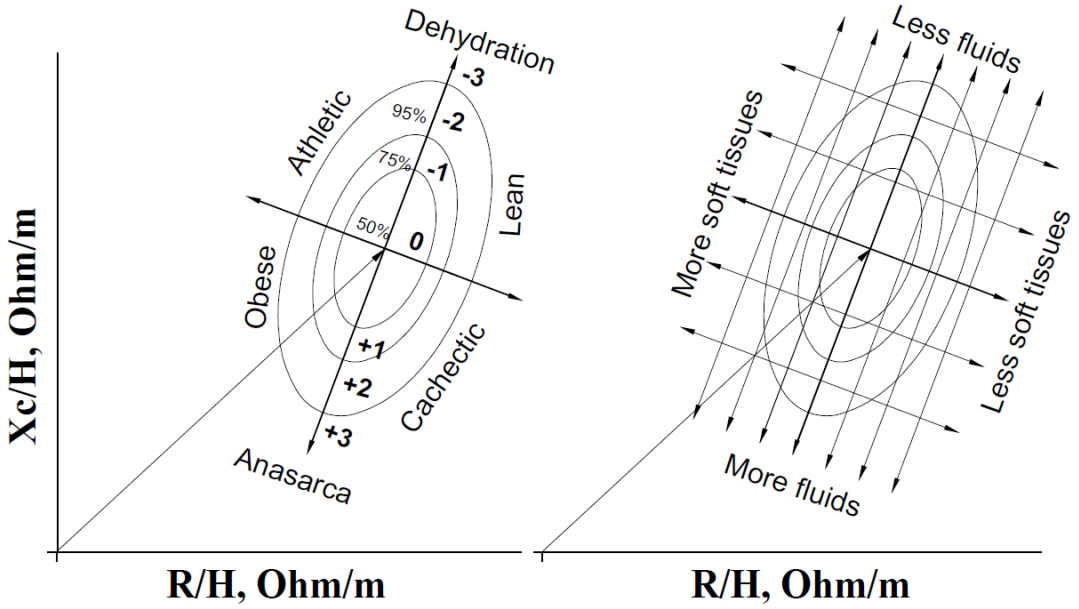

Figure 16. Bio-electric impedance vector analysis (BIVA) patterns on the R/H-Xc/H graph

Vector displacement parallel to the major axis of the tolerance ellipse indicate progressive changes in hydration whereas displacement parallel to the minor axis indicates more (above, left) or less (below, right) cell mass contained in soft tissues. Vectors falling outside the 75% tolerance ellipse indicate abnormal tissue impedance.

Source: Piccoli *et al.* 2002.

The major axis represents variability in hydration where the orthogonal axis represents variability in body cell mass, a proxy for lean body mass.[271,272] The "phase angle" is the angle of each observation relative to the R/H axis. Phase angle is thought to represent both cell mass and cellular health.[272–276] Higher PA is proposed to indicate higher cell mass, cell membrane integrity and better cell function.[272,277] The major limitation of the qualitative BIVA approach is that some authors have suggested that variation in hydration and cell mass sometimes do not translate to the expected vector displacement according to classic BIVA theory.[272]

Implications

A better characterization of body composition in malnourished children could potentially aid in diagnosis and clinical management, and in determining nutritional requirements.[272,278] Yet, very little research on the utility of bio-electric impedance in severe acute malnutrition exists. Grijalva-Eternod *et al.* report differences in body composition between anthropometric phenotypes in SAM, but based on plethysmography, and not bio-electric impedance. They suggest that WHZ and MUAC differ in their ability to index growth and nutritional status. They go on to hypothesize that WHZ is a better index of tissue mass, whereas MUAC is a composite index of poor growth (including stunting, wasting and low adiposity).[70]

9. Aims and objectives

9.1. Research aims

The literature review presented in the *General Introduction* highlights two frustrating challenges that thwart a successful diagnosis and prioritization in SAM. First, diagnosis is based on rudimentary, imperfect anthropometric tools (*i.e.*, MUAC & WHZ). Second, these tools are highly divergent—children identified for admission to therapeutic refeeding programs by either tool are not the same.[11–13,279] Heated debate regarding which of these two tools is a better predictor of mortality is ongoing.[1,2,11,13,24–28] However, hardline advocates argue for the sole use of MUAC for screening and admission (in direct opposition to current WHO recommendations) and many agencies and governments already implement this diagnostic protocol. Doing so bars malnourished children with low WHZ only from access to treatment. Not only are these children at increased risk of subsequent death in the community if left untreated[1,2,13,27,28] but they also represent nearly half of the global burden,[13] a very rough estimate of ~7 million children today.[29] This has created major confusion among policy and decision makers, leading WHO and the scientific community to call for formal investigation into this question.[13,68]

The aim of this dissertation is to help clarify this confusion, and to produce a robust, objective characterization of health and nutrition status linked to diagnostic phenotype in SAM. It is our modest attempt to fill the unacceptably large and overlooked research gap with new evidence—that policy makers urgently require to make to informed decisions on which children to target for treatment.

9.2. Research objectives

The objective of this dissertation is twofold. First, it seeks to describe and compare the pathophysiology and functional severity linked to anthropometric deficit in SAM. In doing so, it asks: are there differences in morbi-mortality risk among (and between) the three phenotypes of SAM (*i.e.*, MUAC-only, MUAC&WHZ, WHZ-only)? In the same vein, the second objective of this dissertation is to pilot the use of emerging indicators of diet, metabolism (stable isotope analysis) and body composition (bio-electric impedance) in this population, asking: can these novel indicators help elucidate the clinical significance of anthropometric deficit?

Very specifically, this dissertation aims to:

1. Describe and compare clinical characteristics, biochemical features and health/nutritional histories of SAM children at admission, among and between phenotype (*i.e.*, MUAC-only, MUAC&WHZ, and WHZ-only);

2. Assess the association between hair $\delta^{13}C$ and $\delta^{15}N$, the diet, and the severity of acute or chronic malnutrition in a representative population of young (6-59 months) children in Bangladesh;

3. Assess the ability of hair $\delta^{15}N$ and $\delta^{13}C$ to differentiate children with SAM, diagnosed with MUAC or WHZ; and

4. Describe and compare the body composition (using quantitative and qualitative bio-electric impedance techniques) of SAM children at admission, among and between phenotype.

III METHODS

10. Methodology

10.1. Cohort study

The OptiDiag project consisted, in large part, of a multi-centric cohort study conducted simultaneously in Bangladesh, Burkina Faso and Liberia between March 2017 and June 2018. Study clinics were nested within CMAM programs run with the technical support of Action Contre la Faim in hospitals and primary health-care centers. Nonedematous, severely acutely malnourished participants aged 6-59 months without medical complications were recruited based on WHO recommendations and treated in an outpatient setting according to the standard of care outlined in the national protocol for SAM management in the country. Participants who developed medical complications were referred to inpatient stabilization centers and excluded from the study at that point.

10.1.1. Data collection

At admission, and at key time points throughout treatment, the following data were collected:

Anthropometry

Anthropometric measurements (weight, height/length, sitting height or laying length) were taken in duplicate as per WHO recommendations.[280] Weight was measured using either a SECA hanging scale or a mother/baby scale. Length or height (change of measuring position at 2 years) was measured using a standard UNICEF wooden measuring board to the nearest 1 mm. MUAC was measured on the left arm with a standard MUAC tape to the nearest 1 mm.

Blood collection, processing, and analysis

In Bangladesh and Liberia, capillary blood (~500 µL) was collected from the non-dominant index finger into a 1.5 mL Eppendorf tube (Eppendorf no. 13094697). In Burkina Faso, the study was nested into a larger trial – Modelling an Alternative Nutrition Protocol Generalizable for Outpatient (MANGO) – and therefore procedures differed slightly; there, venous blood (2-4 mL) was collected from the arm into a vacutainer serum tube (BD no. 366434). One drop was used to test for malaria infection by way of a rapid diagnostic test (RDT, SD-Bioline Combo Standard no. 05FK80). The remaining blood was then transported to the laboratory in a cold box at 2-8°C. Serum was isolated by centrifugation at 2000 × g for 3 minutes and stored at –20°C until shipment to either the Duke Molecular Physiology Institute in Durham, North Carolina for measurement of serum leptin or to VitMin Laboratory in Willstaedt, Germany for measurement of alpha-1-acid glycoprotein (AGP), c-

reactive protein (CRP), retinol binding protein (RBP), body iron stores (BIS), serum ferritin and soluble transferrin receptor (sTfR). Body iron stores (BIS) were estimated based on the ratio of sTfR to serum ferritin (described elsewhere) and expressed in milligram per kilogram body weight.[190,281]

Urine collection, processing, and analysis

In young children, three cotton balls were placed into a clean, disposable diaper for urine collection. Urine was then extracted from the cotton balls.[282] Any cotton balls contaminated with stool were discarded. In older children, urine was collected, if possible, via clean catch. Urinary dipstick analysis was conducted immediately after collection using the CombiScreen 9+Leuko PLUS (Analyticon Biotechnologies AG, Germany reference no. 06885324).

Hair collection, processing and analysis

At the first sample collection, 50 hair follicles were covered in hair gel, twisted into a lock and cut as close to the participant's scalp as possible with sharp, dissecting scissors. The gelled lock was then taped to a sheet of paper and the cut end was labeled and stored at room temperature before further analysis. At subsequent sampling timepoints, the hair that had grown in the presampled section was cut and transferred into Eppendorf tubes. Taped hair samples were cut into 5 mm sub sections and transferred to Eppendorf tubes. Hair gel was removed by two consecutive immersions in 750 µL of ultrapure water for 2 minutes with gentle agitation. Hair samples were then delipidated in 1.5 mL of ether/ethanol (1:1) for 30 minutes, washed in 1.5 mL of ultrapure water for 2 minutes, delipidated once more for 30 minutes, and finally washed thrice consecutively for 2 minutes each, all while being gently agitated. Samples were dried overnight before being transferred into 2 mg tin capsules and analyzed by elemental analysis/isotope-ratio mass spectrometry, using an elemental analyzer (EA Vario Micro Cube, Elementar, Germany) coupled with an isotope-ratio mass spectrometer (Isoprime, VG instruments, Manchester, UK). Tyrosine was used for calibration and drift correction. The natural abundances of ^{15}N and ^{13}C in hair were expressed relative to standards (atmospheric N^2 for $^{15}N/^{14}N$ and Vienna Pee Dee Belemnite for $^{13}C/^{12}C$) using the delta notation, according to the following equation:

$$\delta(‰) = 1000 \times (R_{sample} - R_{standard})/R_{standard}$$

where R_{sample} and $R_{standard}$ stand for the ratio between heavy and light isotope ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) in the sample and standard, respectively.

Bio-electric impedance data collection and analysis

Single frequency BIA was conducted with at 50 kHz using Nutriguard-S instrumentation (Data Input GmbH, Germany). Participants lay supine on an medical examination table with the legs apart an angle of ~45° so that the thighs do not touch, and the arms resting ~30° from the body. Disposable, gelled electrodes were attached to the dominant hand and foot in the standard tetrapolar, ipsilateral

positioning. Injecting leads were attached to electrodes stuck to the dorsal surface of the hand and foot, proximal to the metacarpal phalangeal and metatarsal phalangeal joints, respectively.[249] Measuring leads were attached to electrodes placed at the proximal edge of the ulna head (pisiform prominence of the wrist) and between the medial and lateral malleoli of the ankle[249]). R, Xc, and PA were taken in duplicate, and averaged. Exactly how bio-electric impedance data were analyzed is detailed in **Article 4**.

Clinical examination

Trained research nurses recorded vital signs including temperature, and heart and respiratory rates. The presence or absence of respiratory infection and changes in skin and hair were also recorded. Visible severe wasting, pneumonia and hydration status, and clinical evidence of micronutrient deficiencies (*i.e.*, vitamin A and iron) were assessed based on adapted WHO guidelines for the Integrated Management of Childhood Illness (IMCI) and the Training Course on the Management of Severe Malnutrition: Principles of Care.[283,284]

Caretaker-reported health and nutrition histories

Caretakers were asked to report on recent changes they perceived in their children's general state of health, and whether their children had recently lost weight or eaten less than usual. These data were assessed using a 4-point Likert scale adapted to the context and in the local language. Data were then dichotomized to identify participants who were "much less/a great deal less healthy than usual," or who had lost "a lot (5-15%)/quite a lot (15-25%)" of weight, and ate "a lot less (10-25%)/quite a lot less (25-50%)" food, respectively. Combined indices of these questions were then created to determine which participants had "any 1" or "any 2" of the three aforementioned components.

Additional questionnaires

Questionnaires assessing socio-economic status, infant and young child feeding practices, household food insecurity, and dietary diversity were also conducted.

10.1.2. Study Population

Recruitment

OptiDiag recruitment processes were defined to create the most representative cohort possible—to match global population of children SAM who are detected and referred for treatment present on the field today. SAM children aged 6-59 months were recruited into the study when living in the catchment area at the time of inclusion and following caregiver consent. Children with bilateral pitting edema were excluded, as were those with medical complications requiring inpatient care such as inability to drink or breastfeed, bloody diarrhea, uncontrolled vomiting, convulsions, lethargy, or unconsciousness. Other exclusion criteria included known peanut and/or milk allergies, malformations that affect food intake (such as cleft palate, cerebral palsy, and Down's syndrome), or plans to leave the catchment area within the next 6 months.

Potential participants were identified by way of three screening activities: (1) community health workers (CHWs) conducted active case finding using MUAC <125 mm in the community and referred potential participants to the nearest outpatient therapeutic feeding program (OTP) for a complete anthropometric assessment; (2) health staff passively screened all children under five years of age using both WHZ and MUAC during curative consultations; and (3) health staff passively screened children using both WHZ and MUAC at preventative consultations during child growth monitoring and promotion (GMP) days.

The active screening network in Bangladesh was highly developed and the majority of participants there were identified by CHWs in their communities. Active screening was not routinely conducted in Liberia before the study; the research team only undertook active screening in nearby slum communities to bolster recruitment mid-way through implementation. Thus, the majority of participants in Liberia were identified by way of passive screening during curative consultations. Only in Burkina Faso were potential participants passively screened during growth monitoring activities.

Follow-up (recovery and withdrawal)

After enrollment, participants were meant to return each week until recovery. Recovery was defined as having attained a WHZ ⩾–2 at two consecutive visits for children admitted with at WHZ < –3 or a MUAC ⩾125 mm for children admitted with a MUAC <115mm. Patients were expected to meet discharge criteria within 16 weeks after admission, in the absence of edema and medical complications. Treatment duration was therefore 2 weeks at minimum and 16 weeks at maximum. If discharge criteria are not met within 16 weeks, the child was referred for further examination and/or inpatient treatment.

A caretaker who chose to discontinue the participation of his/her child in the study was free to withdraw at any given time—at which point the caretaker was strongly advised to continue treatment under the CMAM program if the child had not yet successfully reached discharge criteria. For documentation purposes, the reason for withdrawal was determined and recorded. Data collected until the moment of withdrawal from the study was included in analysis unless otherwise requested by the caretaker.

Participants were also withdrawn from the OptiDiag study if they defaulted or were lost to follow-up. After three consecutive missed weekly visits, research staff would visit the child and caretaker at home. Participants that were confirmed alive but whose caretakers were not interested in continuing participation were considered defaulters. Participants who were not located after formal tracing procedures were considered lost to follow-up.

10.1.3. Sample size

The OptiDiag study was exploratory in nature. Thus, it was not considered relevant to calculate the sample size to look at any predefined effect size. Therefore, we employed the general rule of thumb that a minimum sample size n of 30 is sufficient for a normal approximation. To account for loss to follow-up, this number was increased 50, for a minimum of 50 patients per anthropometric

phenotype (n=150). This was then tripled for a total sample size N of 450 patients (*i.e.*, 150 per anthropometric phenotype).

Of note is that patient recruitment was conducted progressively in blocks of 12, meaning that four recruited patients in each anthropometric category were required before moving to the next block, when additional patients could be recruited. In doing this, we ensured an equal representation of anthropometric phenotype over the one-year implementation period, to account for seasonal variability.

10.2. Cross-sectional studies

In addition to the multi-centric cohort study described in the previous section, we conducted a series of cross-sectional studies in an effort to describe the distribution of emerging indicators (*i.e.*, stable isotopes and bio-electric impedance) in a representative population of children. These studies were conducted to generate control data, collected in a reference population, in order to improve the interpretation of emerging indicators collected in children with SAM.

10.2.1. OptiDiag-SMART

A cross-sectional study was within a Standardized Monitoring and Assessment of Relief and Transitions (SMART) evaluation conducted in Ukhiya and Tekaf Upazillas, Cox's Bazar District, Bangladesh in January 2017 (referred to as OptiDiag-SMART).

The primary objective of OptiDiag-SMART was to describe the distribution of (1) bio-electric impedance parameters and (2) stable isotopic ratios of carbon and nitrogen in hair, in a representative population of children aged 6-59 months. Since the distribution of these two indicators in Bangladeshi children is largely unknown, describing that distribution with a given precision was not considered relevant. Thus, the minimum sample size was set to the default SMART sample size (*i.e.*, 704 children in 1093 households). Questionnaires on health and nutrition histories and dietary diversity were administered as well. Collection and analysis of these data were exactly the same as those used in the cohort study.

10.2.2. Bio-electrical impedance sub-studies

We hypothesized that morphological differences in body shape and size might exist between Asian (*i.e.*, Bangladesh) and African (*i.e.*, Burkina Faso and Liberia) children. Because of this, the bio-electric impedance data collected during the OptiDiag-SMART (only in Bangladesh) would not be adequately representative of young children in Burkina Faso and Liberia. Therefore, we conducted additional bio-electric impedance sub-studies in these two countries. Bioimpedance parameters were collected on a sample of 100 age-matched participants (50 boys and 50 girls) without anthropometric deficit (*i.e.*, WHZ > –2 and MUAC >125 mm) were recruited equally into the following four categories, to approximate the age distribution of children with SAM recruited during the cohort study: (1) >6-8 months; (2) >8-12 months; (3) >12-15 months; >15-36 months. Collection and analysis of these data were exactly the same as those described in the previous sections.

10.3. Pilot study and training

Prior to any formal data collection, regardless of the study type, various pilots were carried out to assess feasibility, acceptability and best practices. Research staff were extensively trained on all aspects of study procedures from recruitment, questionnaire administration, measurements, and biological sampling. All procedures were documented in a standard operating procedures (SOPs) manual, which is available upon request.

10.4. Data management

All data were recorded on paper print-outs. Data were double entered in Bangladesh using Microsoft Excel, but entered only once in Liberia. In Burkina Faso most data were collected digitally using tablets and the Open Data Kit (ODK). WHZ and height-for-age *z*-scores (HAZ) were calculated using WHO Growth Standards.[56] Sample data were digitized on the field and uploaded to a cloud on a regular basis and checked for consistency and completeness.

10.5. Data analysis

Data analysis was tailored to each specific research objective and cannot be adequately described in this section. These analytic techniques are detailed in the methods sections of **Article 1**, **Article 2**, **Article 3**, and **Article 4**.

10.6. Ethical considerations

The OptiDiag study was registered at clinicaltrails.gov number NCT03400930 and at isrctn.com number ISRCTN50039021 for the cohort in Burkina Faso. Ethical approval was obtained from: the Ethics Committee at the University Hospital of Antwerp and the University of Antwerp; the National Research Ethics Committee (NERC) at the Bangladesh Medical Research Council (BMRC); the Institutional Review Board at the University of Liberia; and the Ethical Committee for Health Research [Comité d'Éthique pour la Recherche en Santé (CERS)]; the clinical trials board at the Directorate-General of Pharmacy, Medicines and Laboratories [Direction Générale de la Pharmacie, du Médicament et des Laboratoires (DGPML)] in Burkina Faso; and the Institutional Review Board of the Duke University School of Medicine.

Informed consent was obtained from the caretakers of all participants, regardless of the study type. These caretakers were informed about the study in their local language and given an information pamphlet detailing the study specifics as well as a staff phone number. Caregivers who chose to participate were asked to sign a consent form. Illiterate caretakers gave consent by thumbprint in the presence of a witness.

IV ARTICLE 1

Clinical and biochemical markers of functional severity in severe acute malnutrition (SAM): comparative analysis of anthropometric phenotypes from the OptiDiag study

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Abstract: While both weight-for-height *z*-score (WHZ) <–3 SD and mid-upper arm circumference (MUAC) <115 mm are recommended by WHO as independent criteria for diagnosis of severe acute malnutrition (SAM), the links between anthropometric phenotype and functional severity have not been clearly delineated. In Bangladesh, Burkina Faso and Liberia we conducted a multi-centric cohort study in uncomplicated, nonedematous SAM children with low MUAC-only (n=161), low WHZ-only (n=138), and combination of low MUAC and low WHZ (n=152). Alongside routine anthropometric measurements, we collected a wide range of critical indicators of clinical and nutritional status and viability; these included serum leptin, an adipocytokine negatively associated with mortality risk in SAM. We found that median leptin levels at diagnosis are lower in WHZ-only children (215.8 pg/ml) and in those with combined WHZ&MUAC deficits (180.1 pg/ml) than in MUAC-only patients (331.50 pg/ml, p<0.001). The same pattern emerges on a wide range of clinical indicators including visible signs of severe wasting, dehydration, and serum ferritin, care-taker reported health deterioration, and is replicated across study sites. Illustrative of the likely heterogeneous functional severity of the different anthropometric phenotypes of SAM, our results confirm the need to retain low WHZ as an independent diagnostic criterion, in line with WHO recommendations.

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Background

Community-based management of acute malnutrition (CMAM) occupies a unique position between clinical medicine and public health intervention.[198] This standard service delivery model is decentralized, to reduce barriers to access, and employs simplified field procedures conducted by minimally trained health workers in poorly accessible field sites.[285] Simple anthropometric diagnostic methods have been widely and successfully applied to identify and refer malnourished children for treatment.[55] However, anthropometric deficits can result from diverse nutritional, metabolic, and infectious diseases[55] and the links between anthropometric phenotype, functional severity, and long-term morbidity and mortality remain poorly understood.[286]

The current recommendations for the diagnosis of severe acute malnutrition (SAM) are based either on a weight-for-height *z*-score (WHZ) less than –3 standard deviation (SDs) from the international reference median or a mid-upper arm circumference (MUAC) less than 115 mm,[56,68] as well as bilateral pitting edema. By the very nature of these recommendations, SAM children segregate into one of three anthropometric phenotypes: (1) WHZ-only (WHZ <–3 and MUAC ⩾115 mm); (2) both WHZ&MUAC (WHZ <–3 and MUAC <115 mm); and (3) MUAC-only (WHZ ⩾–3 and MUAC <115 mm).

A recent analysis of survey data found that only a minority of SAM children are diagnosed with both low WHZ and low MUAC; most children are selected for treatment on the basis of either WHZ or MUAC alone. This effectively excludes from treatment those who fail to meet criteria for SAM using only one of the diagnostic measures. However, low WHZ and low MUAC likely identify children with varying macro- and micro-nutrient deficiencies, immune dysfunction, secondary infections, and short- and long-term metabolic risks,[67,287,288] and there are dramatic differences in the use of these metrics globally.[13] It is unclear if the divergent anthropometric phenotypes in SAM reflect differences in morbidity and mortality risk and need for treatment. World Health Organization (WHO) and the scientific community have repeatedly called for formal investigation into the pathophysiology and functional severity linked to anthropometric phenotype.[13,60]

The aim of the OptiDiag study was to characterize health and nutrition status, pathophysiology, and functional severity linked to anthropometric phenotype. To that end, we compared the clinical characteristics, biochemical features, and health/nutrition histories of non-edematous, SAM children classified as: (1) low WHZ-only; (2) both low WHZ and low MUAC; and (3) low MUAC-only.

Patients recruited into the OptiDiag study were followed throughout nutritional rehabilitation, and data were collected at key time points during follow-up. However, the results presented in this paper stem from admission data only, when the health/nutrition status in SAM is assumed to be most compromised, and anthropometric deficit at its highest, before therapeutic refeeding begins. We chose serum leptin as our primary outcome measure, based on a comprehensive hormonal and metabolic profile of inpatient SAM children in Uganda that revealed that hypoleptinemia was a robust biochemical predictor of mortality.[3] This association was recently confirmed in an inpatient study in Kenya, which reported that pre-discharge hypoleptinemia was among the strongest determinants of death post-discharge.[4]

Methods

The OptiDiag study followed a multi-centric design and was conducted in Bangladesh, Burkina Faso and Liberia. In Bangladesh the study was conducted in Ukhiya, in 19 primary healthcare facilities located in a rural area on the south-eastern coast. In Burkina Faso, the study was conducted in Fada N'Gourma in 9 primary healthcare clinics in the semi-rural East Region. In Liberia, the study was conducted in the country's capital, in one hospital and two urban clinics.

Study population

SAM children aged 6-59 months were recruited into the study when living in the catchment area at the time of inclusion and following caregiver consent. Children with bilateral pitting edema were excluded, as were those with medical complications requiring inpatient care such as inability to drink or breastfeed, bloody diarrhea, uncontrolled vomiting, convulsions, lethargy, or unconsciousness. Other exclusion criteria included known peanut and/or milk allergies, malformations that affect food intake (such as cleft palate, cerebral palsy, and Down's syndrome), or plans to leave the catchment area within the next 6 months.

Potential participants were identified by way of three screening activities: (1) community health workers (CHWs) conducted active case finding using MUAC <125 mm in the community and referred potential participants to the nearest outpatient therapeutic feeding program (OTP) for a complete anthropometric assessment; (2) health staff passively screened all children under 5 using both WHZ and MUAC during curative consultations; and (3) health staff passively screened children using both WHZ and MUAC at preventative consultations during child growth monitoring and promotion (GMP) days.

The active screening network in Bangladesh was highly developed and the majority of participants there were identified by CHWs in their communities. Active screening was not routinely conducted in Liberia before the study; the research team only undertook active screening is nearby slum communities to bolster recruitment mid-way through implementation, so the majority of participants there were identified by way of passive screening during curative consultations. Only in Burkina Faso were potential participants passively screened during growth monitoring activities.

Data Collection

Patient enrollment and informed consent

The first patient was recruited on March 6th, 2017 and the last on June 8th, 2018. Potential participants were referred to study clinics where their caregivers were informed about the study in their local language and given an information pamphlet detailing the study specifics as well as a staff phone number. Caregivers who chose to participate were asked to sign a consent form; illiterate caretakers gave consent by thumbprint in the presence of a witness.

Treatment

Patients were treated according to standards of care outlined in the national protocol for SAM management for their country and were referred to inpatient stabilization centers (SCs) for any complications requiring medical attention.

Questionnaires and biological samples

Anthropometric measurements (weight, height/length, sitting height or laying length) were taken in duplicate as per WHO recommendations.[280] Weight was measured using either a SECA hanging scale or a mother/baby scale. Length or height (change of measuring position at 2 years) was measured using a standard UNICEF wooden measuring board to the nearest 1 mm. MUAC was measured on the left arm with a standard MUAC tape to the nearest 1 mm.

In Bangladesh and Liberia, capillary blood (~500 µL) was collected from the non-dominant index finger into a 1.5 mL Eppendorf tube (Eppendorf no. 13094697). In Burkina Faso, the study was nested into a larger trial – Modelling an Alternative Nutrition Protocol Generalizable for Outpatient (MANGO) – and therefore procedures differed slightly; there, venous blood (2-4 mL) was collected from the arm into a vacutainer serum tube (BD no. 366434). One drop was used to test for malaria infection by way of a rapid diagnostic test (RDT, SD-Bioline Combo Standard no. 05FK80). The remaining blood was then transported to the laboratory in a cold box at 2-8°C. Serum was isolated by centrifugation at 2000 × g for 3 minutes and stored at –20°C until shipment to either the Duke Molecular Physiology Institute in Durham, North Carolina for measurement of serum leptin or to VitMin Laboratory in Willstaedt, Germany for measurement of alpha-1-acid glycoprotein (AGP), c-reactive protein (CRP), retinol binding protein (RBP), serum ferritin and soluble transferrin receptor (sTfR). Body iron stores (BIS) were estimated based on the ratio of sTfR to serum ferritin described elsewhere and expressed in milligram per kilogram body weight.[190,281]

In young children, three cotton balls were placed into a clean, disposable diaper for urine collection. Urine was then extracted from the cotton balls.[282] Any cotton balls contaminated with stool were discarded. In older children, urine was collected if possible via clean catch. Urinary dipstick analysis was conducted immediately after collection using the CombiScreen 9+Leuko PLUS (Analyticon Biotechnologies AG, Germany reference no. 06885324).

During clinical examinations research nurses measured vital signs including temperature, and heart and respiratory rates. The presence or absence of respiratory infection and changes in skin and hair were recorded. Visible severe wasting, pneumonia and hydration state and clinical evidence of micronutrient deficiencies (*i.e.*, vitamin A and iron) were assessed based on adapted WHO guidelines for the Integrated Management of Childhood Illness (IMCI) and the Training Course on the Management of Severe Malnutrition: Principles of Care.[283,284]

Caretakers were asked to report on recent changes they perceived in their children's general state of health, and whether their children had recently lost weight or eaten less than usual. These data were assessed using a 4-point Likert scale adapted to the context and in the local language. Data were then dichotomized to identify participants who were "much less/a great deal less healthy than usual," or who had lost "a lot (5-15%)/quite a lot (15-25%)" of weight, and ate "a lot less (10-25%)/quite a lot less (25-50%)" food, respectively. Combined indices of these questions were then created to determine which participants had "any 1" or "any 2" of the three aforementioned components.

Biochemical measurements

As noted previously, our primary outcome measure was serum leptin, as it was found to predict acute mortality and post-discharge mortality in malnourished infants and children in Uganda[3] and Kenya.[4] Serum leptin was analyzed by ELISA as previously described.[3] Alpha-1-acid glycoprotein (AGP), c-reactive protein (CRP), retinol binding protein (RBP), serum ferritin, and soluble transferrin receptor (sTfR) were measured using combined sandwich ELISA.[289] With rare exception, all samples were measured in duplicate.

Interpretation of clinical and biochemical data

Leptin levels were compared among the three experimental groups and interpreted in comparison with levels in normal, healthy infants and children.[290] We also compared the percent of children in each group with serum leptin <35 pg/ml, which associates strongly with risk for mortality in children with SAM.[3,4] Because RBP and ferritin are known to inaccurately reflect nutrition status in the presence of subclinical inflammation, correction factors were calculated to adjust their values.[125] The thresholds for defining abnormal biochemical parameters in blood and urine were as follows: AGP >1 g/L;[291] CRP >5 mg/L;[291] adjusted SF <12 µg/L;[125] unadjusted SF <12 µg/L or <30 µg/L in the presence of infection;[166,180] sTfR >8.3 mg/L;[289] adjusted and unadjusted RBP ⩽0.7 µmol/L and ⩽1.05 µmol/L for vitamin A deficiency (VAD) and vitamin A insufficiency (VAI), respectively;[292,293] ascorbic acid ⩾0.2 g/L (20 mg/dL); bilirubin ⩾15 µmol/L (1 mg/dL); blood ⩾5-10 ery/µL; glucose ⩾2.8 mol/L (50 mg/dL); ketones ⩾1 mmol/L (10mg/dL); leukocytes ⩾25 leu/µL; and urobilinogen ⩾35 µmol/L (2 mg/dL). Protein or nitrites present in the urine were considered abnormal. Clinical cutoffs were defined as follows: fever, temperature ⩾37.5°C;[283] tachycardia, >160 beats/minute for children <12 months and >120 beats/minute for children ⩾12 months;[294] and tachypnea, ⩾50 breaths/minute for children <12 months and ⩾40 breaths/minute for children ⩾12 months.[283]

Data handling and statistical analysis

Data were double entered in Bangladesh using Microsoft Excel, but entered only once in Liberia. In Burkina Faso most data were collected digitally using tablets and the Open Data Kit (ODK). WHZ and height-for-age *z*-scores (HAZ) were calculated using WHO Growth Standards.[56]

Characteristics of the study population were summarized as percentages, means±SDs, or medians and interquartile ranges (IQRs) if not normally distributed. Differences in clinical features, health/nutrition histories, and biochemical data among the three phenotypic groups were assessed using χ^2 and Kruskal-Wallis tests; when statistical differences were detected ($p<0.05$), the following three pairwise comparisons were conducted to determine how the groups differed: (1) WHZ-only vs. MUAC-only; (2) both WHZ&MUAC vs. MUAC-only; and (3) All-MUAC (MUAC <115 mm with/without WHZ <–3) vs. WHZ-only. The first comparison tests differences between children with low WHZ and low MUAC. The second comparison tests the added effect of low WHZ in the presence of low MUAC. The final comparison identifies abnormalities in low WHZ children who would not be targeted for treatment using a low MUAC-only screening program.

Bivariate and multivariate logistic regression were used to examine the associations between each significant feature and the odds of being in one diagnostic group compared to the other. Quintile, median regression was also used for continuous variables to account for skew.[295] Confounding and/or effect modification by age (±24 months), sex, stunting (HAZ ±–2), and sitting-standing ratio (SSR) was assessed; we computed SSR and generated individual *z*-scores (SSRZ) as described elsewhere [12]. SSRZ was dichotomize to identify children with 'longer legs' (SSRZ in the lower tertile vs. SSRZ in upper 2 tertiles). Adjusted models included any or all of the aforementioned covariates when confounding (magnitude of confounding ⩾20%) or interaction (Cochran-Mantel-Haenzel test, $p<0.05$) was detected. Variance inflation factors (VIFs) were used to quantify multicollinearity, and then covariates in adjusted models were removed to reduce it (VIF <3). The performance of the resulting adjusted models was evaluated for statistical improvement compared to the unadjusted alternative (Hosemer-Lemeshow goodness-of-fit test, $p<0.05$) when the significance of the independent variable of interest was either lost or gained. To determine if a multi-level modelling approach was necessary to control for country-level cohort effects we examined intraclass correlation coefficients (ICCs) and found that it was not. However, a country variable was included in all adjusted models as either a covariate (to account for residual confounding) or an interaction term (when effect modification was detected). Correction for multiple testing was applied using a step-up, false-discovery rate (FDR) method.[296–298] All analyses were performed using STATA version 13.0 and $p<0.05$ was used to determine statistical significance.

Approval

The OptiDiag study was registered at clinicaltrails.gov number NCT03400930 and at isrctn.com number ISRCTN50039021 for the cohort in Burkina Faso. Ethical approval was obtained from: the Ethics Committee at the University Hospital of Antwerp and the University of Antwerp; the National Research Ethics Committee (NERC) at the Bangladesh Medical Research Council (BMRC); the

Institutional Review Board at the University of Liberia; and the Ethical Committee for Health Research [Comité d'Éthique pour la Recherche en Santé (CERS)]; the clinical trials board at the Directorate-General of Pharmacy, Medicines and Laboratories [Direction Générale de la Pharmacie, du Médicament et des Laboratoires (DGPML)] in Burkina Faso; and the Institutional Review Board of the Duke University School of Medicine.

Results

A total of 473 patients were recruited between March 2017 and June 2018. However, 22 patients (4.7%) were excluded from analysis because of inclusion error—4 patients (0.9%) were younger than 6 months at the time of admission and 18 patients (3.8%) were only moderately malnourished. The median [IQR] age of participants was 10.7 [7.4-17.5] months, with 86.5% younger than 24 months (**Table 6**). There was a nearly equal proportion of girls to boys. The distribution of patients across phenotype groups was as follows: 138 (30.6%) low WHZ-only, 152 (33.7%) both low WHZ and low MUAC, and 161 (35.7%) low MUAC-only.

Table 6. Demographic and anthropometric characteristics on admission*

Characteristic	WHZ-only (n=138)	Both-WHZ&MUAC (n=152)	MUAC-only (n=161)
Age, months	15.5 [10.55–25.61]	10.2 [7.36–16.47]	8.0 [6.71–11.8]
6–11 months	33.3 (46)	61.2 (93)	76.4 (123)
12–23 months	36.9 (51)	29.6 (45)	21.1 (32)
24–59 months	29.7 (41)	9.2 (14)	3.7 (6)
Male	68.1 (94)	44.7 (68)	32.9 (53)
Anthropometry			
WHZ	-2.77 [-2.92– -2.63]	-3.54 [-3.98– -3.25]	-2.38 [-2.69– -1.88]
MUAC, mm	120 [116.5–124]	111 [107–113]	113 [110–114]
HAZ	-1.82 ± 1.31	-2.46 ± 1.44	-1.82 ± 1.31
HAZ < –2	42.8 (59)	58.6 (89)	70.2 (113)

Abbreviations: HAZ, height-for-age z-score; MUAC, mid-upper arm circumference; WHZ, weight-for-height z-score.

* Values are % (n) for categorical variables, means ±SDs for continuous variables with a normal distribution, or medians [IQRs] for continuous variables with a skewed distribution.

Median age was lowest in low MUAC-only children (8 months; IQR, 6.71-11.8), followed by both low WHZ and low MUAC children (10.2 months; IQR, 7.36-16.27) and highest in low WHZ-only children (15.5 months; IQR, 10.55-25.61, p<0.0001, **Table 6**). The proportion of boys was highest in low WHZ-only patients (68.1%), followed by both low WHZ and low MUAC patients (44.7%), and lowest in low MUAC-only patients (32.9%, p<0.001, **Table 6**). Severe stunting (<–3 SD) was most prevalent in low MUAC-only children (40.4%), followed by both low WHZ and low MUAC children (34.2%) and least prevalent in low WHZ-only children (16.7%, p<0.001, **Table 6**).

Biochemical data, clinical features, and health/nutrition histories, among phenotypes at admission are shown in **Table 7**, **Table 8**, and **Table 9** respectively. A number of significant differences were observed and prompted pairwise comparisons, beginning with leptin, the primary outcome measure for our study. Unadjusted and adjusted analyses of continuous data are presented in **Table 10**. Unadjusted, bivariate pairwise analyses of categorical data are presented in **Table 11** followed by adjusted, multivariate models including age, sex, stunting, SSR and country as covariates in **Table 12**.

Table 7. Biochemical data at admission*

	No. (%) of patients by anthropometric category						
	WHZ-only (n = 138)		WHZ&MUAC (n = 152)		MUAC-only (n = 161)		p†
Biochemical features							
Serum leptin, pg/mL	215.80	[122.0-346.6]	180.10	[87.7-346.8]	331.50	[159.0-560.1]	0.000
Serum leptin ⩽35 pg/mL	5	(7)	9	(14)	3	(5)	0.063
Acute-phase proteins							
AGP, g/L	1.25	[0.83-1.69]	1.25	[0.88-1.82]	1.13	[0.79-1.81]	0.413
AGP >1 mg/L	66	(85)	66	(94)	56	(87)	0.133
CRP, mg/L	1.51	[0.50-12.44]	1.655	[0.63-8.29]	2.67	[0.68-10.14]	0.252
CRP >5 mg/L	34	(44)	32	(45)	38	(59)	0.485
AGP >1 mg/L and/or CRP >5 mg/L	67	(86)	69	(98)	62	(96)	0.455
Iron deficiency							
Body irons stores, mg/kg body weight	5.99	[1.82-8.17]	5.68	[1.8-8.34]	4.56	[1.87-7.92]	0.231
Body irons stores <0 mg/kg body weight	25	(32)	17	(24)	14	(21)	0.043
Adjusted serum ferritin, µL/L	27.74	[8.77-48.44]	33.99	[16.42-67.71]	33.60	[15.93-54.27]	0.042
Adjusted serum ferritin <12 µg/L	30	(39)	18	(26)	19	(29)	0.026
sTfR, mg/L	8.34	[6.91-11.24]	8.8	[8.8-11.65]	8.7	[7.21-12.96]	0.410
sTfR >8.3 mg/L	52	(67)	54	(76)	57	(88)	0.695
Vitamin A insuffiency/deficiency							
Adjusted RBP, µmol/L	1.01	[0.77-1.24]	0.98	[0.78-1.22]	1	[0.77-1.25]	0.987
Adjusted RBP <1.05 µmol/L	55	(71)	58	(83)	56	(87)	0.880
Adjusted RBP ⩽0.7 µmol/L	20	(25)	15	(22)	19	(29)	0.644
Urinalysis							
Ascorbic acid excretion, ⩾0.2 g/L (20 mg/dL)	44	(39/88)	53	(49/92)	48	(41/86)	0.478
Bilirubinuria, ⩾15 µmol/L (1 mg/dL)	19	(17/88)	18	(17/92)	6	(5/86)	0.019
Hematuria, ⩾5-10 ery/µL	9	(8/88)	17	(15/90)	19	(16/86)	0.172
Glycosuria, ⩾2.8 mol/L (50 mg/dL)	1	(1/88)	3	(3/91)	1	(1/85)	0.479
Ketonuria, ⩾1 mmol/L (10mg/dL)	16	(14/88)	8	(7/92)	5	(4/84)	0.189
Leukocyturia, ⩾25 leu/µL	14	(12/88)	30	(28/92)	22	(19/85)	0.026
Nitrituria	25	(22/88)	31	(28/91)	40	(34/86)	0.117
Proteinuria	8	(7/88)	10	(9/92)	8	(7/85)	0.895
Urobilinogenuria, ⩾35 µmol/L (2 mg/dL)	3	(3/88)	1	(1/92)	1	(1/86)	0.434

Abbreviations: AGP, α-1-acid glycoprotein; CRP, C-reactive protein; ery, erythrocyte leu, leukocyte; MUAC, mid-upper arm circumference; RBP, retinol binding protein; sTfR, soluble transferrin receptor; WHZ, weight-for-height z-score.

* Values are % (n), denominators lower than n indicate missing data.

† For 3 group comparison (WHZ-only, both WHZ&MUAC, and MUAC-only) by Pearson's χ^2 test (or Fisher's exact tests for small sample sizes) for categorical data and Kruskal-Wallis tests for skewed, continuous data.

Table 8. Clinical characteristics admission*

	No. (%) of patients by anthropometric category			
	WHZ-only (n = 138)	WHZ&MUAC (n = 152)	MUAC-only (n = 161)	p†
Clinical features				
Cough/difficult breathing				
Cough	48 (66)	56 (85)	45 (73)	0.152
Nasal discharge	31 (43/137)	37 (56)	28 (45)	0.238
Tachypnea‡	17 (24)	25 (38)	21 (34)	0.286
Difficult breathing	4 (6)	9 (13)	4 (7)	0.194
Subcostal indrawing	2 (3)	2 (3)	1 (2)	0.809
Stridor	1 (2)	3 (4)	4 (7)	0.320
WHO/IMCI: pneumonia or severe pneumonia	19 (26/137)	28 (42)	24 (39)	0.223
Dehydration				
Slow/very slow skin pinch	17 (15/90)	21 (21/99)	4 (4/97)	0.002
Sunken eyes	32 (29/90)	30 (30/99)	14 (14/97)	0.008
Restlessness/irritability	8 (7/90)	16 (16/99)	3 (3/97)	0.006
Some or severe dehydration	18 (16/89)	22 (22/99)	6 (6/96)	0.006
WHO/IMCI: Some or severe dehydration	5 (4/86)	6 (6/98)	2 (2/96)	0.351
Dermatosis	9 (12)	18 (27)	7 (12)	0.008
Hair changes	9 (13)	4 (6/150)	7 (11/157)	0.184
Iron deficiency				
Conjunctival and/or palmar pallor	34 (47)	41 (62/151)	27 (43)	0.028
Fever	15 (21)	18 (28)	18 (29)	0.737
Malaria	8 (11/136)	14 (20/148)	21 (33/157)	0.007
Visible severe wasting				
Visible ribs	67 (60/90)	76 (75/99)	53 (51/97)	0.003
Loose skin on arms or thighs	14 (19/90)	24 (37/99)	9 (14/97)	0.001
Visible back ribs or shoulder bones	58 (52/90)	69 (68/99)	46 (45/97)	0.004
Flesh missing/folds of skin on buttocks and/or ‘baggy pants’	6 (5/90)	17 (17/99)	3 (3/97)	0.001
WHO/IMCI: severe or extreme wasting	67 (60/90)	77 (76/99)	57 (55/97)	0.012

Abbreviations: MUAC, mid-upper arm circumference; WHO/IMCI, World Health Organization/integrated management of childhood illness recommendations; WHZ, weight-for-height z-score.

* Values are % (n), denominators lower than n indicate missing data.

† For 3 group comparison (WHZ-only, both-WHZ&MUAC, and MUAC-only) by Pearson’s χ^2 test (or Fisher’s exact tests for small sample sizes) for categorical data and Kruskal-Wallis tests for skewed, continuous data.

‡ 50 breaths/minute in children 2 to 12 months of age, and >40 breaths/minute in children ⩾1 year of age

Table 9. Health and nutrition histories at admission*

	No. (%) of patients by anthropometric category			
	WHZ-only (n = 138)	WHZ&MUAC (n = 152)	MUAC-only (n = 161)	p†
Recent health and nutrition history (caretaker-reported)				
Eaten less‡	49 (67)	38 (58)	37 (59)	0.081
Decreased health status§	42 (58)	43 (65)	29 (47)	0.021
Weight loss, ⩾5%	63 (87)	62 (94)	52 (83/160)	0.092
Any 1‖	75 (104)	72 (109)	62 (100)	0.035
Any 2¶	57 (79)	48 (73)	41 (65/160)	0.017
Diarrhea	22 (31)	27 (40/150)	21 (33/159)	0.453
Vomiting	13 (18/135)	23 (34/149)	16 (25/158)	0.088
Fever	54 (74/136)	60 (89/148)	50 (78/157)	0.186
Cough/difficult breathing	45 (62)	47 (71/150)	44 (70/159)	0.835
Any 1††	70 (95/136)	77 (114/149)	68 (108/159)	0.223
Any 2‡‡	47 (64/135)	52 (77/148)	41 (65/157)	0.175

Abbreviations: MUAC, mid-upper arm circumference; WHZ, weight-for-height z-score.

* Values are % (n), denominators lower than n indicate missing data.
† For 3 group comparison (WHZ-only, MUAC&WHZ, and MUAC-only) by Pearson's χ^2 test (or Fisher's exact tests for small sample sizes) for categorical data and Kruskal-Wallis tests for skewed, continuous data.
‡ ⩾10% less
§ "much less" healthy than usual
‖ Eaten ⩾10% less, ⩾"much less" healthy than usual, or lost ⩾5% weight
¶ Any two of: eaten ⩾10% less, ≥"much less" healthy than usual, or lost ⩾5% weight
†† Diarrhea, vomiting, fever, cough/difficult breathing
‡‡ Any two of: diarrhea, vomiting, fever, cough/difficult breathing

Table 10. Unadjusted and adjusted median serum leptin and serum ferritin by comparison*

	Comparison 1							Comparison 2							Comparison 3						
	WHZ only			MUAC-only				MUAC-only			MUAC&WHZ				WHZ-only			All-MUAC			
	Median	95%	CI	Median	95%	CI	*p*	Median	95%	CI	Median	95%	CI	*p*	Median	95%	CI	Median	95%	CI	*p*
Serum leptin, pg/mL																					
Unadjusted median†	215.8	122.0	346.6	331.5	159.0	560.1	0.001	331.5	159.0	560.1	180.1	87.7	346.8	<0.001	215.8	122.0	346.6	245.8	117.0	482.0	0.212
Adjusted median	232.1	193.9	263.0	372.8	226.4	380.0	0.002	352.0	229.8	414.3	217.6	133.2	238.0	<0.00001	220.1	193.9	309.0	262.0	223.0	331.4	0.462
Serum ferritin, µL/L																					
Unadjusted median†	27.7	8.8	48.4	33.6	15.9	54.3	0.064	33.6	15.9	54.3	34.0	16.4	67.7	0.488	27.7	8.8	48.4	33.9	16.1	60.2	0.015
Adjusted median	31.9	7.1	34.4	32.9	24.0	51.0	0.829	31.1	27.4	34.9	34.3	30.6	38.6	0.404	22.3	18.3	26.7	33.8	33.3	37.8	0.017

Abbreviations: MUAC, mid-upper arm circumference; WHZ, weight-for-height *z*-score.

* Adjusted for age, sex, stunting, SSR and country.

† The Wilcoxon rank-sum test is used for equality median when the unadjusted median is presented

Table 11. Unadjusted, bivariate logistic regression analysis by comparison

	Comparison 1: WHZ-only vs. MUAC-only				Comparison 2 MUAC-only vs. WHZ&MUAC				Comparison 3 WHZ-only vs. all-MUAC			
	UOR	(95%	CIs)	*p*	UOR	(95%	CIs)	*p*	UOR	(95%	CIs)	*p*
Biochemical features												
Iron deficiency												
Body iron stores, <0 mg/kg body weight	2.11	1.15	3.88	0.016	1.29	0.68	2.43	0.435	0.54	0.32	0.90	0.017
Adjusted serum ferritin, <12 µg/L	1.89	1.09	3.28	0.024	0.97	0.54	1.74	0.908	0.52	0.32	0.84	0.007
Urinalysis												
Bilirubinuria, ⩾15 µmol/L (1 mg/dL)	3.88	1.36	11.05	0.011	3.67	1.29	10.45	0.015	0.59	0.29	1.18	0.134
Leukocyturia, ⩾25 leu/µL	0.55	0.25	1.21	0.138	1.52	0.77	2.99	0.225	2.29	1.14	4.58	0.019
Clinical Features												
Slow/very slow skin pinch	4.65	1.48	14.60	0.008	6.26	2.06	19.01	0.001	0.73	0.36	1.47	0.377
Sunken eyes	2.82	1.37	5.78	0.005	2.58	1.27	5.24	0.009	0.61	0.35	1.06	0.080
Restlessness/irritability	2.64	0.66	10.55	0.169	6.04	1.70	21.46	0.005	1.27	0.51	3.15	0.601
Some or severe dehydration	3.29	1.22	8.83	0.018	4.29	1.65	11.11	0.003	0.76	0.39	1.50	0.435
Dermatosis	1.18	0.51	2.72	0.694	2.68	1.31	5.51	0.007	1.49	0.76	2.95	0.247
Iron deficiency												
Conjunctival and/or palmar pallor	1.42	0.86	2.33	0.168	1.91	1.19	3.08	0.008	0.98	0.64	1.50	0.933
Malaria	0.33	0.16	0.68	0.003	0.59	0.32	1.08	0.086	2.39	1.21	4.74	0.013
Visible severe wasting												
Visible ribs	1.80	1.00	3.26	0.051	2.82	1.53	5.18	0.001	0.61	0.35	1.06	0.080
Loose skin on arms or thighs	1.59	0.74	3.39	0.234	3.54	1.76	7.11	0.000	1.27	0.51	3.15	0.601
Visible back ribs or shoulder bones	1.65	0.92	2.94	0.090	2.64	1.47	4.73	0.001	0.76	0.39	1.50	0.435
Flesh missing/folds of skin on buttocks and/or ‘baggy pants’	1.84	0.43	7.95	0.412	6.50	1.84	22.96	0.004	1.93	0.70	5.32	0.203
WHO/IMCI: severe or extreme wasting	1.53	0.84	2.77	0.163	2.52	1.36	4.67	0.003	1.01	0.59	1.71	0.977
Recent health and nutrition history												
Decreased General state of health status	1.76	1.09	2.84	0.021	1.74	1.08	2.79	0.022	0.77	0.51	1.16	0.208
Any 1*	1.87	1.13	3.08	0.015	1.55	0.96	2.49	0.072	0.66	0.42	1.03	0.069
Any 2†	1.96	1.23	3.11	0.004	1.35	0.86	2.11	0.189	0.59	0.40	0.89	0.011

Abbreviations: CI, confidence interval; leu, leukocyte; MUAC, mid-upper arm circumference; UOR, unadjusted odds ratio; WHO/IMCI, World Health Organization/integrated management of childhood illness recommendations; WHZ, weight-for-height z-score.

* Eaten ⩾10% less, ⩾"much less" healthy than usual, or lost ⩾5% weight.

† Any two of: eaten ⩾10% less, ⩾"much less" healthy than usual, or lost ⩾5% weight.

Table 12. Adjusted, multivariate logistic regression analysis by comparison*

	Comparison 1 WHZ-only vs. MUAC-only				Comparison 2 MUAC-only vs. WHZ&MUAC				Comparison 3 WHZ-only vs. all-MUAC			
	AOR	(95%	CIs)	*p*	AOR	(95%	CIs)	*p*	AOR	(95%	CIs)	*p*
Biochemical features												
Iron deficiency												
Body iron stores <0 mg/kg body weight	2.25	1.10	4.60	0.026	1.29	0.68	2.44	0.430	0.50	0.28	0.90	0.021
Adjusted serum ferritin <12 µg/L	2.13	1.11	4.09	0.024	0.97	0.54	1.75	0.920	0.35	0.21	0.60	0.000
Urinalysis												
Bilirubinuria, ≥15 µmol/L (1 mg/dL)	2.51	0.67	9.36	0.171	3.96	1.38	11.42	0.011	0.61	0.31	1.24	0.174
Leukocyturia, ≥25 leu/µL	0.55	0.25	1.21	0.136	1.46	0.73	2.91	0.286	1.77	0.86	3.66	0.123
Clinical Features												
Slow/very slow skin pinch	4.58	1.45	14.39	0.009	6.36	2.09	19.35	0.001	0.74	0.37	1.48	0.390
Sunken eyes	2.88	1.22	6.76	0.015	2.58	1.25	5.34	0.011	0.57	0.32	1.00	0.049
Restlessness/irritability	1.78	0.42	7.52	0.436	6.72	1.86	24.30	0.004	1.06	0.41	2.76	0.899
Some or severe dehydration	2.51	0.90	6.97	0.079	4.29	1.65	11.12	0.003	0.76	0.39	1.49	0.426
Dermatosis	1.25	0.54	2.92	0.605	4.56	1.72	12.10	0.002	3.43	0.95	12.46	0.061
Clinical iron deficiency												
Conjunctival and/or palmar pallor	1.42	0.86	2.33	0.166	1.93	1.20	3.12	0.007	0.99	0.65	1.52	0.967
Malaria	0.24	0.10	0.56	0.001	0.58	0.31	1.06	0.078	2.34	1.18	4.65	0.015
Visible severe wasting												
Visible ribs	2.44	0.73	8.20	0.149	10.00	2.71	36.85	0.001	0.59	0.20	1.74	0.338
Loose skin on arms or thighs	1.78	0.74	4.27	0.198	3.66	1.81	7.40	0.000	1.13	0.60	2.11	0.714
Visible back ribs or shoulder bones	2.88	1.07	7.81	0.037	8.36	2.98	23.44	0.000	0.79	0.32	1.91	0.595
Flesh missing/folds of skin on buttocks and/or ‘baggy pants’	2.04	0.46	9.00	0.345	6.46	1.81	23.02	0.004	1.25	0.43	3.63	0.676
WHO/IMCI: severe or extreme wasting	1.28	0.51	3.25	0.601	9.17	2.48	33.87	0.001	0.64	0.21	1.95	0.436
Recent health and nutrition history (caretaker-reported)												
Recent health deterioration	1.79	1.11	2.91	0.017	5.73	1.91	17.25	0.002	0.75	0.50	1.14	0.180
Any 1†	1.29	0.53	3.15	0.574	1.55	0.93	2.58	0.089	0.86	0.40	1.88	0.713
Any 2‡	1.13	0.55	2.36	0.735	6.12	2.17	17.27	0.001	0.93	0.52	1.66	0.796

Abbreviations: AOR, adjusted odds ratio; leu, leukocyte; MUAC, mid-upper arm circumference; WHO/IMCI, World Health Organization/integrated management of childhood illness recommendations; WHZ, weight-for-height *z*-score.

* Adjusted for age, sex, stunting, SSR and/or country

† Eaten ⩾10% less, ⩾"much less" healthy than usual, or lost ⩾5% weight

‡ Any two of: eaten ⩾10% less, ⩾"much less" healthy than usual, or lost ⩾5% weight

Serum leptin levels and anthropometric phenotype in SAM

Among the three anthropometric phenotypes, median serum leptin was lowest in patients with both low WHZ and low MUAC (180.10 pg/mL; IQR, 87.7-346.8), followed by those with low WHZ-only (215.80 pg/mL; IQR, 122.0-362.6), and highest in those with low MUAC-only (331.50 pg/mL; IQR, 159.0-560.1). Adjusted and non-adjusted models for pairwise comparisons further confirmed the statistical significance of the observed differences between the low MUAC-only patients and the two other groups (**Table 10**). As illustrated in **Figure 17**, this pattern was highly consistent across the three study contexts.

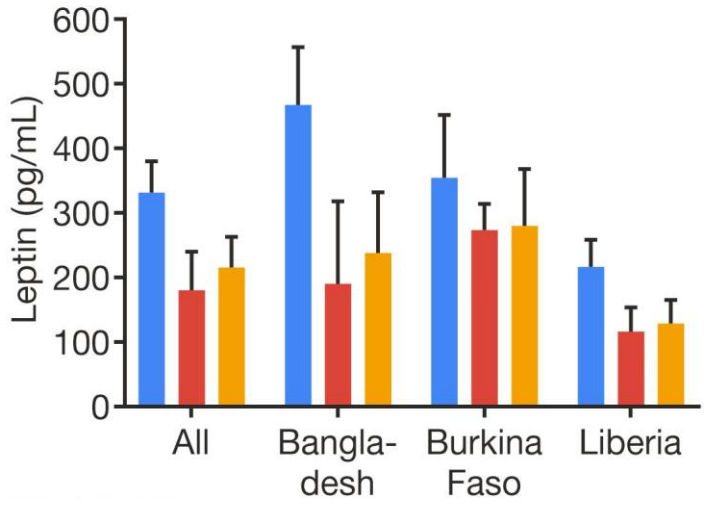

Figure 17. Median leptin (pg/mL) with IQR by anthropometric phenotype combined and by country

Abbreviations: MUAC, mid-upper arm circumference; WHZ, weight-for-height z-score.

MUAC-only; Both WHZ&MUAC; WHZ-only

There were relatively few children with serum leptin ⩽35 pg/mL; however the proportions of children under this cut-off followed a similar trend, with the highest rate of severe hypoleptinemia in the low WHZ and low MUAC category (**Table 7**). Leptin levels among all patients correlated far more strongly with WHZ (r=0.287, p<0.001) than with MUAC (r=0.102, p=0.043, **Figure 18**).

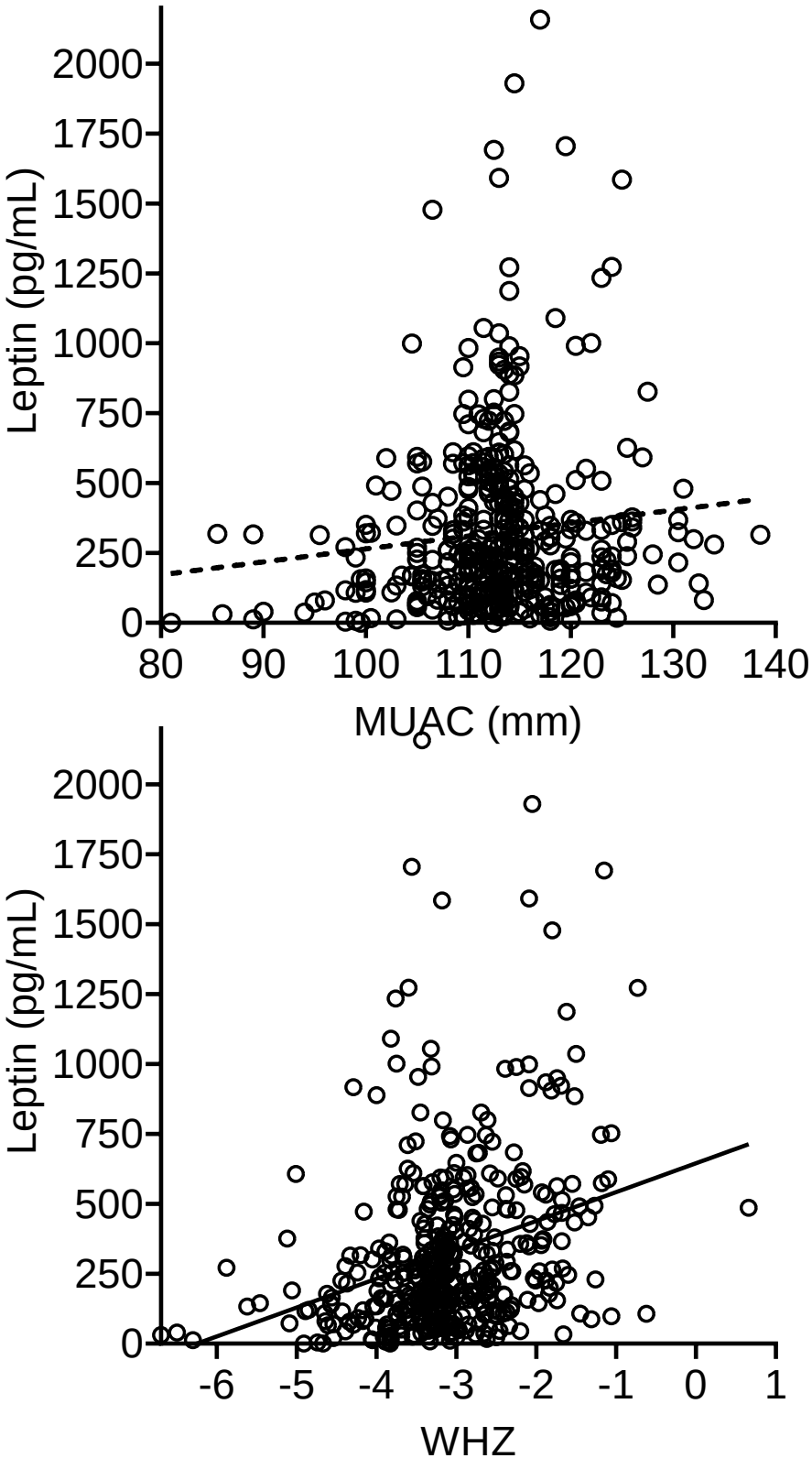

Figure 18. Linear regression fitted to leptin by WHZ and MUAC

Abbreviations: MUAC, mid-upper arm circumference; WHZ, weight-for-height z-score.

- - - - - MUAC, r=0.102, p=0.043,
--------- WHZ, r=0.287, p<0.001

Comparison 1: low WHZ-only vs. low MUAC-only

This comparison tests differences between children with low WHZ and low MUAC. Unadjusted median serum leptin was significantly lower (p=0.0009) in WHZ-only patients than in MUAC-only patients, and remained so after adjustment (p=0.002, **Table 10**).

WHZ-only children also had the lowest median adjusted ferritin levels (27.74 µL/L; IQR, 8.77-48.44, p=0.002, **Table 10**) and the highest proportions of iron deficiency as evidenced by low body iron stores (25% <0 mg/kg body weight, **Table 7**) and low adjusted serum ferritin (30% <12 µg/L, **Table 10**). WHZ-only patients were more likely to present with iron deficiency than MUAC-only patients, evidenced by both low body iron stores [unadjusted odds ratio (UOR, 2.11; p=0.016)] and low adjusted serum ferritin (UOR, 1.89; p=0.024). WHZ-only children also had a greater odds of bilirubinuria (UOR, 3.88; p=0.011) compared to MUAC-only children (**Table 11**).

Clinical features of dehydration, namely delayed skin pinch (UOR, 4.65; p=0.008), sunken eyes (UOR, 2.82; p=0.005) and finally some/severe dehydration itself (UOR, 3.29; p=0.018) were more common in children in the low WHZ-only group than in the low MUAC group (**Table 11**). After adjustment, WHZ-only patients were more likely to have visible ribs (AOR, 2.88; p=0.037) as well (**Table 12**).

Finally, caretakers of low WHZ-only children were more likely to report a recent deterioration in the health status of their children (UOR, 1.76; p=0.021); combined health/nutrition history indices showed that the caretakers were also more likely to respond 'yes' to 'any 1' (UOR, 1.87; p=0.015) or 'any 2' (UOR, 1.96; p=0.004) of the questions assessed (**Table 11**).

Conversely, MUAC-only children were three times more likely to present with malarial infection (UOR^{-1}, 0.33; p=0.003 than WHZ-only children (**Table 11**).

Comparison 2: combined low WHZ and low MUAC vs. low MUAC-only

This comparison tests the added effect of low WHZ in the presence of low MUAC. Children with both low WHZ and low MUAC had the lowest unadjusted median serum leptin of any diagnostic phenotype—significantly lower than that of MUAC-only children both before (p<0.001 and after adjustment (p<0.00001, **Table 10**).

Likewise, children with both low WHZ and low MUAC were more likely to exhibit manifestations of severe wasting (**Table 11**), including: visible ribs (UOR, 2.82; p=0.001), loose skin on the arms and/or thighs (UOR 3.54, p=0.000), visible back ribs and/or shoulder bones (UOR, 2.68; p=0.001), flesh missing from the buttocks, folds of skin on the buttocks and thighs, and/or 'baggy pants' (UOR 6.50, p=0.004), and severe or extreme wasting itself (UOR, 2.52; p=0.003).

Caretakers of those with both-low WHZ and low MUAC were more likely to report a recent deterioration in the health status of their children (UOR, 1.74; p=0.022, **Table 11**); after adjustment, combined health/nutrition history indices showed these caretakers were also likely to respond "yes" to "any 2" (AOR, 1.96; p=0.004, **Table 12**) of the questions assessed.

Children with both low WHZ and low MUAC were also more likely than low MUAC-only children to exhibit every clinical component of dehydration that we assessed (**Table 11**), including: delayed skin pinch (UOR, 6.26, p=0.001), sunken eyes (UOR, 2.58; p=0.009), restlessness/irritability (UOR, 6.04; p=0.005) as well as some/severe dehydration itself (UOR 2.68, p=0.007). Those with both low WHZ and low MUAC were also more likely to have dermatosis (UOR 2.68, p=0.007, **Table 11**) and conjunctival and/or palmar pallor (UOR, 1.91, p=0.008, **Table 11**).

Finally children with both low WHZ and low MUAC were more likely to present with bilirubinuria (UOR, 3.67; p=0.015, **Table 11**) than those with low MUAC alone.

Comparison 3: WHZ-only vs. all-MUAC

The final comparison identifies abnormalities in low WHZ children who would not be targeted for treatment within the framework MUAC-only screening program. Children with low WHZ-only presented with very similar profiles to the group of all children with low MUAC ("all-MUAC") on most clinical indicators.

Median serum leptin was not statistically different between these two phenotypes (**Table 10**). However, adjusted logistic analysis revealed that adjusted median serum ferritin was lower in WHZ-only participants (27.7 µL/L; IQR, 8.8-48.4) compared to all-MUAC participants (33.9 µL/L; IQR16.1-60.2], p=0.015, **Table 10**), and remained so after adjustment. Patients with low WHZ only were more likely to present with iron deficiency than all-MUAC patients, as evidenced by both low body iron stores (UOR^{-1}, 1.85; p=0.017) and low adjusted serum ferritin (UOR^{-1}, 1.92; p=0.007, **Table 10**).

Conversely, all-MUAC children were more likely to present with malarial infection (UOR, 2.39; p=0.013, **Table 11**) than low WHZ-only children.

Impaired nutrition and health status in all diagnostic groups

Biochemical profiles showed evidence of immunological stress and acute phase response (APR, *i.e.*, elevated AGP and/or CRP) in nearly two thirds (65.9%) of all patients, regardless of phenotype. More than half (54.6%) of all children had elevated sTfR. As determined by measurement of adjusted RBP, vitamin A insufficiency and deficiency were prevalent in 57.7% and 18.9% of children, respectively. Proteinuria (30.3%), hematuria (15.2%), glycosuria (1.9%) and urobilinogenuria (1.8%) were comparable among the three phenotypic groups

Clinical features of cough and/or difficult breathing were similar across the diagnostic phenotypes; the prevalence of cough (49.7%), nasal discharge (32.0%) and tachypnea (21.3%) were high while difficult breathing was relatively low (5.3%), and stridor (2.9%) and indrawing (1.8%) very low. Nearly one-third (31.2%) of children were diagnosed with pneumonia or severe pneumonia. Changes in hair color and/or consistency were relatively uncommon (6.7%) among all children. Eye infection, Bitot's spots, tachycardia and hypothermia were rare (**eTable 1** in **Supplement 1**). Groups were largely

similar in terms of history of present illness, with a high prevalence of diarrhea (23.3%), vomiting (17.4%), fever (54.6%).

False discovery rate correction for multiple testing

Unadjusted and adjusted analyses of continuous and categorical data, corrected for false discovery rates, are presented in **eTable 2**, **eTable 3**, and **eTable 4** in **Supplement 1**. FDR correction did not change the statistical significance of any unadjusted or adjusted analyses of continuous variables (*e.g.*, leptin and ferritin, **eTable 2**). For unadjusted analyses of categorical data, after FDR correction, one association was no longer statistically significant: comparison 3, 'any 2' (p=0.165, **eTable 3**). Finally, for adjusted analyses of categorical data, after FDR correction, two associations became marginally statistically significant (p<0.1): (1) comparison 1, sunken eyes, p=0.064; (2) comparison 1, 'general state of health,' p=0.064, (**eTable 4**); and three associations were no longer statistically significant: (1) comparison 1, 'visible back ribs or shoulder bones,' p=0.111; (2) comparison 3, 'sunken eyes,' p=0.305; (3) comparison 3, 'malaria,' p=0.225 (**eTable 4**).

Discussion

In this study, we described and compared biochemical features, clinical characteristics, and health/nutritional histories among children with SAM in an effort to clarify the pathophysiology and functional severity linked to anthropometric phenotype. We chose serum leptin as our primary outcome measure, based on our previous finding that hypoleptinemia in malnourished Ugandan infants and children was a robust biochemical predictor of acute mortality.[3] In our current study, generally all children with SAM (*i.e.*, with low WHZ and/or low MUAC), regardless of phenotype, presented with clinical evidence of nutritional deprivation and micronutrient deficiencies, but with significant heterogeneities on key criteria, including leptin. The trends were consistent across all three countries studied, lending credibility to our findings.

While the pathophysiology and functional severity associated with the different anthropometric phenotypes of SAM remain unclear,[13] policy makers and government stakeholders need robust scientific evidence to produce evidence-based guidance to appropriately target and prioritize SAM children for treatment. The use of MUAC as a single anthropometric assessment tool for SAM case finding and admission to therapeutic feeding programs, and the abandonment of WHZ, is increasingly promoted and applied.[83,299–301] Beyond practical arguments related to the attractive simplicity of the use of the MUAC tape, as compared with the determination of WHZ, it has long been assumed that SAM children presenting with WHZ <-3 but MUAC $\geqslant 115$ mm are not as gravely ill as children with low MUAC and have a higher risk of death.[25] Our findings challenge these assumptions.

We first compared clinical and biochemical markers of illness in children with low WHZ-only to those in children with low MUAC-only. We found that serum leptin was significantly lower in WHZ-only infants and children than in MUAC-only infants and children and far lower than in normally nourished children.[290] Leptin is produced by white adipocytes and its levels in circulation reflect the mass of subcutaneous, and to a lesser extent visceral, white adipose tissue. A low level of leptin suggests white adipose tissue depletion. Since white adipose represents the major bodily storage form of mobilizable energy, a low level of leptin likely represents a deficiency of body energy stores. Under conditions of severe malnutrition and other severe illnesses and stress, a lack of white adipose may limit the ability to sustain energy production for cardiopulmonary function and gluconeogenesis and thereby increase the risk of death.[3,4,219] Leptin is also a stimulus for immune cell development and function; a lack of leptin production or action in patients with mutations in leptin or the leptin receptor is associated with increased risk of infectious diseases.[302,303] Thus hypoleptinemia in infants

and children with SAM may impair immune function and predispose to mortality from sepsis and other major infectious diseases.[304–306]

There were additional biochemical and clinical differences between the WHZ-only and MUAC-only children. WHZ-only patients were more likely to present with biochemical evidence of iron deficiency (low ferritin or low body iron stores). Both low serum ferritin and low body iron stores are found frequently in SAM and represent depleted tissue iron reserves.[307,308] Severe iron deficiency and anemia have been linked to increased mortality in SAM children.[309]

In addition, WHZ-only patients were more often dehydrated than MUAC-only patients. We recognize the difficulty in assessing dehydration status in SAM children.[197] Nevertheless, clinical signs of severe dehydration have been linked to high mortality in hospitalized children with SAM in Bangladesh, Ethiopia and Kenya.[135,310–312]

Caretakers are able to perceive changes in their children's health and nutrition status,[313–316] concern over which leads them to seek care.[317] However, few studies have evaluated caretaker perception in undernourished children. In our study, caretakers of WHZ-only children were more likely to report a recent deterioration in their health status (with or without either recent weight loss and/or a recent reduction in food intake), in line with the aforementioned quantitative and objective indicators of nutritional and health status.

In sum, higher rates of dehydration, low iron stores, hypoleptinemia and deterioration in overall health status place low WHZ children at high risk of acute and long-term morbidity and mortality.

Next we examined the added effect of low WHZ in the presence of low MUAC. To that end, we compared children with both low WHZ and MUAC to those with low MUAC alone.

Among all diagnostic phenotypes, children with both low WHZ and low MUAC had the lowest serum leptin, significantly lower than low MUAC-only patients, and were more likely to present with bilirubinuria, suggesting more severe impairments in bile acid homeostasis.[318] Likewise children with both low WHZ and low MUAC were more dehydrated and visibly wasted than children with low MUAC alone. As noted previously, severe dehydration has been linked to high mortality in children with SAM in a number of settings in Asia and Africa.[135,310–312] Likewise, an inpatient study of SAM in Kenya showed that children with visible wasting and WHZ <–4 had highest risk of death.[140]

In sum, these findings suggest that children with both low WHZ and low MUAC are at higher risk of morbidity and mortality than children with low MUAC alone.

Finally, we compared children with low WHZ-alone to all-MUAC children (those with low MUAC with or without low WHZ); this comparison identifies abnormalities in low WHZ children who would be excluded from treatment in a low MUAC-only screening program.

While median serum leptin levels were lowest in children with both low WHZ and low MUAC, the levels in the broader all-MUAC group were not significantly different than those in children with low WHZ alone. Hypoleptinemia is a consequence of adipose depletion and is therefore observed in children with low WHZ alone as well as children with both low MUAC and low WHZ. On the other

hand median serum ferritin was significantly lower in WHZ-only children than in all-MUAC children; moreover, low body iron stores and low adjusted serum ferritin were significantly more prevalent in this phenotype. This observation suggests that iron deficiency was more prevalent in WHZ-only patients. Caretakers of WHZ-only children more frequently reported indexed health decline and/or weight loss or reduced food intake compared to all-MUAC children. Thus WHZ-only children are more likely to appear visibly compromised. These findings are supported by a range of clinical studies[7,24] as well as by direct observation of mortality risks in cohorts of patients[1] and in large community cohorts.[2,87] In line with our results, recent studies indicate that SAM children with low WHZ have risks of death that are comparable to, or higher than, those with low MUAC.[2,87] These observations, and the findings of our study, provide compelling evidence that malnourished children with low WHZ require intensive nutritional intervention even if MUAC exceeds 115 mm. Moreover, our findings suggest that children with both low MUAC and low WHZ are more vulnerable and at higher risk of mortality than children with low WHZ or low MUAC alone. Arguing in favor of the prioritization of children with both low WHZ and low MUAC seems justified, especially in situations of dramatically constrained resources such as sudden shortages in drugs or food commodities. The identification and adequate treatment of children with both low WHZ and low MUAC in resource-restricted situations would require measurement of WHZ alongside MUAC.

The only clinical characteristic that displayed an opposite trend was malaria prevalence: malaria was more common in low-MUAC children than in low-WHZ only children. This is consistent with observations made in a past study describing the profiles of the different sub-populations of SAM children.[24] Of note, malaria deaths were not related to anthropometrical status in a recent re-analysis of mortality risks associated with SAM case definitions, based on 87 deaths.[2] Studies that explore death due to malaria and anthropometric status are inconsistent.[36,319] Malaria diagnosis in community based-settings is unreliable because fever, the main symptom of malaria, is nonspecific. It is also unclear if low anthropometric status is a protective or risk factor for malaria.[320,321]

Outside these differences, our results reveal that all anthropometric phenotypes of SAM present with biochemical and clinical profiles characteristic of severe nutritional deprivation, marked by clinical dehydration and wasting, inflammation, and micronutrient deficiencies.

Limitations

This study has some limitations. First, heterogeneity in screening processes among countries most likely contributed to slight differences in age range, extent of anthropometric deficit, and clinical profiles in our cohort. An expansive, effective, community screening network in Bangladesh favored the timely detection and referral of very young MUAC-only children (median age 7.1 months; IQR, 6.16-9.94) with moderate MUAC deficit (median MUAC 112 mm; IQR 110-113); nearly 2/3rds of the sample in Bangladesh was identified by active screening. MUAC <115 mm has been shown to be strongly associated with young age.[7,11,12,71,73,77,78] In Burkina Faso, the research team recruited approximately 50% of participants by way of passive screening at growth monitoring and promotion visits, which primarily focus on children under 2 years of age.[322] Because of this, 90% of participants recruited were 24 months old or younger in Burkina Faso, compared to 45 months old or younger in

Bangladesh. The age range in Liberia is very similar to that of Burkina Faso. However, in Liberia, half of all patients were treated in either one urban hospital or in two urban community clinics that serve the catchment area encompassing the biggest slum in the country's capital: West Point. Systematic passive screening for SAM is mandatory for all children under five that enter the premises of a health center or hospital in Liberia. Because of this, a vast majority of patients were identified and referred to our study because their caretakers had sought medical attention for them. In an effort to address this potential selection bias, we created an artificial active community-screening network because no community screening activities existed at time of study implementation. Trained nursing students conducted door-to-door active screening in the West Point slum community, and referred all children with MUAC <125 mm for full anthropometric screening by both MUAC and WHZ at our nearest study clinic. These screening procedures may have introduced selection biases in our sample. Yet, the multi-centric design of our study aimed to create a cohort of children with SAM that matches the population detected and referred to treatment in the catchment areas of CMAM programs globally. Active community screening, growth monitoring and promotion, and passive screening are all major routes that lead children with SAM to care. We consider these heterogeneities to accurately represent the screening procedures used for SAM diagnosis (and their limits), and to contribute to the generalizability of our results.

A second limitation is that we focused recruitment on uncomplicated SAM cases only. By strict definition, uncomplicated SAM refers to patients without fever, infection, or complicated disease according to the Integrated Management of Childhood Illness guidelines, and with an appetite sufficient to consume ready-to-use therapeutic food (RUTF); uncomplicated patients represent about 80% of all SAM children.[323] Our sample included patients with fever and infection, but did not include patients with IMCI danger signs (such as inability to drink and/or breastfeed, uncontrolled vomiting, convulsions, lethargy and/or unconsciousness) or anorexia. We excluded these patients for two reasons: (1) we assumed that the superimposition of life-threatening illnesses in complicated SAM would complicate interpretation of biochemical data; and (2) uncomplicated SAM represents 80% of SAM worldwide. This restriction, however, limits the generalizability of our results to this less severe, uncomplicated sub-group of SAM.

Conclusions

The use of MUAC as a single assessment tool for identification of infants and children with SAM, and the abandonment of WHZ, is increasingly promoted and applied in developing countries around the world.[83,299–301] Our results challenge this widespread operational and advocacy strategy. We find that children with low WHZ have deficits in nutritional status, hydration, and iron balance more severe than those in children with low MUAC and they have lower levels of leptin, a marker of mortality risk in SAM. The addition of WHZ to MUAC is required to identify a sub-population of case at highest risks of acute and post-discharge morbidity and death.

A research priority should be to harness technological capacity to develop innovative diagnostic methods to identify low WHZ children in the community; work on this is already underway.[324,325] Until then, both WHZ and MUAC should be retained as independent diagnostic criteria for SAM, in line with repeated WHO recommendations.

Supplement 1

Supplementary tables

eTable 1. Supplementary clinical characteristics at admission*

	No. (%) of patients by anthropometric category			
	WHZ-only (n=138)	WHZ&MUAC (n=152)	MUAC-only (n=161)	p†
Eye infection	1 (2/136)	1 (2)	1 (2/160)	0.986
Bitot's spots	1 (1)	1 (2)	1 (1)	0.783
Tachycardia	7 (9)	5 (8)	6 (9)	0.894
Hypothermia (<36.0°C)	4 (5)	5 (8)	2 (4)	0.433

Abbreviations: MUAC, mid-upper arm circumference; WHZ, weight-for-height z-score.

* Values are % (n).

† For 3 group comparison (WHZ-only, both-WHZ&MUAC, and MUAC-only) by Pearson's χ^2 test (or Fisher's exact tests for small sample sizes) for categorical data and Kruskal-Wallis tests for skewed, continuous data.

eTable 2. FDR corrected unadjusted and adjusted median serum leptin and serum ferritin by comparison*

	Comparison 1							Comparison 2							Comparison 3						
	WHZ only			MUAC only				MUAC-only			MUAC&WHZ				WHZ-only			All-MUAC			
	Median	95%	CI	Median	95%	CI	*p*	Median	95%	CI	Median	95%	CI	*p*	Median	95%	CI	Median	95%	CI	*p*
Serum leptin, pg/mL																					
Unadjusted median†	215.8	122.0	346.6	331.5	159.0	560.1	0.002	331.5	159.0	560.1	180.1	87.7	346.8	<0.0001	215.8	122.0	346.6	245.8	117.0	482.0	0.212
Adjusted median	232.5	193.7	263.0	365.0	226.4	372.8	0.004	352.0	226.6	410.1	212.0	139.0	239.8	<0.00001	220.1	193.9	309.0	262.0	223.0	331.4	0.462
Serum ferritin, µL/L																					
Unadjusted median†	27.7	8.8	48.4	33.6	15.9	54.3	0.064	33.6	15.9	54.3	34.0	16.4	67.7	0.488	27.7	8.8	48.4	33.9	16.1	60.2	0.015
Adjusted median	33.5	8.7	34.7	33.0	23.3	51.0	0.809	31.2	28.2	36.6	36.1	30.7	41.2	0.219	28.5	20.6	32.7	37.2	25.0	41.8	0.250

Abbreviations: CI, confidence interval; FDR, false discovery rate; MUAC, mid-upper arm circumference; WHZ, weight-for-height *z*-score.

* Adjusted for age, sex, stunting, SSR and country.

† The Wilcoxon rank-sum test is used for equality median when the unadjusted median is presented.

eTable 3. FDR corrected unadjusted, bivariate logistic regression analysis by comparison

	Comparison 1: WHZ-only vs. MUAC-only				Comparison 2 MUAC-only vs. WHZ&MUAC				Comparison 3 WHZ-only vs. all-MUAC			
	UOR	(95%	CIs)	*p*	UOR	(95%	CIs)	*p*	UOR	(95%	CIs)	*p*
Biochemical features												
Iron deficiency												
Body iron stores, <0 mg/kg body weight	2.11	1.15	3.88	0.024	1.29	0.68	2.43	0.870	0.54	0.32	0.90	0.017
Adjusted serum ferritin, <12 µg/L	1.89	1.09	3.28	0.024	0.97	0.54	1.74	0.908	0.52	0.32	0.84	0.014
Urinalysis												
Bilirubinuria, ⩾15 µmol/L (1 mg/dL)	3.88	1.36	11.05	0.022	3.67	1.29	10.45	0.030	0.59	0.29	1.18	0.134
Leukocyturia, ⩾25 leu/µL	0.55	0.25	1.21	0.138	1.52	0.77	2.99	0.225	2.29	1.14	4.58	0.038
Clinical Features												
Slow/very slow skin pinch	4.65	1.48	14.60	0.032	6.26	2.06	19.01	0.005	0.73	0.36	1.47	0.593
Sunken eyes	2.82	1.37	5.78	0.024	2.58	1.27	5.24	0.012	0.61	0.35	1.06	0.299
Restlessness/irritability	2.64	0.66	10.55	0.211	6.04	1.70	21.46	0.010	1.27	0.51	3.15	0.694
Some or severe dehydration	3.29	1.22	8.83	0.045	4.29	1.65	11.11	0.008	0.76	0.39	1.50	0.593
Dermatosis	1.18	0.51	2.72	0.694	2.68	1.31	5.51	0.012	1.49	0.76	2.95	0.529
ID												
Conjunctival and/or palmar pallor	1.42	0.86	2.33	0.211	1.91	1.19	3.08	0.012	0.98	0.64	1.50	0.977
Malaria	0.33	0.16	0.68	0.024	0.59	0.32	1.08	0.092	2.39	1.21	4.74	0.593
Visible severe wasting												
Visible ribs	1.80	1.00	3.26	0.095	2.82	1.53	5.18	0.005	0.61	0.35	1.06	0.299
Loose skin on arms or thighs	1.59	0.74	3.39	0.270	3.54	1.76	7.11	0.005	1.27	0.51	3.15	0.694
Visible back ribs or shoulder bones	1.65	0.92	2.94	0.151	2.64	1.47	4.73	0.005	0.76	0.39	1.50	0.593
Flesh missing/folds of skin on buttocks and/or ‘baggy pants’	1.84	0.43	7.95	0.442	6.50	1.84	22.96	0.008	1.93	0.70	5.32	0.520
WHO/IMCI: severe or extreme wasting	1.53	0.84	2.77	0.211	2.52	1.36	4.67	0.008	1.01	0.59	1.71	0.977
Health and nutrition history												
General state of health	1.76	1.09	2.84	0.045	1.74	1.08	2.79	0.028	0.77	0.51	1.16	0.520
Any 1*	1.87	1.13	3.08	0.044	1.55	0.96	2.49	0.083	0.66	0.42	1.03	0.299
Any 2†	1.96	1.23	3.11	0.024	1.35	0.86	2.11	0.189	0.59	0.40	0.89	0.165‡

Abbreviations: CI, confidence interval; FDR, false discovery rate; leu, leukocyte; MUAC, mid-upper arm circumference; UOR, unadjusted odds ratio; WHO/IMCI, World Health Organization/integrated management of childhood illness recommendations; WHZ, weight-for-height *z*-score.

* Eaten ≥10% less, ≥"much less" healthy than usual, or lost ≥5% weight.

† Any two of: eaten ≥10% less, ≥"much less" healthy than usual, or lost ≥5% weight.

‡ No longer statistically significant after FDR correction.

eTable 4. FDR corrected, adjusted, multivariate logistic regression analysis by comparison*

	Comparison 1 WHZ-only vs. MUAC-only				Comparison 2 MUAC-only vs. WHZ&MUAC				Comparison 3 WHZ-only vs. all-MUAC			
	AOR	(95%	CIs)	*p*	AOR	(95%	CIs)	*p*	AOR	(95%	CIs)	*p*
Biochemical features												
Iron deficiency												
Body iron stores <0 mg/kg body weight	2.25	1.10	4.60	0.026	1.29	0.68	2.44	0.860	0.50	0.28	0.90	0.021
Adjusted serum ferritin <12 µg/L	2.13	1.11	4.09	0.026	0.97	0.54	1.75	0.920	0.35	0.21	0.60	0.000
Urinalysis												
Bilirubinuria, ⩾15 µmol/L (1 mg/dL)	2.51	0.67	9.36	0.171	3.96	1.38	11.42	0.060	0.61	0.31	1.24	0.174
Leukocyturia, ⩾25 leu/µL	0.55	0.25	1.21	0.171	1.46	0.73	2.91	0.225	1.77	0.86	3.66	0.174
Clinical Features												
Slow/very slow skin pinch	9.47	2.07	43.25	0.028	6.36	2.09	19.35	0.002	0.74	0.37	1.48	0.818
Sunken eyes	2.88	1.22	6.76	0.064§	2.58	1.25	5.34	0.012	0.57	0.32	1.00	0.305‖
Restlessness/irritability	1.78	0.42	7.52	0.544	6.72	1.86	24.30	0.006	1.06	0.41	2.76	0.963
Some or severe dehydration	2.51	0.90	6.97	0.196	4.29	1.65	11.12	0.005	0.76	0.39	1.49	0.818
Dermatosis	1.25	0.54	2.92	0.648	2.68	1.29	5.60		3.43	0.95	12.46	0.305
Clinical iron deficiency												
Conjunctival and/or palmar pallor	1.42	0.86	2.33	0.297	1.93	1.20	3.12	0.010	0.99	0.65	1.52	0.967
Malaria	0.24	0.10	0.56	0.015	0.58	0.31	1.06	0.084	2.34	1.18	4.65	0.225‖
Visible severe wasting												
Visible ribs	2.44	0.73	8.20	0.297	10.00	2.71	36.85	0.002	0.59	0.20	1.74	0.818
Loose skin on arms or thighs	1.78	0.74	4.27	0.297	3.66	1.81	7.40	0.002	1.13	0.60	2.11	0.892
Visible back ribs or shoulder bones	2.88	1.07	7.81	0.111	8.36	2.98	23.44	0.001	0.79	0.32	1.91	0.892
Flesh missing/folds of skin on buttocks and/or 'baggy pants'	2.04	0.46	9.00	0.470‖	6.46	1.81	23.02	0.006	1.25	0.43	3.63	0.892
WHO/IMCI: severe or extreme wasting	2.28	0.68	7.71	0.297	9.17	2.48	33.87	0.002	0.64	0.21	1.95	0.818
Health and nutrition history												
General state of health	1.79	1.11	2.91	0.064§	6.82	2.21	21.00	0.002	0.75	0.50	1.14	0.675
Any 1†	1.29	0.53	3.15	0.648	1.55	0.93	2.58	0.089	0.86	0.40	1.88	0.892
Any 2‡	1.13	0.55	2.36	0.735	6.12	2.17	17.27	0.002	0.93	0.52	1.66	0.918

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; FDR, false discovery rate; leu, leukocyte; MUAC, mid-upper arm circumference; WHO/IMCI, World Health Organization/integrated management of childhood illness recommendations; WHZ, weight-for-height *z*-score.

* Adjusted for age, sex, stunting, SSR and/or country
† Eaten ⩾10% less, ⩾"much less" healthy than usual, or lost ⩾5% weight
‡ Any two of: eaten ⩾10% less, ⩾"much less" healthy than usual, or lost ⩾5% weight
§ Marginally statistically significant (p<0.1) after FDR correction
‖ No longer statistically significant after FDR correction

V ARTICLE 2

Weaning and stunting affect nitrogen and carbon stable isotope natural abundances in the hair of young children

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Abstract: Natural abundances of stable nitrogen and carbon isotopes ($\delta^{15}N$ and $\delta^{13}C$) can vary with both dietary intake and metabolic (specifically catabolic) state. In low-income countries, weaning is a period of dietary transition from milk to plant-based foods and a high-risk period for malnutrition. We explored how diet and malnutrition impact hair $\delta^{15}N$ and $\delta^{13}C$ in young children by an observational, cross-sectional study in Cox's Bazar District, Bangladesh [255 children, 6-59 months with 19.6% wasted (7.1% severely) and 36% stunted (9.8% severely)]. Hair $\delta^{15}N$ and $\delta^{13}C$ exhibited exponential decreases with age, with the loss of one trophic level (3.3‰ and 0.8‰, respectively) from 6 to 48 months, which we associate with the shift from exclusive breastfeeding to complete weaning. After adjustment for age and breastfeeding status, hair isotopic values were unaffected by wasting but lower in severe stunting (–0.45‰ to –0.6‰, $p<0.01$). In this population of young children, whose isotopic values in hair primarily depended on age, we failed to observe any effect of wasting, likely due to opposite, compensating effects between the dietary and metabolic changes involved. In contrast, we evidenced low $\delta^{15}N$ and $\delta^{13}C$ values in severely stunted children that likely indicate chronic exposure to diets low in animal products.

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Introduction

The natural abundances of stable carbon and nitrogen heavy isotopes ($\delta^{13}C$ and $\delta^{15}N$) in body proteins reflect those of the diet, plus a small discrimination factor, known as the trophic step, which is due to the preferential elimination of light isotopes and is smaller for carbon (+1‰) than for nitrogen (+3-4‰).[233,235] Since $\delta^{13}C$ and $\delta^{15}N$ values in the body primarily reflect those of the diet, they have long been used in ecology and archaeology to reconstruct trophic webs and characterize ancient diets.[326–329] More recently, they have been used in epidemiology as biomarkers to assess the consumption of fish, animal products, and sugar.[236,239,330–333] In this regard, measuring isotopic natural abundances in hair has several advantages. Hair is easily and non-invasively sampled and its isotopic values correlate well with those in other body proteins. Moreover, hair keratin is an ideal isotopic archive because it is produced sequentially and is inert after synthesis,[246,247] provided that a sufficient number of follicles is sampled to limit the potential bias due to the presence of a small proportion of hair in the telogen phase (*i.e.*, not growing).[247,334,335] Considering an average hair growth rate of 1 cm per month and a one-week delay between hair synthesis and bulb exit, the 5 mm of hair closest to the scalp contains isotopic information for the period between –3 and –1 weeks before sampling.[247]

Moreover, it is now recognized that the isotopic discrimination between the body and the diet (*i.e.*, the trophic step) varies between subjects according to their particular metabolic orientation, leading to changes in $\delta^{13}C$ and $\delta^{15}N$ in body proteins. For example, an anabolic shift, as observed during pregnancy, is associated with a decrease in hair $\delta^{15}N$ due to a decrease in the nitrogen trophic step.[244] Conversely, a catabolic shift with weight loss, as observed during anorexia nervosa or in women experiencing nausea during early pregnancy, has been associated with an increase in $\delta^{15}N$ and a decrease in $\delta^{13}C$ in hair.[245–247] In rats submitted to prolonged caloric restriction with maintained protein intake, we recently evidenced tissue $\delta^{15}N$ and $\delta^{13}C$ variations resulting from metabolic adaptations; in particular, we observed that increased amino acid orientation towards the transamination and deamination pathways fueling gluconeogenesis, and the mobilization of muscle ^{15}N-enriched amino acids, resulted in an increase in $\delta^{15}N$.[243] Thus, beyond simple markers of dietary exposure, hair $\delta^{13}C$ and $\delta^{15}N$ measurements could also be used to identify a catabolic state characterized by the mobilization of body proteins for new protein synthesis, which is believed to be part of the natural history of acute malnutrition in children.

Early childhood is a period of dietary transition, with the gradual shift from infant-specific, milk-based feeding to family foods. In low-income countries, weaning is known to be a high-risk period for malnutrition, in particular because of the introduction of inadequate complementary foods with

low protein content and low energy density. In these countries, poor sanitary conditions are associated with a high risk of infection during early childhood, which further increases the risk of malnutrition.[99,336–339] Malnutrition can occur in two forms: (1) wasting (*i.e.*, acute malnutrition) is a rapid loss of fat and muscle mass due to recent food deprivation and/or disease; (2) stunting (*i.e.*, chronic malnutrition) is the consequence of a progressive alteration in linear growth, caused by long-term insufficient nutrient intake and recurrent infections. Stunting has been recently described as a consequence of repeated episodes of wasting.[89] Although archaeologists frequently use $\delta^{13}C$ and $\delta^{15}N$ measurements in hair or bone collagen to identify weaning practices in ancient civilizations,[340–343] few data are available on the evolution of $\delta^{13}C$ and $\delta^{15}N$ during this period in contemporary populations.[344,345] Additionally, to what extent hair $\delta^{13}C$ and $\delta^{15}N$ values are affected by wasting or stunting during the weaning period remains to be defined.

In this context, the aim of the present study was to assess the association between hair $\delta^{13}C$ and $\delta^{15}N$, the diet and the severity of wasting or stunting in a representative population of young (6-59 months) children in Bangladesh.

Methods

Ethical considerations

Ethical approval was obtained from the Ethics Committee at the University Hospital of Antwerp and the University of Antwerp (B300201627243) and the National Research Ethics Committee (NREC) at the Bangladesh Medical Research Council (BMRC) (BMRC/NREC/2016-2019/1463). All parents and/or legal guardians of participants were asked to sign an informed consent form and those who were illiterate indicated consent by inked thumbprint in the signature space. All research described in this manuscript was performed in accordance with relevant guidelines and regulations.

Population

This observational, cross-sectional study was conducted on a sub-sample of children randomly drawn from a larger nutritional survey following the Standardized Monitoring and Assessment of Relief and Transitions (SMART) methodology and conducted in Ukhiya and Teknaf Upazillas, in the Cox's Bazar District, Bangladesh. Sample size was calculated based on the SMART two-stage sampling methodology, using ENA software, in order to produce a precise estimate of the prevalence of Global Acute Malnutrition (GAM, defined as weight-for-height *z*-score (WHZ) < –2 SD and/or bilateral pitting edema).[346] A total of 1,095 households and 728 children were reached in the SMART survey; in this study, only 665 caretakers agreed that their children participate in the stable isotope analysis (SIA) sub-study, of which only 655 children had hair at the time of the investigation. A random sample of 255 children was selected for isotopic analysis from these 665 participants. However, because the number of severely wasted children in the SMART study was very low (28 or 4.3%, **eTable 5**), we decided to oversample this category of children to reach a sufficient group size to allow for statistical comparisons. Therefore all severely wasted children recruited in the SMART survey with enough hair to perform isotopic analyses (n=18) were included in the final sample. The prevalences of stunting and wasting in the final sample were close to those measured in the SMART survey, conducted on a representative sample of children in Cox's Bazar, and close to the prevalences of the joint malnutrition estimates for south Asia issued by UNICEF/WHO/World Bank in 2019.[29]

Data handling

All data were collected using structured questionnaires written in Bengali, and information was recorded on children's morbidity and diet from immediate caregivers, which were usually mothers. The dietary diversity score (DDS) was calculated based on 7 food groups (grains roots and tubers,

legumes and nuts, dairy products, flesh foods, eggs, vitamin A-rich fruits and vegetables, other fruits and vegetables) by summing the number of unique food groups eaten by the child during the last 24 hours. Breastfeeding status was assessed based on current breastfeeding and DDS over the past 24 hours, as follows: (1) exclusive breastfeeding: currently breastfed and DDS=0; (2) partially breastfeeding: currently breastfed and DDS >0; (3) fully weaned: not currently breastfed. Anthropometric measurements were conducted by trained investigators based on WHO standardized procedures.[280] Naked or minimally clothed children were weighed to the nearest 0.1 kg using portable Salter spring scales while recumbent length or standing height was measured to the nearest 0.1 cm using standard UNICEF height boards. Mid-upper arm circumference (MUAC) was measured on the left arm to the nearest 1 mm using a standard MUAC tape. Data were recorded on a mix of hard-copy, paper print-outs (then entered into EPI Info and Microsoft Excel) and tablets using Open Data Kit (ODK). Anthropometric indices (*i.e.*, BMI, HAZ, WAZ, and WHZ) were calculated using WHO Growth Standards.[56] Stunting (chronic malnutrition) was defined as HAZ < –2 SD and severe stunting as HAZ < –3 SD. Wasting (acute malnutrition) was defined as WHZ < –2 SD and/or MUAC < 125 mm and severe wasting as WHZ < –3 SD and/or MUAC < 115 mm.

Hair collection and measurement of isotopic natural enrichments

In these children, a sample of 50 hair follicles was covered in hair gel, twisted into a lock and cut as close to the patient's scalp as possible with sharp, dissecting scissors. The gelled lock was then taped to a sheet of paper and the cut end was labeled and stored at room temperature before further analysis.

Two consecutive segments of 5 mm on the cut end of the lock were removed and transferred into Eppendorf tubes. Hair gel was removed by two consecutive immersions in 750 µL of ultrapure water for 2 minutes with gentle agitation. Hair samples were then delipidated in 1.5 mL of ether/ethanol (1:1) for 30 minutes, washed in 1.5 mL of ultrapure water for 2 minutes, delipidated once more for 30 minutes, and finally washed thrice consecutively for 2 minutes each, all while being gently agitated. Samples were dried overnight before being transferred in 2 mg tin capsules and analyzed by elemental analysis/isotope-ratio mass spectrometry, using an elemental analyzer (EA Vario Micro Cube, Elementar, Germany) coupled with an isotope-ratio mass spectrometer (Isoprime, VG instruments, Manchester, UK). Tyrosine ($\delta^{15}N$ = 10 ‰ and $\delta^{13}C$ = –23.20‰) was used for calibration and drift correction. The natural abundances of ^{15}N and ^{13}C in hair were expressed relative to standards (atmospheric N_2 for $^{15}N/^{14}N$ and Vienna Pee Dee Belemnite for $^{13}C/^{12}C$) using the delta notation, according to the following equation:

$$\delta\ (‰) = 1000 \times (R_{sample} - R_{standard})/R_{standard}$$

where R_{sample} and $R_{standard}$ stand for the ratio between heavy and light isotope ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) in the sample and standard, respectively. The measurement precision was very good, as assessed by repeated measurements of standards with SD of 0.07 ‰ for both $\delta^{15}N$ and $\delta^{13}C$.

Data analysis

All statistical analyses were performed using statistical analysis software (SAS 9.4, Cary, NC, USA). Unless otherwise specified, all data are presented as mean ± SD or as *n* (frequency). The only exceptions are data adjusted for age and/or breastfeeding status. In this case, we present least-square means estimated from linear models where age and/or breastfeeding status were included as covariates, and their associated standard error (that estimate the accuracy of the estimation of these least-square means). Frequency comparisons between non stunted/wasted children (controls) and (severely) stunted/wasted children were conducted using χ^2 tests. Pearson bivariate correlations were as used to assess the associations between isotopic measurements and the anthropometric characteristics of the subjects. Bivariate and multivariate logistic regression was used to assess the link between age, weaning status, $\delta^{15}N$ and $\delta^{13}C$ and the odds of being (severely) stunted or wasted. The Gauss-Newton method was used for non-linear adjustment of $\delta^{15}N$ and $\delta^{13}C$ with age. The effect of (severe) stunting or wasting on continuous variables was assessed using analysis of variance with post-hoc Tukey-Kramer tests. Regarding the effect of (severe) stunting or wasting on $\delta^{15}N$ and $\delta^{13}C$, age and breastfeeding status were included as covariates in the model as they are major determinants of hair $\delta^{15}N$ and $\delta^{13}C$. The validity of covariance analyses was verified by inspecting the normality of model residuals using the Shapiro-Wilk tests. Significance tests were two-sided and performed at the $\alpha=0.05$ or $\alpha=0.01$ levels.

Statistical analyses for $\delta^{15}N$ and $\delta^{13}C$ were conducted using isotopic data obtained from the 5 mm hair segment closest to the scalp and the mean of the two adjacent 5 mm segments closest to the scalp. These two analyses lead to very similar numerical results and identical conclusions. Therefore, only results from the analysis of isotopic data on the 5 mm segment closest to the scalp are presented in this article. A mixed model for repeated data was also used to analyze the evolution of $\delta^{15}N$ and $\delta^{13}C$ along the hair follicle and look for a possible influence of stunting or wasting on this evolution. This analysis and its main results are described in the **Supplementary Analyses** section of **Supplement 2.**

Results

Population description

The final number of children with hair isotopic measurements was 255, with a mean age of 33.7 months, a sex ratio close to 50%, and approximately one third of the population partially or exclusively breastfed (**Table 13**).

Table 13. Main characteristics of the population*

	Population characteristics (n=255)
Age (months)	33.7 ± 15.4
Height-length (cm)	86.7 ± 10.8
Weight (kg)	11.2 ± 2.6
Female	120 (47.1%)
Breastfed	85 (33.3%)
Wasted	50 (19.6%)
Severely wasted	18 (7.1%)
Stunted	93 (36.5%)
Severely stunted	25 (9.8%)

* Values are mean ± SD or n (%).

Moreover, ~20% of the population was wasted (~7% severely) and ~36% was stunted (~10% severely). The characteristics of our study population were similar to those of the representative sample of children surveyed during the overarching nutritional survey (n=654), from which our population was randomly sub-sampled (**eTable 5**), except for a higher proportion of children with severe acute malnutrition (7.1% *vs.* 4.3%).

In accordance with the respective anthropometric criteria by which they were diagnosed, wasted children had a lower mid-upper arm circumference (MUAC) and/or weight-for-height z-score (WHZ) than non-wasted children, while stunted children had a lower height-for-age z-score (HAZ) than non-stunted children (**Table 14**).

Table 14. Characteristics of children with wasting or stunting

Characteristic	No wasting (n=205)	Wasting (n=50)	Severe wasting (n=18)
Female	103 (49.7 %)	17 (34 .0 %)*	8 (44.4%)
Weaned	145 (70.7 %)	25 (50.0 %)*	7 (38.9%)*
Age (months)	34.6 ± 14.9	29.4 ± 16.7	25.5 ± .17.0*
DDS	4.7 ± 1.6	4.1 ± 1.7	3.7 ± 2.0*
WAZ	–1.20 ± 0.96	–2.88 ± 0.87*	–3.10± 1.07*
HAZ	–1.48 ± 1.13	–1.93 ± 1.50*	–1.43 ± 1.67
MUAC (mm)	147.0 ± 10.3	125.9 ± 11.1**	120.8 ± 15.0**
WHZ	–0.51 ± 0.90	–2.52 ± 0.68**	–3.13 ± 0.57**
Stunting	70 (34%)	23 (46.0%)	5 (27.8%)
Characteristic	No stunting (n=162)	Stunting (n=93)	Severe stunting (n=25)
Female	76 (46.9 %)	44 (47.3%)	13 (52.0%)
Weaned	104 (64.2 %)	66 (70.9 %)	18 (72.0%)
Age (months)	32.2 ± 16.6	36.1 ± 12.8	35.9 ± 13.1
DDS	4.5 ± 1.8	4.6 ± 1.5	4.8 ± 1.4
WAZ	–1.14 ± 1.12	–2.21 ± 0.87**	–2.80 ± 0.90**
HAZ	–0.92 ± 0.91	–2.73 ± 0.74**	–3.71 ± 0.83**
MUAC (mm)	144.3 ± 14.2	140.5 ± 11.7	137.0 ± 15.7*
WHZ	–0.84 ± 1.21	–0.96 ± 1.09	–0.96 ± 1.15
Wasting	27 (16.6%)	23 (24.7%)	9 (36.0%)

Abbreviations: DDS, dietary diversity score; WAZ, weight-for-age z-score; HAZ, height-for-age z-score; MUAC, mid-upper arm circumference; WHZ, weight-for-height z -score.

Values are mean ± SD or n (%). ** and *, significant different versus control (p<0.01 and p<0.05, respectively). Children with severe wasting or severe stunting represent sub-populations of children with wasting or stunting, respectively

In addition, compared to non-wasted children, wasted children were also younger, more often boys and fully or partially breastfed, and had a lower dietary diversity score (DDS), a lower HAZ and a lower weight-for-age z-score (WAZ). In contrast, compared to non-stunted children, stunted children were similar in terms of age, sex ratio and DDS but had a lower WAZ and MUAC (for severely stunted only).

In bivariate logistic regression, age was weakly and negatively associated with the risk of wasting while still being breastfed was a strong determinant for wasting. Neither age nor weaning status were associated with the risk of stunting (**eTable 6**).

Hair $\delta^{15}N$ and $\delta^{13}C$: links with anthropometric parameters

The measured C/N ratios in hair (3.14 ± 0.17, mean ± SD) were close to the theoretical value of keratin, confirming that sample preparation (*i.e.*, cleaning and delipidation) was carried out with care, and were not statistically different across conditions (breastfeeding, wasting or stunting).

For the 255 children included, hair $\delta^{15}N$ and $\delta^{13}C$ values were 8.74 ± 1.25‰ and -22.18 ± 0.75‰ (mean ± SD), respectively, and did not vary between boys and girls (**eTable 7**). For the 7 exclusively breastfed children (breastfed and DDS =0), the $\delta^{15}N$ and $\delta^{13}C$ values (11.45 ± 0.69 ‰ and –22.01 ± 0.44‰, respectively) were significantly higher compared to those of the 170 weaned children (8.19

± 0.78 ‰ and –22.32 ± 0.76‰, **eTable 7**). Both $\delta^{15}N$ and $\delta^{13}C$ were negatively correlated with age, height and weight (**Table 15**).

Table 15. Pearson correlations coefficients between the hair natural abundances of stable nitrogen and carbon isotopes ($\delta^{15}N$ and $\delta^{13}C$) and age and anthropometric indicators (n=255)

	$\delta^{15}N$	$\delta^{13}C$
Age	-0.63***	-0.32***
Height	-0.60***	-0.23**
Weight	-0.55***	-0.24***
MUAC	-0.29***	-0.08
Head circumference	-0.34***	-0.12
BMI	0.11	0.01
HAZ	0.21**	0.22**
WHZ	-0.06	-0.07

Abbreviations: HAZ, height-for-age z-score; MUAC, mid-upper arm circumference; WHZ, weight-for-height z-score.

***p<0.0001, **p<0.01.

Hair $\delta^{15}N$ was also negatively correlated with head circumference and MUAC. Moreover, both $\delta^{15}N$ and $\delta^{13}C$ were positively correlated with HAZ.

Hair $\delta^{15}N$ and $\delta^{13}C$: trajectories during weaning

Plotting $\delta^{15}N$ or $\delta^{13}C$ against age showed an exponential decay between 6 and 60 months (**Figure 19**).

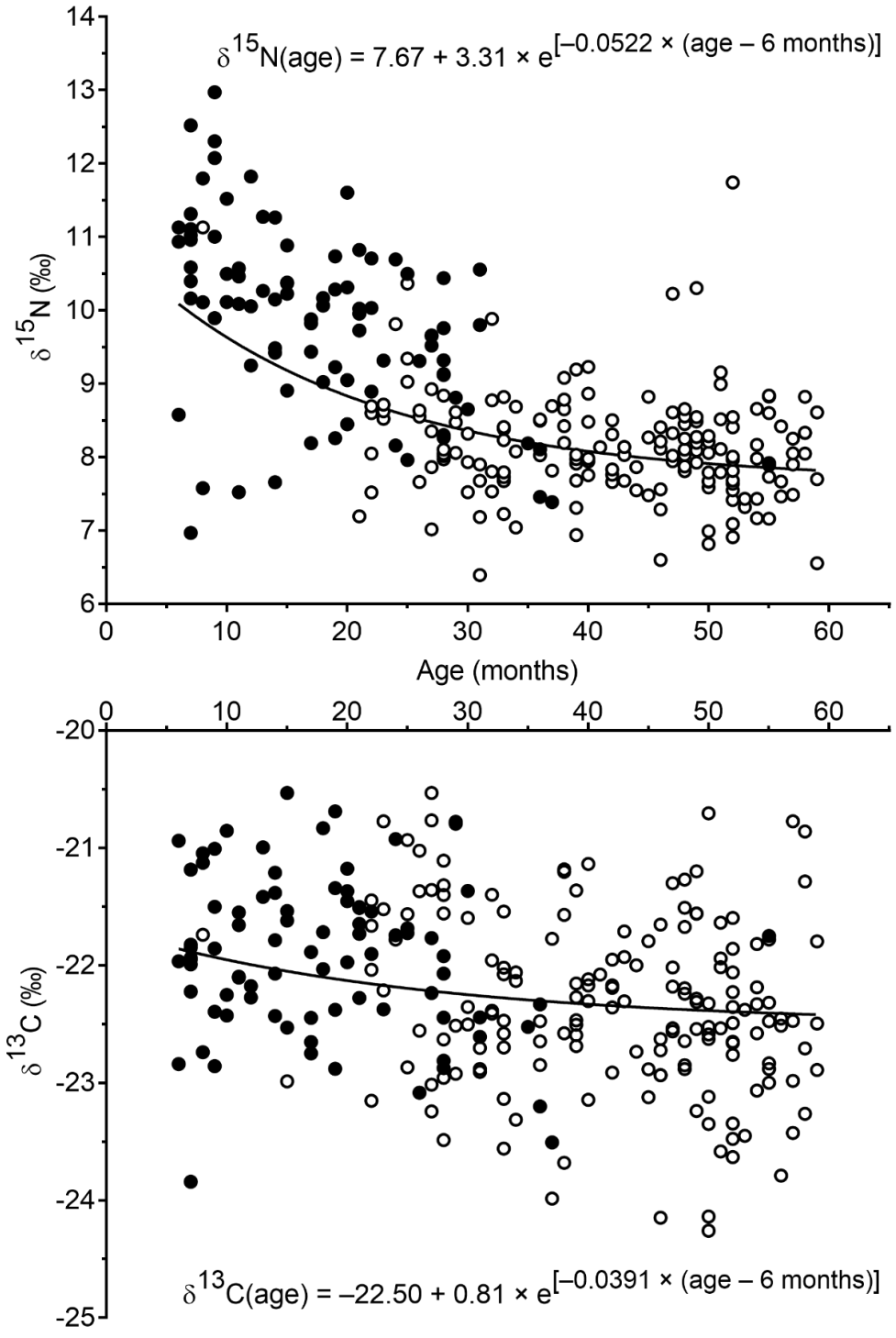

Figure 19. $\delta^{15}N$ and $\delta^{13}C$ in hair as a function of age in children aged 6 to 60 months (n=255).

Empty circles represent children who were fully weaned and filled circles represent children who were partially or exclusively breastfed

These decreases were fit using the following 3-parameter equation:

$$\delta X(\text{age}) = \delta X_{\infty} + \Delta \times e^{-k \times (\text{age} - 6\ \text{months})}$$

where X stands for either ^{15}N or ^{13}C, δX_{∞} (‰) represents the plateau $\delta^{15}N$ or $\delta^{13}C$ value at the final isotopic steady state (*i.e.*, the expected value in adults), Δ (‰) is the difference between the $\delta^{15}N$ or $\delta^{13}C$ value measured at 6 months of age and the corresponding expected value in adults (*i.e.*, the weaning trophic level decrease), and k (%/month) represents the rate of decrease in $\delta^{15}N$ or $\delta^{13}C$ (*i.e.*, the isotopic turnover rate).

Because the dispersion of $\delta^{13}C$ values at a given age was large compared to the variation of $\delta^{13}C$ with age, a two-step procedure was used to estimate the three parameters for ^{13}C. The $\delta^{13}C_{\infty}$ value was first estimated as the mean $\delta^{13}C$ value for fully weaned children ⩾48 months of age (our closest proxy for the value in adults) and this value was then forced into the model to estimate the other two parameters.

The weaning trophic level decrease (Δ) was much higher for $\delta^{15}N$ than for $\delta^{13}C$ (3.31‰ *vs.* 0.81‰) but the isotopic turnover rate (*k*) rate was similar for the two isotopes (**Table 16**).

Table 16. Parameter estimates [95% confidence intervals] for $\delta^{15}N$ and $\delta^{13}C$ fitted trajectories during weaning (exponential decay between 6 and 60 months of age)

	$\delta^{15}N$		$\delta^{13}C$	
δX_{∞} (‰)	7.67	[7.21 – 8.13]	-22.50	[-22.70 – 22.30]
Δ (‰)	3.31	[2.85 – 3.76]	0.81	[0.56 – 1.05]
k (%: months)	5.22	[3.05 – 7.39]	3.91	[2.20 – 5.62]

Variables: δX_{∞}, final isotopic steady state; Δ, weaning trophic level decrease; *k*, isotopic turnover rate.

The time needed to reach 90% of the final isotopic equilibrium ($t0.9 = -\ln(1 - 0.9)/k$) was 52 months, from the initial age of 6 months, on average, for both isotopes.

After adjustment for age, $\delta^{15}N$ was lower in fully weaned children compared to children fully or partially breastfed (8.42 ± 0.08 vs 9.39 ± 0.13, $p<0.0001$) while no difference was observed for $\delta^{13}C$, indicating that at least for $\delta^{15}N$, the effect of age and breastfeeding status were not statistically confounded.

Hair $\delta^{15}N$ and $\delta^{13}C$: effects of wasting and stunting

Before adjustment for age and breastfeeding status, hair $\delta^{15}N$ was higher in wasted children and lower in stunted children compared to non-wasted and non-stunted children, respectively. Hair $\delta^{13}C$ was also lower in severely stunted children compared to non-stunted children (**eTable 7**). After adjustment for age and breastfeeding status, there was no difference in hair $\delta^{15}N$ and $\delta^{13}C$ between wasted children and their controls. However, both $\delta^{15}N$ (-0.57‰, $p<0.01$) and $\delta^{13}C$ (-0.44‰, $p<0.01$) were significantly lower in stunted children (**Figure 20**).

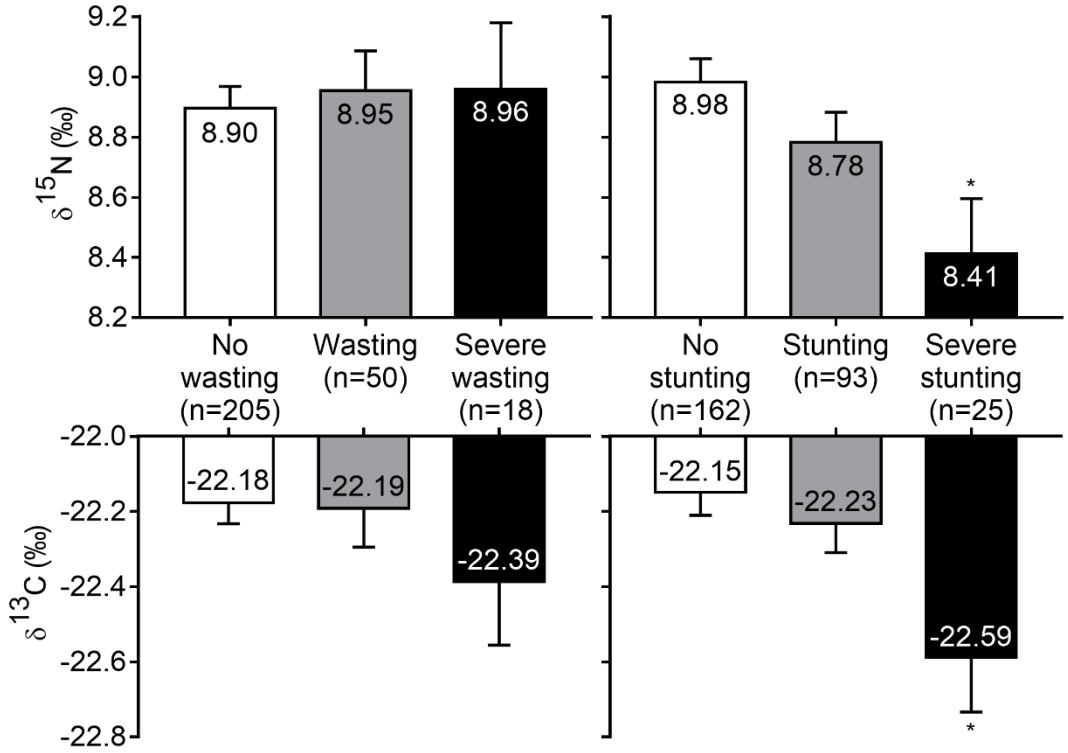

Figure 20. Effect of wasting and stunting on age- and breastfeeding-adjusted $\delta^{15}N$ and $\delta^{13}C$ in hair of children (6-60 months)

Data are least-square means ± SE; * different from control (non-wasted or non-stunted) children, $p<0.01$.

In bivariate logistic regression, $\delta^{15}N$ but not $\delta^{13}C$ was weakly and positively associated with the risk of being wasted. This association was no longer observed in multi-adjusted models. In contrast, $\delta^{15}N$, and to a lesser extent $\delta^{13}C$, were negatively associated with the risk of being stunted. In multi-adjusted models, $\delta^{15}N$ was still negatively associated with the risk of being severely stunted whereas the association was marginally significant for $\delta^{13}C$ (**eTable 8**).

Discussion

In the present study, we showed, with high statistical power due to the large sample of children, that hair $\delta^{15}N$ and $\delta^{13}C$ values decreased between 6 months and 4 years of age, with little variation thereafter—a consequence of the weaning process and the gradual switch from breast milk to plant-based family meals of lower $\delta^{15}N$ and $\delta^{13}C$ values.[221,347] In this population of young children, we failed to observe any association between hair $\delta^{15}N$ and $\delta^{13}C$ and wasting, but highlighted that both were associated with stunting, a new result that has not been previously reported. Indeed, compared to non-stunted children, severely stunted children exhibited lower hair $\delta^{15}N$ (–0.6‰) and $\delta^{13}C$ (–0.4‰) values that likely reflect chronic exposition to diets low in animal source food (ASF).

One of the most important results of this study is our report of an exponential decrease in hair $\delta^{15}N$ and $\delta^{13}C$ between 6 months and 5 years of age, which was more pronounced for ^{15}N than for ^{13}C. Although many archaeological studies rely on the hypothesis of a decrease in protein $\delta^{15}N$ and $\delta^{13}C$ during weaning to reconstruct the practices of ancient civilizations,[329,340–343,348] there are very little data on the evolution of hair $\delta^{15}N$ and $\delta^{13}C$ with age in contemporary children. Fogel *et al.*, using periodic fingernail clippings sampled from mother-infant pairs, were among the first to report a $\delta^{15}N$ increase between birth and 6 months of age due to breastfeeding, followed by a gradual $\delta^{15}N$ decrease after weaning initiation.[344] More recently, and again using fingernail clippings from mother-infant pairs collected during breastfeeding *versus* formula-feeding, Fuller *et al.* reported that breastfeeding resulted in gradual increases in both $\delta^{15}N$ and $\delta^{13}C$ until weaning onset, when infants' enrichments (relative to their mothers') reached 2-3‰ for ^{15}N and a 1‰ for ^{13}C, followed by gradual decreases during weaning, which were more pronounced for ^{15}N than for ^{13}C.[345] However, these two studies remain largely qualitative, as the limited number of participants did not allow for statistical analysis, and the follow-up periods were restricted to the early phase of weaning (up to 1.5 years of age) and not to complete weaning. Even though we did not measure hair $\delta^{15}N$ and $\delta^{13}C$ values in mothers, nor in children under 6 months of age, our data shed light on the impact of breastfeeding and weaning on $\delta^{15}N$ and $\delta^{13}C$ variations during infancy and early childhood (up to 5 years of age). Firstly, aside from age, we observed that hair $\delta^{15}N$ and $\delta^{13}C$ were higher in fully breastfed children than in weaned children no longer receiving breast milk. Secondly, analysis of hair $\delta^{15}N$ and $\delta^{13}C$ kinetics between 6 months and 5 years of age supports the hypothesis of an underlying trophic level effect. Considering that the isotopic values at the plateau correspond with those of adults, and assuming that data from children at 6 months of age are a proxy for the maximum values reached at the end of breastfeeding period, the Δ parameters in our equations correspond to the maximum isotopic enrichment in infants relative to their mothers, *i.e.*, the breastfeeding trophic-level shift equivalent to the weaning trophic

level decrease. The second assumption is supported by the lack of difference between our model predictions (by curve-fitting) for $\delta^{15}N$ and $\delta^{13}C$ at 6 months and our observations in the 7 exclusively breastfed children in our study population. Our estimates for Δ, *i.e.*, 3.3‰ [2.8-3.8] for ^{15}N and 0.8‰ [0.6-1.0] for ^{13}C, correspond with the magnitude of a trophic level shift reported in the literature, *i.e.*, 3-4‰ for ^{15}N and 1‰ for ^{13}C.[221] Therefore, our data support the hypothesis that after birth, exclusive breastfeeding induces a gradual increase in $\delta^{15}N$ and $\delta^{13}C$ in infants compared to their mothers, up to a difference on the order of one trophic level around the age of 6 months. These increases are then reversed during weaning as the proportion of breastmilk in the infant diet is gradually reduced. A state close to isotopic equilibrium with the family diet is reached around 4 years of age, with little change in $\delta^{15}N$ and $\delta^{13}C$ afterwards. While the dietary transition associated with weaning is undoubtedly the underlying cause of the $\delta^{15}N$ and $\delta^{13}C$ decrease with age, the effects of age and breastfeeding status were not fully confounded in our study, at least for $\delta^{15}N$, for two reasons. First, we assume that in our study breastmilk consumption varied in partially breastfed children—and children were likely to receive less and less breast milk with age. Second, after a diet transition, hair progressively integrates the isotopic signature of the new diet because endogenous amino acids released during the renewal of body proteins (mainly muscle) represent a significant supply to the keratin precursor pool; at the time of dietary transition, these endogenous amino acids still carry the isotopic signature of the former diet. The isotopic differences between endogenous and exogenous amino acids mitigate the signature of the new diet detectable in hair, at least until a new isotopic steady state is reached in muscle tissue. It should be also noted that, in our study, the ratio between the SD of the isotopic abundance for the whole population and the predicted weaning trophic level was higher for ^{13}C (0.93) compared to ^{15}N (0.38). This indicates that the exponential model left more variability unexplained for $\delta^{13}C$ than for $\delta^{15}N$. The significant variability in $\delta^{13}C$ (in children of all ages) likely reflects variability in proportion of ^{13}C-rich C4 plants in the family diets in our sample. Rice (a C3 plant) is the staple food consumed by resource-poor Bangladeshis. Yet, some families may have received food aid in the form of corn (a C4 plant). However, we do not know the proportion of corn consumption in the diet of each family. In fully or partially breastfed children, the proportion of C4 plants in the family diet has an indirect effect by modifying the level of ^{13}C enrichment of breast milk. Last, it is also worth noting that in our study, $\delta^{15}N$ and $\delta^{13}C$ estimates at the plateau were lower than those previously reported by Fuller *et al.* in adults,[345] but close to those previously reported for modern vegetarians and vegans[328,332]—suggesting a low consumption of ASF in the low-income Bangladeshi population.[349]

Assuming that isotopic variations in $\delta^{15}N$ and $\delta^{13}C$ values between 6 months and 5 years reflect a progressive dietary shift during the weaning process, the correlations between these isotopic values and age-related parameters (*e.g.*, height, weight, head circumference, and MUAC) are likely indirect consequences of this diet shift as well. This explanation is consistent with the fact that hair $\delta^{15}N$ and $\delta^{13}C$ values were not correlated with anthropometric parameters that are weakly or unrelated to age (*e.g.*, BMI, WHZ or WAZ) with the notable exception of HAZ. HAZ was found to be weakly and positively correlated with both $\delta^{15}N$ and $\delta^{13}C$. Indeed, $\delta^{15}N$ and $\delta^{13}C$ values were significantly lower in severely stunted children (HAZ <-3) compared to control children (HAZ $\geqslant -2$), even after adjusting for age and breastfeeding status. Despite strong statistical significance ($p<0.01$), these

effects remain small compared to the trophic level decrease during weaning, especially for $\delta^{15}N$. Consequently, the risk of archaeologically mischaracterizing a severely stunted child for a child in process of weaning is limited, but non-negligible. Conversely, in a contemporary population where children's ages and breastfeeding statuses are usually known, $\delta^{15}N$ and $\delta^{13}C$ measurements in hair could help identify those with severe chronic malnutrition. Such low isotopic values observed in severely stunted children could be due to the consumption of a diet of low isotopic value by either: (1) children after weaning; and/or (2) their mothers during pregnancy and lactation. A dietary explanation is likely, since diets consisting mainly of plant protein (*i.e.*, poor in ASF) have low $\delta^{15}N$ and $\delta^{13}C$ values,[350] and very low dietary diversity with almost no ASF consumption has been identified as a risk factor for stunting.[351] In the present study, we found no difference in dietary diversity scores (DDS) between severely stunted and non-stunted children. However, DDS assessment was based on caretaker declaration and did not take into account the quantities consumed for each of the groups declared. Therefore, it is not possible to exclude a lower ASF consumption in severely stunted children compared to children with no stunting. In this regard, hair $\delta^{15}N$ and $\delta^{13}C$ measurements could be used as alternatives to assess sub-optimal breastfeeding practices, DDS and ASF consumption which occurred in the past, with a lower risk of bias. Alternatively, the early origin of severe stunting could also constitute a possible explanation for the low values of $\delta^{15}N$ and $\delta^{13}C$. Intrauterine growth retardation (IUGR) linked to the poor nutritional status of mothers is recognized as a risk factor for severe stunting.[338,352] Low dietary diversity of mothers during pregnancy could lead to IUGR and low $\delta^{15}N$ and $\delta^{13}C$ values in newborns, although experimental evidence of this is not available to date. These low values at birth may, in part, explain the low values observed after 6 months of age in our population. These two potential causes are not mutually exclusive and can all contribute to the low hair $\delta^{15}N$ and $\delta^{13}C$ values measured in severely stunted children.

Quite surprisingly, in this study, we failed to observe a specific isotopic signature for wasted children. Yet, it has been previously reported that muscle wasting in anorexia was associated with an increase in hair $\delta^{15}N$[246,247] because of a higher trophic step due to a metabolism more oriented towards catabolism. A first possible explanation is that, in our population, because acute malnutrition mostly occurred between 6 and 24 months of age, at a period of rapid decline in $\delta^{15}N$ in hair due to the introduction of plant foods during weaning, any $\delta^{15}N$ increase due to muscle wasting was blurred by an antagonistic $\delta^{15}N$ decrease due to weaning. Emaciated children, just like stunted children, are likely to be weaned on a diet of poor nutritional quality, almost devoid of ASF, and become emaciated as a consequence of recent diseases or deprivation. This hypothesis of antagonistic effects of diet quality and muscle wasting on natural isotopic abundance in hair is supported by the observation that compared to children with severe stunting, children with severe wasting have similar low $\delta^{13}C$ values that are characteristic of ASF-poor diets, but higher $\delta^{15}N$ values. In addition, it has been shown that in children with non-edematous acute malnutrition, food deprivation is not associated with an increase of body protein breakdown beyond that measured in well-nourished children.[142] The mechanisms responsible for wasting are thus quite different from those involved in weight loss in adolescents or adults with anorexia, whose lean body mass is initially much higher than that of children. These two hypotheses could explain why, unlike anorexia in adolescents and adults, acute malnutrition in children is not associated with an increase in hair $\delta^{15}N$.

The main strength of this study is the sample size and age distribution that, for the first time, models the evolution of $\delta^{15}N$ and $\delta^{13}C$ across the entire weaning period in humans. In addition, the high prevalence of chronic malnutrition in our population provides sufficient power to highlight its isotopic signature in the hair. This study has also some limitations. Information on children's food consumption is limited, qualitative, and caretaker-reported. Additionally, we did not measure the natural ^{15}N and ^{13}C abundances in locally consumed staple foods. This limits our ability to ascertain the links between hair $\delta^{15}N$ and $\delta^{13}C$ values and dietary diversity and ASF consumption. Lastly, since each 5 mm segment corresponds to a 2-week period, it would have been interesting to have isotopic measurements over a greater number of consecutive segments along the hair follicle to trace each child's nutritional history and to highlight, at the individual level, the isotopic trajectory related to dietary transition during weaning, and its possible deviation by an episode of acute malnutrition.

Supplement 2

Supplemental Tables

eTable 5. SMART sample description

Variable	Sample size	
Age (month)	654	30.7 ± 15.6
Height/length (cm)	655	84.5 ± 11.0
Weight (kg)	655	10.9 ± 2.6
Female	656	318 (48.5%)
Breastfed	653	265 (40.6%)
Wasted	656	104 (15.8%)
Severely wasted	656	28 (4.3%)
Stunted	656	243 (37.0%)
Severely stunted	656	76 (11.6%)

Values are mean ± SD or n (%)

eTable 6. Effect of age and breastfeeding status on the risk of being wasted or stunted

	Age (months)		Breastfeeding status	
	OR	p	OR	p
Wasted	0.98 [0.96-1.00]	<0.05	2.42 [1.29-4.56]	<0.01
Severely wasted	0.96 [0.93-0.99]	<0.05	3.80 [1.42-10.76]	<0.01
Stunted	1.02 [1.00-1.03]	NS	0.73 [0.42-1.27]	NS
Severely stunted	1.02 [0.99-1.04]	NS	0.70 [0.26-1.70]	NS

OR, odds ratio [95% CI]. Breastfeeding status was assessed based on caregiver declaration. For breastfeeding status, two categories were considered: children exclusively or partially breastfed (breastfeeding status =1) and fully weaned children (breastfeeding status = 0).

eTable 7. Effects of sex, breastfeeding status, wasting and stunting on the raw (unadjusted) $\delta^{15}N$ and $\delta^{13}C$ data

	$\delta^{15}N$	$\delta^{13}C$
	Sex	
Boys (n=135)	8.71 ± 1.18	-22.16 ± 0.76
Girls (n=120)	8.79 ± 1.33	-22.21 ± 0.73
	Breastfeeding status	
Fully breastfed (n=7)	11.45 ± 0.70	-22.02 ± 0.45
Partially breastfed (n=78)	9.73 ± 1.22**	-21.92 ± 0.68
Fully weaned (n=170)	8.19 ± 0.78**	-22.32 ± 0.76*
	Wasting	
Not wasted (n=205)	8.67 ± 1.22	-22.20 ± 0.78
Wasted (n=50)	9.08 ± 1.34*	-22.13 ± 0.61
Severely wasted (n=18)	9.31 ± 1.39*	-22.26 ± 0.73
	Stunting	
Not stunted (n=162)	8.89 ± 1.32	-22.14 ± 0.73
Stunted (n=93)	8.51 ± 1.10*	-22.28 ± 0.78
Severely stunted (n=25)	8.13 ± 0.85**	-22.63 ± 0.94**

$\delta^{15}N$ and $\delta^{13}C$ values (‰) are mean ± SD.
* and ** indicate differences from the first line of each block, $p<0.05$ and $p<0.01$.

eTable 8. Raw and age- and breastfeeding-adjusted odd ratios for the risk of being wasted or stunted according to hair $\delta^{15}N$ or $\delta^{13}C$ values.

		Unadjusted		Adjusted	
		OR [CI 95%]	*P*	OR [CI 95%]	*P*
Wasted	$\delta^{15}N$	1.28 [1.01-1.62]	0.04	1.08 [0.78-1.51]	NS
	$\delta^{13}C$	1.13 [0.75-1.74]	NS	0.95 [0.60-1.52]	NS
Severely wasted	$\delta^{15}N$	1.44 [1.01-2.02]	0.04	1.11 [0.70-1.78]	NS
	$\delta^{13}C$	0.90 [0.48-1.69]	NS	0.61 [0.29-1.24]	NS
Stunted	$\delta^{15}N$	0.77 [0.62-0.96]	0.02	0.79 [0.58-1.06]	NS
	$\delta^{13}C$	0.78 [0.55-1.10]	NS	0.90 [0.62-1.31]	NS
Severely stunted	$\delta^{15}N$	0.54 [0.32-0.82]	<0.01	0.53 [0.29-0.91]	0.02
	$\delta^{13}C$	0.43 [0.24-0.76]	<0.01	0.55 [0.28-1.02]	0.06

OR, odds ratio [95% CI].

Supplementary Analyses

Using a mixed procedure for repeated data (PROC MIXED, SAS 9.14, Cary, NC), we assessed the change in $\delta^{15}N$ and $\delta^{13}C$ between two consecutive segments of hair, the first and second adjacent 5 mm segments closest to the scalp. The first (proximal) segment corresponds with the more recent period, from -3 to -1 weeks before sampling. The second (distal) segment corresponds with the earlier period, from -5 to -3 weeks before sampling. Because the data presented in **Figure 19** and **Table 17.** Anthropometric and isotopic values according to the three anthropometric phenotypes of SAM of the manuscript suggest that the age of children at inclusion may affect the change in $\delta^{15}N$ and $\delta^{13}C$ between the two segments, age at inclusion was included as a covariate in the model.

Our results indicate a slight but significant (-0.03) decrease in both $\delta^{15}N$ and $\delta^{13}C$ from the distal to the proximal segment, corresponding to a more recent period of 2 weeks (**eTable 9**).

eTable 9. $\delta^{15}N$ and $\delta^{13}C$ evolution between two consecutive hair segments

Fixed effects of the mixed model†	$\delta^{15}N$	$\delta^{13}C$
Segment	$p<0.05$	$p<0.05$
Age at inclusion	$p<0.0001$	$p<0.0001$
Post-hoc comparisons	**Distal segment (-5 to -3 weeks)‡**	**Proximal segment (-3 to -1 weeks)‡**
$\delta^{15}N$	8.78 ± 0.06	8.75 ± 0.06*
$\delta^{13}C$	-22.15 ± 0.05	-22.18 ± 0.04*

†Type 3 fixed effects of the mixed model
‡δ15N and δ13C values (‰) are least-square means ± SE
* Significantly different from the older segment, P<0.05

These results are in line with a progressive decrease in $\delta^{15}N$ and $\delta^{13}C$ observed between 6 months and 5 years, due to the weaning process.

We evaluated the effect of wasting and stunting on this isotopic change and did not find evidence of interaction between wasting or stunting and the changes in $\delta^{15}N$ and $\delta^{13}C$ between distal and proximal segments (**eTable 10**).

eTable 10. Effect of stunting and wasting on $\delta^{15}N$ and $\delta^{13}C$ evolution between two consecutive hair segments

Fixed effects and interaction mixed models†		$\delta^{15}N$	$\delta^{13}C$
Model for wasting	Segment	$p<0.05$	$p=0.10$
	Age at inclusion	$p<0.0001$	$p<0.0001$
	Wasting	NS	NS
	Segment*Wasting	NS	NS
Model for severe wasting	Segment	$p=0.06$	$p=0.05$
	Age at inclusion	$p<0.0001$	$p<0.0001$
	Severe wasting	NS	NS
	Segment*Severe wasting	NS	NS
Model for stunting	Segment	$p<0.05$	$p<0.05$
	Age at inclusion	$p<0.0001$	$p<0.0001$
	Stunting	NS	NS
	Segment*Stunting	NS	NS
Model for severe stunting	Segment	$p<0.01$	NS
	Age at inclusion	$p<0.0001$	$p<0.0001$
	Severe stunting	$p<0.01$	$p<0.01$
	Segment*Severe stunting	NS	NS

†Type 3 fixed effects and interaction of the mixed model.

This suggests that the low $\delta^{15}N$ and $\delta^{13}C$ values observed in severely stunted children are due to dietary or metabolic changes that occurred before the one-month period covered by the two consecutive hair segments.

VI ARTICLE 3

Preliminary stable isotope analyses in the hair of young children with SAM at admission and during nutritional rehabilitation

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Abstract: In addition to the cross-sectional study presented in **Article 2**, we also measured natural isotopic abundance of carbon and nitrogen in the hair of children with SAM, from the Bangladesh cohort. The first objective of these additional analyses was to assess the ability of hair $\delta^{15}N$ and $\delta^{13}C$ to differentiate children with severe acute malnutrition (SAM), diagnosed with mid-upper arm circumference (MUAC) <115 mm) or weight-for-height *z*-score (WHZ) <-3. An additional objective was to evaluate the ability of sequential measurements of $\delta^{15}N$ and $\delta^{13}C$ along the hair follicle to indicate weight gain during nutritional rehabilitation. The analysis of these data is still ongoing. In the following pages I present the preliminary results of our $\delta^{15}N$ and $\delta^{13}C$ measurements that correspond to the 2 weeks immediately preceding SAM diagnosis, and subsequent admission for nutritional rehabilitation.

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Methods

This study is nested in the Bangladesh cohort of the OpitDiag study, which includes 142 children with a diagnosis of SAM according to the MUAC criterion and/or the WHZ. The OptiDiag study was registered at clinicaltrails.gov number NCT03400930. Ethical approval was obtained from: the Ethics Committee at the University Hospital of Antwerp and the University of Antwerp; the National Research Ethics Committee (NERC) at the Bangladesh Medical Research Council (BMRC).

Anthropometric measurements (weight, height/length, sitting height or laying length) were taken in duplicate as per WHO recommendations.[280] Weight was measured using either a SECA hanging scale or a mother/baby scale. Length or height (change of measuring position at 2 years) was measured using a standard UNICEF wooden measuring board to the nearest 1 mm. MUAC was measured on the left arm with a standard MUAC tape to the nearest 1 mm. In this cohort, 48 children with SAM were diagnosed by MUAC < 115 mm only (MUAC-only), 49 by WHZ < –3 only (WHZ-only) and 45 had both anthropometric deficits (WHZ & MUAC).

Immediately after diagnosis, children were referred to the outpatient therapeutic feeding center and received ready-to-use therapeutic food (RUTF) for roughly 8 weeks, with monitoring of their nutritional status every week. At admission and at two weeks intervals, a sample roughly 50 hair follicles was covered in hair gel, twisted into a lock and cut as close to the patient's scalp as possible with sharp, dissecting scissors. The gelled lock was then taped to a sheet of paper and the cut end was labeled and stored at room temperature before further analysis. Repeated samples were taken from the same sampling zone as to recover the hair that had grown over the course of the two weeks between sample collection.

Five consecutive segments of 5 mm were prepared and transferred into separate Eppendorf tubes. Hair gel was removed by two consecutive immersions in 750 µL of ultrapure water for 2 minutes with gentle agitation. Hair samples were then delipidated in 1.5 mL of ether/ethanol (1:1) for 30 minutes, washed in 1.5 mL of ultrapure water for 2 minutes, delipidated once more for 30 minutes, and finally washed thrice consecutively for 2 minutes each, all while being gently agitated. Samples were dried overnight before being transferred in 2 mg tin capsules and analyzed by elemental analysis/isotope-ratio mass spectrometry, using an elemental analyzer (EA Vario Micro Cube, Elementar, Germany) coupled with an isotope-ratio mass spectrometer (Isoprime, VG instruments, Manchester, UK). Tyrosine was used for calibration and drift correction. The natural abundances of 15N and 13C in hair were expressed relative to standards (atmospheric N_2 for $^{15}N/^{14}N$ and Vienna Pee Dee Belemnite for $^{13}C/^{12}C$) using the delta notation, according to the following equation:

$$\delta\ (‰) = 1000\ x\ (R_{sample} - R_{standard})/R_{standard}$$

where R_{sample} and $R_{standard}$ stand for the ratio between heavy and light isotope ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) in the sample and standard, respectively.

The average hair growth rate being approx. 2.5 mm per week, each segment corresponds to a period of about 2 weeks. Therefore, the sample collected at admission is expected to record the dietary and metabolic isotopic signatures of the period immediately preceding the diagnosis. The results presented in this additional chapter concern the isotopic measurements performed on this segment collected at admission.

Results

Anthropometric characteristics according to the different SAM phenotypes

Children diagnosed with WHZ were older, higher, heavier and have a higher height-for-age *z*-score (HAZ) than children diagnosed with MUAC or WHZ & MUAC (**Table 17**).

Table 17. Anthropometric and isotopic values according to the three anthropometric phenotypes of SAM

	MUAC-only	WHZ&MUAC	WHZ-only
N	43	41	43
Age (months)	8.4 ± 4.0[a]	15.2 ± 8.8[b]	28.7 ± 17.7[c]
Height (cm)	62.5 ± 4.3[a]	70.1 ± 8.6[b]	83.1 ± 12.8[c]
Weight (kg)	5.4 ± 0.8[a]	6.0 ± 1.3[a]	8.6 ± 2.2[b]
MUAC (mm)	111 ± 3[a]	122 ± 5[b]	109 ± 6[c]
BMI (kg/m^{-2})	13.7 ± 0.7[a]	12.2 ± 0.8[b]	12.3 ± 0.7[b]
HAZ	-3.06 ± 1.48[a]	-2.58 ± 1.39[a]	-1.66 ± 1.13[b]
WAZ	-3.52 ± 0.87[a]	-4.03 ± 0.71[b]	-3.22 ± 0.59[a]
WHZ	-2.16 ± 0.60[a]	-3.65 ± 0.59[b]	-3.39 ± 0.27[b]

Data are mean ± SD; means with different letters are different, $p<0.05$

Correlation between anthropometric parameters and natural isotopic abundances in hair

As previously reported, $\delta^{15}N$, and to a lesser extent $\delta^{13}C$, were negatively correlated with age and age-related anthropometric parameters such as height or weight (**Table 18**).

Table 18. Pearson correlation coefficients and associated P values between $\delta^{15}N$ or $\delta^{13}C$ and age or anthropometric parameters

	$\delta^{15}N$	$\delta^{13}C$
Age (months)	-0.61 (P<0.0001)	-0.24 (P<0.01)
Height (cm)	-0.53 (p<0.0001)	-0.24 (P<0.01)
Weight (kg)	-0.52 (p<0.0001)	-0.24 (P<0.01)
MUAC (mm)	-0.16 (NS)	-0.20 (P<0.05)
BMI (kg/m^{-2})	0.39 (P<0.0001)	0.05 (NS)
HAZ	0.05 (NS)	-0.03 (NS)
WAZ	0.14 (NS)	-0.09 (NS)
WHZ	0.21 (P<0.05)	0.02 (NS)

Moreover, $\delta^{15}N$ was marginally, positively correlated with BMI and WHZ.

Effect of SAM phenotypes on natural isotopic abundances in hair

In a second step, we tested the effect of the anthropometric phenotype (WHZ-only, MUAC-only and WHZ&MUAC) on the values of $\delta^{15}N$ and $\delta^{13}C$, with or without adjustment for covariates, using the general linear model (GLM) procedure.

Before adjustment, hair $\delta^{15}N$ was higher in MUAC-only children (10.10 ± 1.17‰) compared to WHZ&MUAC children (9.32 ± 1.11‰) or WHZ-only children (9.13 ± 1.44‰). There was no difference between groups regarding unadjusted $\delta^{13}C$ values (data not shown).

After adjustment for age, there was still a small but significant effect of SAM phenotype on hair $\delta^{15}N$ ($p<0.05$) but not on $\delta^{13}C$. Age-adjusted $\delta^{15}N$ was higher in WHZ-only group compared the WHZ&MUAC group (+0.65‰, $p<0.05$) (**Figure 21**).

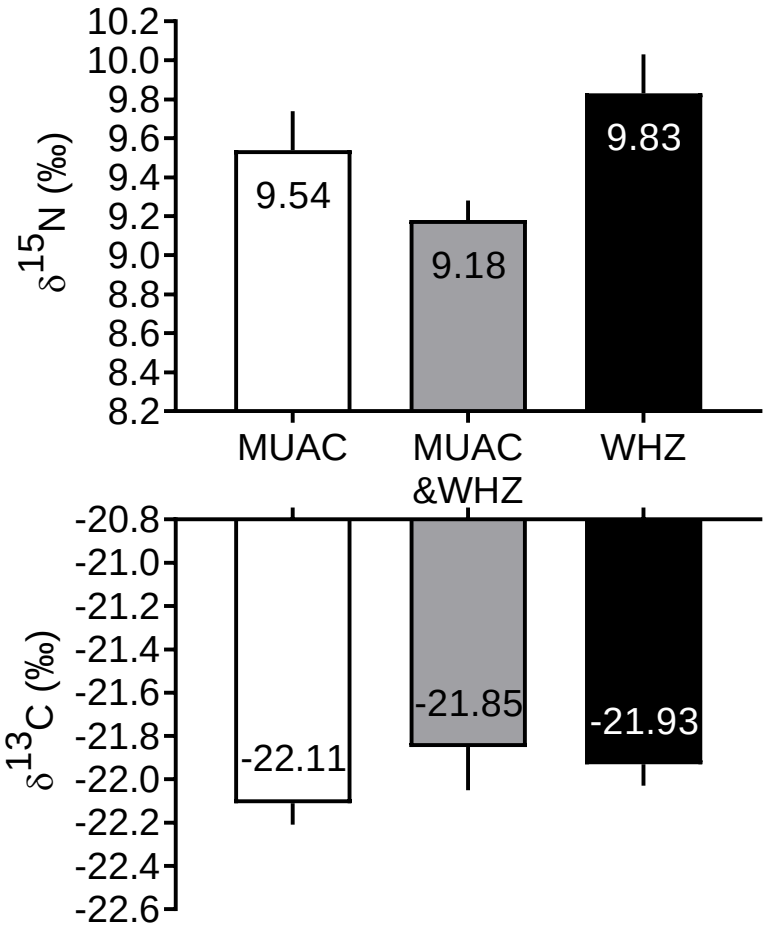

Figure 21. Age-adjusted hair $\delta^{15}N$ and $\delta^{13}C$ according to the different SAM phenotypes

Different letters indicate statistically significant differences in isotopic values ($p<0.05$).

Further adjustment of hair $\delta^{15}N$ for HAZ or WAZ resulted in the disappearance of the phenotype effect (**Table 19**).

Table 19. Main and covariate effects on hair $\delta^{15}N$ and $\delta^{13}C$ using different adjustment

Effects	$\delta^{15}N$	$\delta^{13}C$
Unadjusted model		
Phenotype	$p<0.01$ (MUAC > others)	NS
Age-adjusted model		
Phenotype	$p<0.05$ (WHZ > WHZ&MUAC)	NS
Age	$\beta = -0.062$, $p<0.0001$	$\beta = -0.017$, $p< 0.01$
Age and WAZ-adjusted model		
Phenotype	NS	NS
Age	$\beta = -0.062$, $p<0.0001$	$\beta = -0.017$, $p< 0.01$
WAZ	$\beta = -0.304$, $p<0.05$	NS
Age and HAZs-adjusted model		
Phenotype	NS	NS
Age	$\beta = -0.061$, $p< 0.0001$	$\beta = -0.017$, $p< 0.01$
HAZ	$\beta = -0.144$, $p< 0.05$	NS

Abbreviations: HAZ, height-for-age- z-score; MUAC, mid-upper arm circumference; NS, not significant; WAZ, weight-for-height z-score.

Hair $\delta^{15}N$ and risk WHZ or MUAC SAM diagnosis

Using logistic regressions, we also explored at the relationship between $\delta^{15}N$ and the risk of being diagnosed as SAM, based on either WHZ or MUAC, in bivariate models or models adjusted for other factors such as age, WAZ or HAZ (**Table 20**).

Table 20. OR and confidence interval for the risk of being severely acutely malnourished according to MUAC (<115 mm) or WHZ (<–3) as a function of hair $\delta^{15}N$ in unadjusted and adjusted models

	Risk of MUAC <115 mm		Risk of WHZ ratio <–3	
Factor	OR	[CI 95%]	OR	[CI 95%]
Unadjusted bivariate model				
$\delta^{15}N$	0.35	[0.06-0.66]	0.57	[0.40-0.77]
Age-adjusted model				
$\delta^{15}N$	0.56	[0.34-0.89]	0.86	[0.58-1.28]
Age	0.87	[0.82-0.92]	1.19	[1.10-1.31]
Age and WAZ-adjusted model				
$\delta^{15}N$	0.63	[0.36-1.04]	0.91	[0.60-1.38]
Age	0.86	[0.81-0.92]	1.19	[1.10-1.31]
WAZ	0.34	[0.16-0.66]	0.82	[0.46-1.41]
Age and HAZ-adjusted model				
$\delta^{15}N$	0.60	[0.35-1.01]	0.62	[0.36-1.01]
Age	0.87	[0.81-0.92]	1.23	[1.12-1.38]
HAZ	0.51	[0.33-0.14]	2.06	[1.42-3.20]

In unadjusted and age-adjusted models, $\delta^{15}N$ was negatively associated with the risk of having a MUAC < 115 mm. This association was no longer observed when WAZ or HAZ were entered in the regression model as covariates

Only in the unadjusted bivariate model was $\delta^{15}N$ negatively associated with the risk of having a WHZ <–3. This association was no longer observed after adjustment for age. Further introduction of WAZ or HAZ in the regression model had no effect.

Discussion

In this study of 123 children with SAM, we measured the natural isotopic abundances of nitrogen and carbon in the hair and examined the relationship between these abundances and diagnostic phenotype. Our results show that $\delta^{13}C$ hair values do not vary according to SAM diagnosis (either by MUAC or WHZ). Conversely, hair $\delta^{15}N$ values do vary between anthropometric phenotype of SAM.

These differences are in part, but not entirely, explained by age differences between groups. In line with results from **Article 2**, hair $\delta^{15}N$ values were strongly and negatively correlated with age. This correlation is linked to the progressive transition from breastmilk to family foods that occurs during the weaning period. In the present study, MUAC-only SAM children were younger than WHZ & MUAC children (–6.8 months) as well as WHZ-only children (–20.3 months). Accordingly, crude $\delta^{15}N$ hair values were higher in MUAC-only children, than in WHZ & MUAC and WHZ-only children. Age-adjusted $\delta^{15}N$ hair values were higher in WHZ-only children, compared to the WHZ & MUAC phenotype; MUAC-only children had intermediate values $\delta^{15}N$ values.

Age is not the only factor associated with differences in $\delta^{15}N$ values among the three phenotypes of SAM. We also observed that children with a MUAC <115 mm (including children belonging to both MUAC-only and WHZ & MUAC groups) had lower HAZ scores, compared to children with MUAC ⩾115 mm. Significant differences in $\delta^{15}N$ between phenotypes disappeared after adjustment for HAZ—suggesting that a low HAZ may in part account for the lower $\delta^{15}N$ observed in children with a low MUAC compared to children with MUAC ⩾115 mm.

Similarly, the low WAZ scores observed in the WHZ & MUAC phenotype may also, in part, account for low $\delta^{15}N$ (because $\delta^{15}N$ differences between these groups were no longer significant after adjustment for both age and WAZ). A HAZ <–2 (–3) is the diagnostic criterion for (severe) chronic malnutrition and a low WAZ is considered a hallmark of both acute and chronic malnutrition.[52] Thus, differences in $\delta^{15}N$ values among the three phenotypes of SAM may be explained by a difference in growth retardation (stunting) between groups. In **Article 2,** severe stunting was associated with a decrease in age-adjusted $\delta^{15}N$. This finding is supported in the present study because the phenotypes with lower mean HAZ and WAZ were those with the lower age-adjusted $\delta^{15}N$ values.

At present, the mechanisms responsible for decreased $\delta^{15}N$ values in stunted children remain elusive. $\delta^{15}N$ in hair are known to reflect dietary isotopic exposures; $\delta^{15}N$ values in hair are equal to the $\delta^{15}N$ values of the diet, plus a small discrimination factor known as trophic step, that is due to the

preferential elimination of light isotopes during catabolic processes.[233,235] It has been shown that people with a diet low in animal source foods (ASF) have lower $\delta^{15}N$ values compared to fish or meat-eaters.[332] Lastly, it has been shown that the trophic step magnitude varies according to the relative catabolic activity, and periods of weight-loss (such as in anorexia nervosa or morning sickness during early pregnancy) are associated with an increase in hair $\delta^{15}N$.[245,246]

In the absence of information regarding children's diets, and the extent of weight loss over the period before the admission to therapeutic refeeding for SAM, it is not possible to decide between these two potential mechanisms. Further studies are needed to better understand the significance of hair $\delta^{15}N$ differences according to diagnostic phenotypes of SAM.

VII ARTICLE 4

Preliminary bio-electric impedance (vector) analyses in young children with SAM at admission to nutritional rehabilitation

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Abstract: In addition to the biochemical, clinical and isotopic data presented thus far we also measured bio-electric impedance, first in a representative age- and sex- matched community cohort of children, and then in children with SAM. The objective of this analysis was to describe and compare body composition among and between different phenotypes of SAM. The analysis of these results is ongoing. In the following pages I present the preliminary results of these data. These include the references generated from the representative population as well as bio-impedance measurements in children with SAM at admission to treatment.

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Introduction

A better characterization of body composition in malnourished children could potentially aid in diagnosis, clinical management and in determining nutritional requirements.[272,278] Bio-electric impedance analysis is a useful tool in assessing body composition in this group because it is portable, non-invasive, and can be conducted with relative ease in the field setting.

Analysis of bio-electric impedance (often shortened to bioimpedance) data is generally conducted one of two ways. The first is the conventional bioimpedance analysis (BIA) approach whereby impedance (Z), adjusted for height (H), is used to estimate total body water (TBW), then fat-free mass (FFM), and finally fat mass (FM) based on predefined equations.[353,354] These equations usually predict TBW or FFM from the impedance index (HT^2/Z), ideally using a calibration equation generated in the same population. Fat-free mass and fat mass are then often indexed by the square of height (H^2), which produce fat-free mass index (FFMI) and fat-mass index (FMI).

The second approach is bioimpedance vector analysis (BIVA). Developed by Piccoli, this qualitative approach separates impedance into its two components: resistance (R) and reactance (Xc). R and Xc are then divided by height (H) and plotted on a Cartesian plane called the "R/H-Xc/H graph." The data form an ellipse bisected by two axes (**Figure 22**) that are proposed to indicate the level of hydration and cell mass.[271]

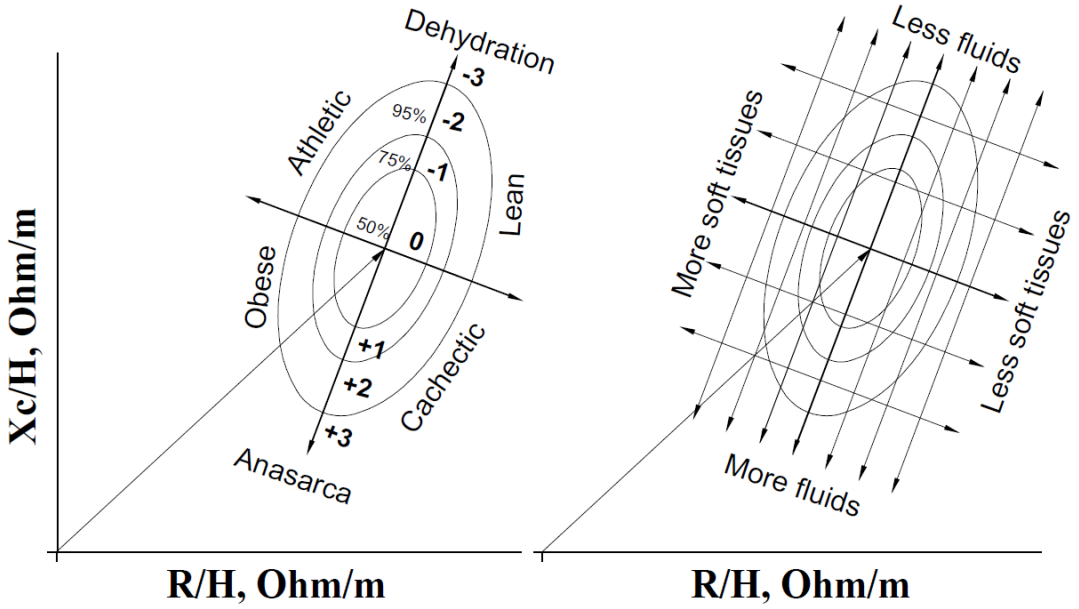

Figure 22. Bio-electric impedance vector analysis (BIVA) patterns on the R/H-Xc/H graph

Vector displacement parallel to the major axis of the tolerance ellipse indicate progressive changes in hydration whereas displacement parallel to the minor axis indicates more (above, left) or less (below, right) cell mass contained in soft tissues. Vectors falling outside the 75% tolerance ellipse indicate abnormal tissue impedance.

Source: Piccoli *et al.*, 2002.

Specifically, the diagonal axis represents variability in hydration where the orthogonal axis represents variability in body cell mass, a proxy for lean body mass.[271,272] The "phase angle" (PA) is the angle of each observation relative to the "R/H" axis. Phase angle is thought to represent a marker of both cell mass and cellular health.[272–276] Higher PA is proposed to indicate higher cell mass, cell membrane integrity and better cell function.[272,277]

Studies on different age groups have shown that vector parameters vary with both age and sex.[355] One solution to this issue is to generate standard deviation scores (SDS) for raw or height-adjusted BIVA parameters, to remove sources of variability linked to age and sex.[272] This approach uses data on a population of normal healthy individuals to generate reference centile curves with which subjects can be identified as 'unusual' if their particular measurement value (*i.e.*, R/H, Xc/H, etc.) lies in one or another tail of the reference distribution.[356] Centile curves (compared to a simple reference range) are particularly useful when the measurement is strongly dependent on a continuous covariate (which most often is age) and the reference range changes with that covariate;[356] such is the case for BIVA parameters. Reference centile curves are widely used in medical practice[356] and are increasingly used in bioimpedance analysis.[272,357]

Methods

Study design and setting

This multicentic, longitudinal observational study was conducted within the framework of the larger OptiDiag study, in Bangladesh, Burkina Faso and Liberia (Clinical trials NCT03400930). In brief, the OptiDiag study aimed to characterize the health and nutrition status, pathophysiology and functional severity linked to anthropometric phenotype. To that end, we compared the bioimpedance parameters (R, Xc, PA, R/H, Xc/H, TBW, FFM, FM, FFMI and FMI) of non-edematous, SAM children classified as: (1) low WHZ-only; (2) both-low WHZ and low MUAC; or (3) low MUAC-only. Patients were followed throughout nutritional rehabilitation, and data were collected at key time points during follow-up. The results presented in this paper come from admission data only, when the health and nutrition status is expected to be the most degraded, before the beginning of nutritional rehabilitation.

Participants

Children aged 6-59 months with SAM, currently residing in the catchment area and after caregiver consent, were screened for eligibility. Children meeting any of the following criteria were excluded: bilateral pitting edema; medical complications requiring inpatient care; inability to drink or breastfeed; bloody diarrhea; uncontrolled vomiting; convulsions; lethargy; unconsciousness; known peanut and/or milk allergies; malformations that affect food intake (such as cleft palate, cerebral palsy, and Down's syndrome); plans to leave the catchment area within the next 6 months. For comparison, healthy age- and sex-matched community controls children (n = 196) were recruited for to produce normalized, control data.

Data collection

Anthropometric measurements (weight, height/length) were taken in duplicate as per WHO recommendations (WHO and UNICEF 2019). Weight was measured using either a SECA hanging scale or a mother/baby scale. Length or height (change of measuring position at 2 years) was measured using a standard UNICEF wooden measuring board, to the nearest 1 mm.

Single frequency BIA was conducted at 50 kHz using Nutriguard-S instrumentation (Data Input GmbH, Germany). Participants lay supine on an medical examination table with the legs apart at an angle of ~45° so that the thighs did not touch, and the arms resting ~30° from the body. Disposable,

gelled electrodes were attached to the dominant hand and foot in the standard tetrapolar, ipsilateral positioning. Injecting leads were attached to electrodes placed on the dorsal surface of the hand and foot, proximal to the metacarpal phalangeal and metatarsal phalangeal joints, respectively.[249] Measuring leads were attached to electrodes placed at the proximal edge of the ulna head (pisiform prominence of the wrist) and between the medial and lateral malleoli of the ankle.[249] R, Xc, and PA were recorded in duplicate, and the results averaged. Trained nursing staff rated the position of the child on a 5-point Likert scale in **Table 21**.

Table 21. BIA quality scale

Rating	Description
Ideal	Limbs not touching and straight • Child lying on his/her back, arms towards the lower body, straight elbows and legs, thighs apart, arms not touching the body, calm. All limbs resting on the ground.
Good	Limbs not touching, very slight bends in arms and legs • Small variations from the ideal position: slight bends in the knees or the elbows, but overall the child is lying flat, relatively calm and most importantly the arms are not touching the torso and the thighs are not touching each other either (no touching whatsoever).
Fair	Limbs not touching but bent • Limbs separate, not touching, legs or arms maybe bent and/or arms until elbow on the ground but from elbow onwards possibly in the air or towards the upper body.
Bad	Limbs touching • Arms touching the torso and/or thighs or feet touching each other but child still at the moment of the measurement.
Catastrophic	Child moving • Child crying, moving, contracted, not laying on his/her back (sitting, or on the side), caretaker touching the child.

Only measurements rated fair, good or ideal were used in analyses. R and Xc were standardized for height (H),[358] expressed as R/H and Xc/H in Ω/m. Prior to analyses, individuals were included based on the following criteria: PA >8.0°; | duplicates difference | >0.5 for PA; | duplicates difference | ⩾6.0 for R/H and Xc/H.[272]

Data analysis

We employed the following analytical techniques to interpret our data:

1. Standard Deviation Scores (SDS)

First, sex-specific reference centile curves were produced for all bioimpedance outcomes using the lambda-mu-sigma (LMS) method (LMS Chart Maker, Medical Research Council) on community control data. This statistical approach is used to obtain smoothed centile curves from cross-sectional data that indicate the pattern of growth in the population.

Then, to control for age and sex, all bioimpedance outcomes for SAM patients (R, Xc, PA, R/H, Xc/H, H^2/Z, and 1/Z) were converted to standard deviation scores (SDS) by month of age, using the reference centile curves.

Total body water (TBW) and fat free mass (FFM) were predicted using the following equations generated by Wells *et al.*,[354] in Gambian infants, using isotope dilution as the reference method:

$$TBW\ (kg) = 1.596 + 0.521(H^2/Z)$$

$$FFM\ (kg) = 1.930 + 0.669(H^2/Z)$$

Fat mass (FM) was calculated by subtracting weight from FFM. Finally, FM and FFMI were divided by the square of height in meters to calculate the FM index (FMI) and FFMI index (FFMI).

ANOVA was used to compare means among anthropometric phenotypes for normally distributed data and Kruskal-Wallace tests were used to compare median for skewed data.

2. Bioimpedance Vector Analysis (BIVA)

Confidence and tolerance ellipses were obtained using Piccoli's software.[359] Confidence ellipses for anthropometric phenotypes were compared using Hotelling's t-test.

A first "tolerance" categorical variable was created based on the position of each individual observation (*i.e.*, SAM patient) within the concentric, tolerance ellipses ($0 \leqslant 50\%$; $50\% \leqslant 1 < 75\%$; $75\% \leqslant 2 < 95\%$; $95\% \leqslant 3$). A second "quadrant" categorical variable was created based on the position of each observation between the two axes (*i.e.*, Piccoli's *et al.* "tissue hydration" major axis and "soft tissue mass" minor axis[359]) by subdividing the plane into 4 counterclockwise quadrants, according to the mathematic custom (I=lean; II=athletic; III=obese; IV=cachectic). Note that, however, these naming conventions are not directly applicable for use in infants and young children. I report them here for easier reference to the way Piccoli names the quadrants in **Figure 22**. Bivariate and logistic regression were used to examine the associations between each "tolerance" or "categorical" positioning and the odds of being in one diagnostic group compared to the other.

All analyses were performed using STATA version 13.0 and $p<0.05$ was used to determine statistical significance.

Results

A total of 196 non-malnourished (MUAC ⩾125 and WHZ ⩾ –3) individuals provided age- and sex-matched community control data to produce BIVA charts. Average age and [IQR] was 12.7 [8-16] months. **Figure 23**, **Figure 24**, **Figure 25**, and **Figure 26** illustrate BIVA centile charts for each sex.

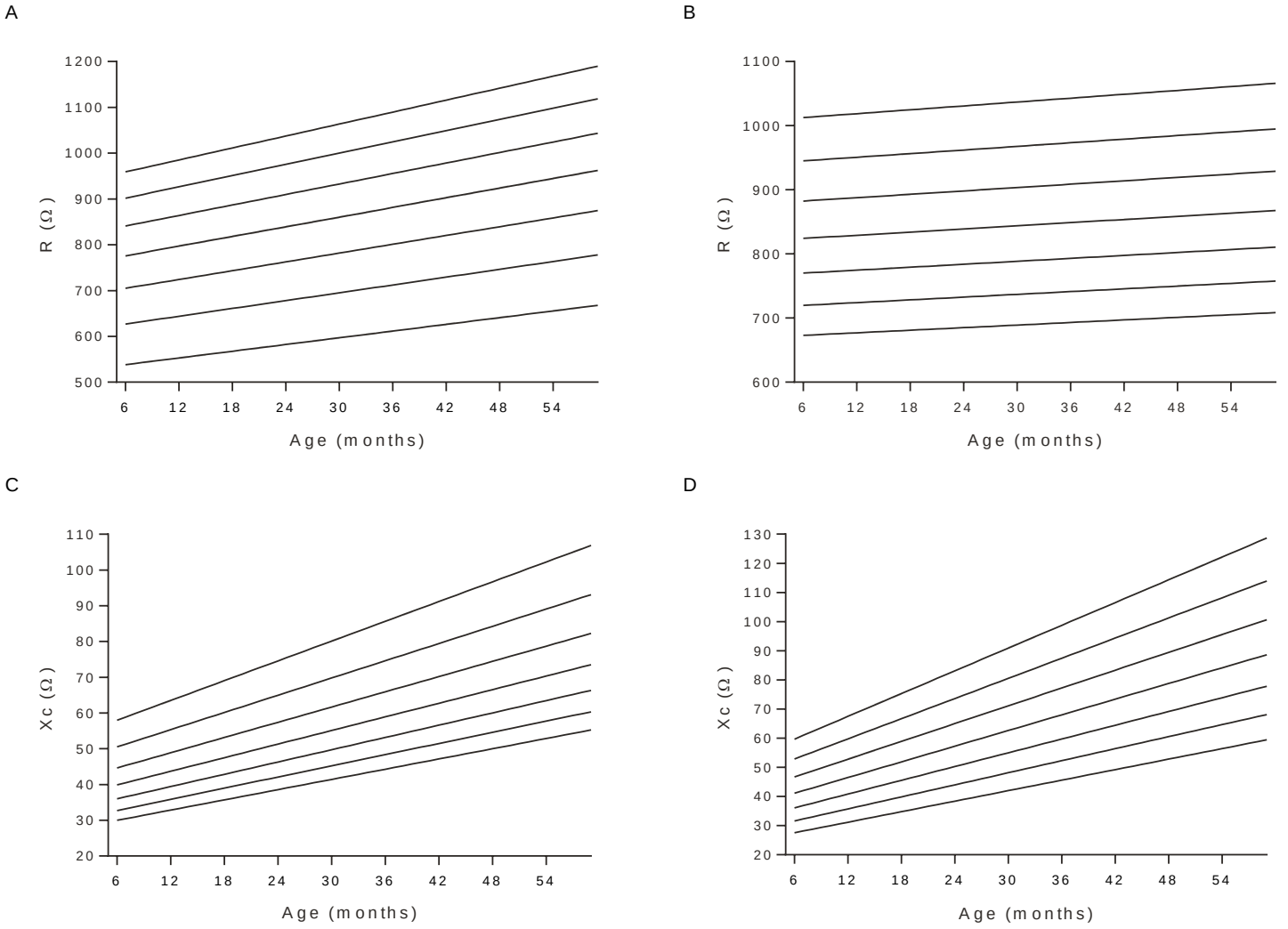

Figure 23: Centile charts for BIVA outputs R and Xc (left column males, right column females)

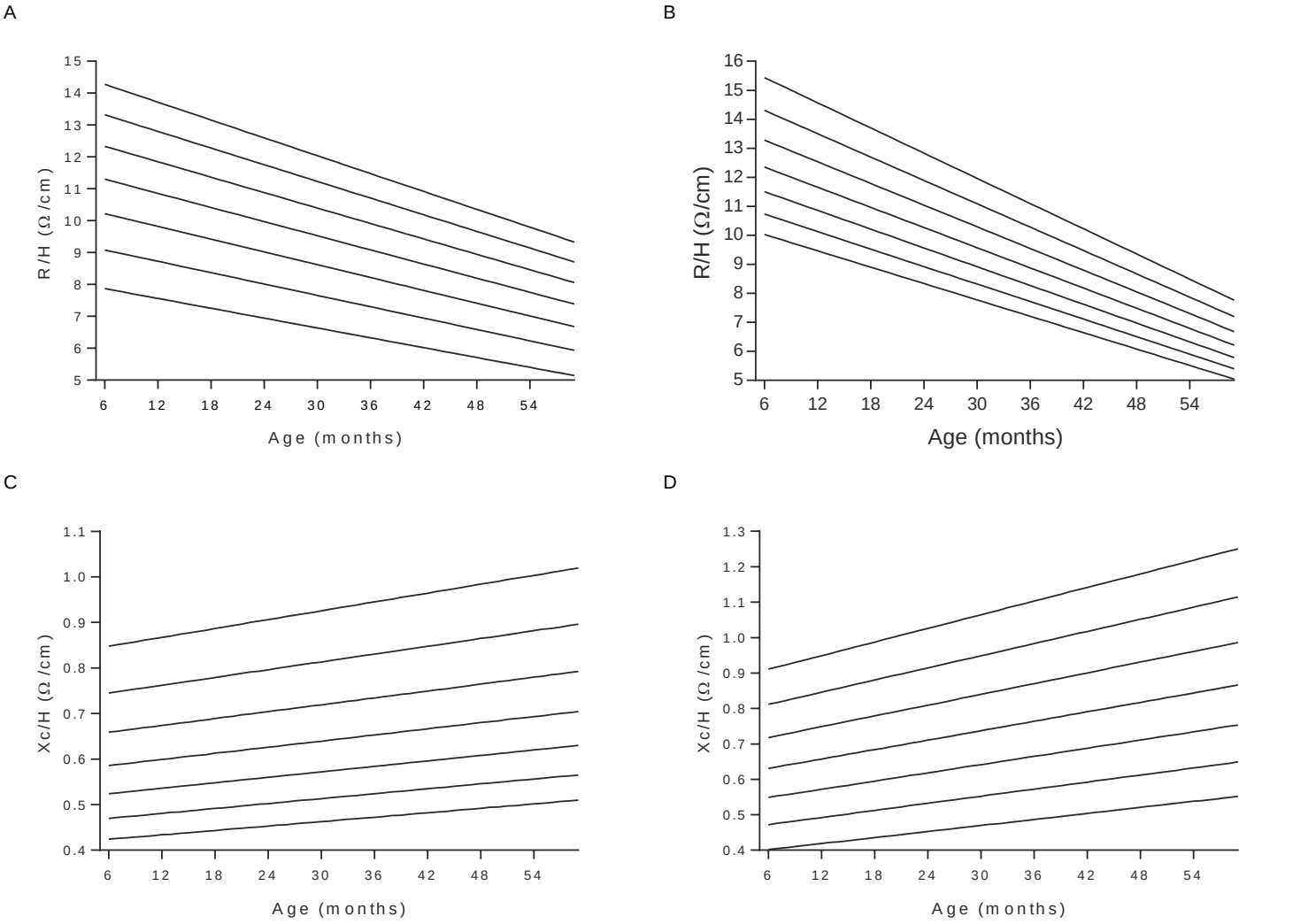

Figure 24: Centile charts for BIVA outputs R/H and Xc/H (left column males, right column females)

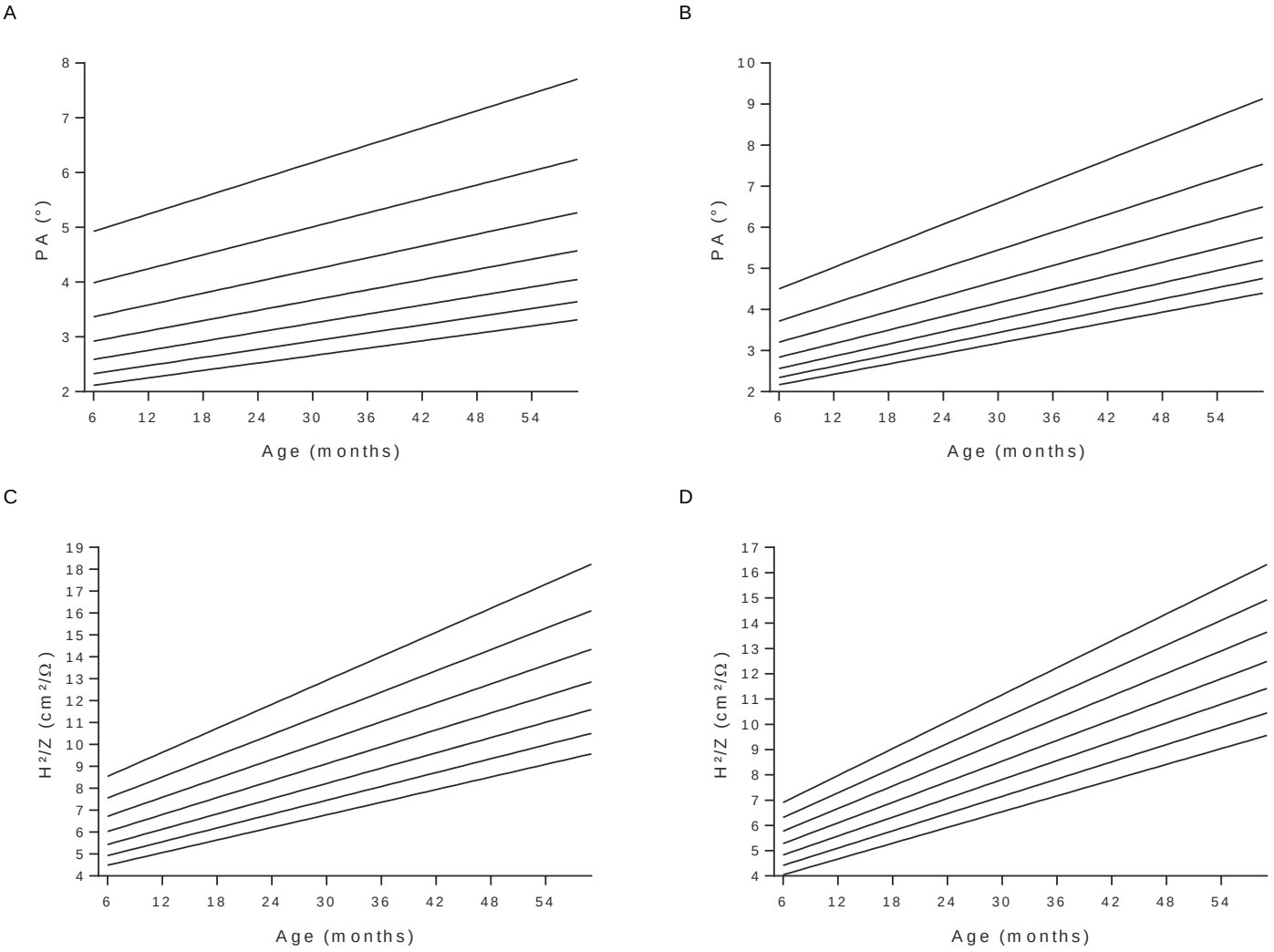

Figure 25: Centile charts for BIVA outputs PA and H²/Z (left column males, right column females)

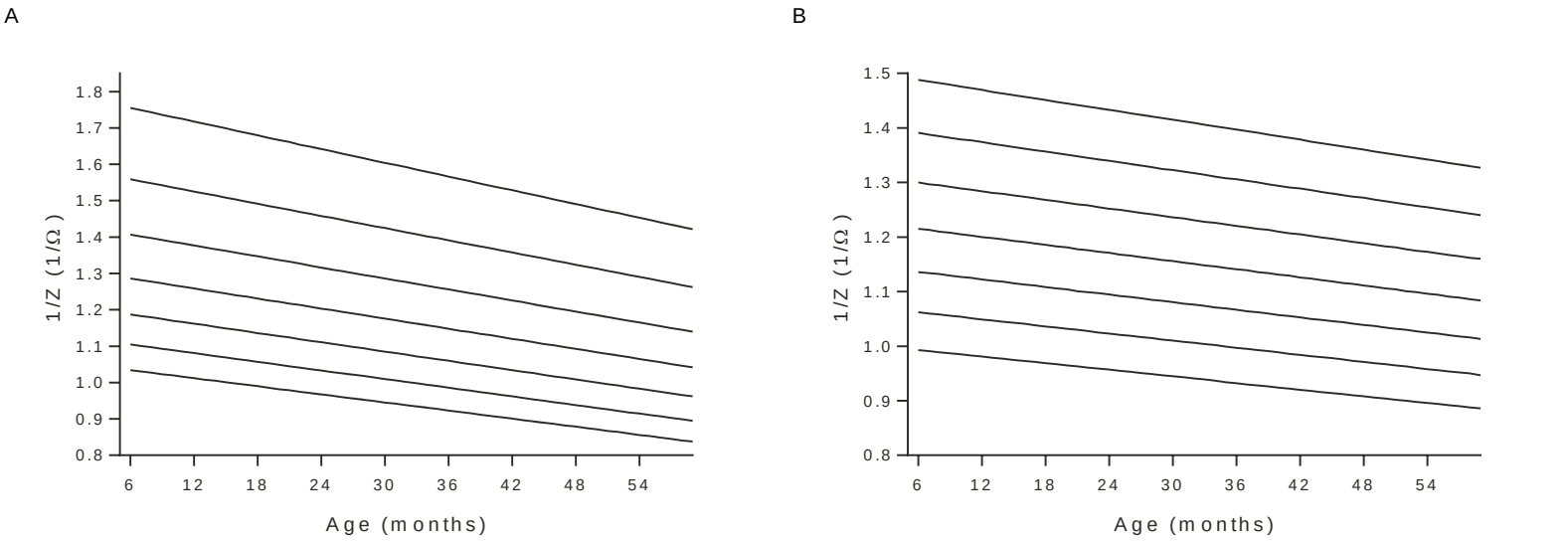

Figure 26: Centile charts for BIVA output 1/Z (left column males, right column females)

R, Xc, Xc/H, PA, and H²/Z increased, while R/H and 1/Z decreased, in a linear manner with age; for both sexes, each BIVA output behaved relatively similarly.

Mean (and median) BIA parameters for each anthropometric phenotype at admission are shown in **Table 22**.

Table 22. Mean (and median) BIVA parameter SDS and absolute body composition from BIA

	WHZ-only (n=138)		WHZ&MUAC (n=152)		MUAC-only (n=161)		p
R-SDS (n=243)	1.12	± 1.43	1.41	± 1.79	0.80	± 1.48	0.0464
Xc-SDS (n=226)	0.05	± 1.13	0.35	± 1.40	–0.18	± 1.40	0.0516
R/H-SDS (n=242)	1.49	± 1.40	1.76	± 1.62	1.44	± 1.53	0.3272
Xc-SDS (n=233)	0.13	[–0.81 –0.74]	0.57	[–0.56 - 1.35]	0.06	[–0.68 - 1.07]	0.1355
PA-SDS (n=201)	-0.62	[–2.03 - –0.28]	–1.02	[–1.86 - –0.10]	–0.77	[–2.02 - –0.06]	0.9870
H²-SDS (n=234)	-1.13	± 1.16	–1.67	± 1.25	–1.59	± 1.32	0.0152
1/Z-SDS (n=154)	-72.48	[–73.33 - –71.47]	–72.27	[–73.49 - –71.06]	–71.65	[–72.59 - –70.70]	0.0368
TBW (n=234)	4.70	[4.23 - 5.82]	4.15	[3.85 - 4.57]	4.13	[3.76 - 4.56]	0.0001
FFM (n=234)	5.93	[5.33 - 7.37]	5.23	[4.84 - 5.77]	5.21	[4.73 - 5.76]	0.0001
FM (n=234)	1.20	± 0.99	0.45	± 0.78	0.70	± 0.84	0.0000
FFMI (n=234)	10.39	[9.48 - 11.62]	11.38	[10.46 - 12.54]	12.28	[11.02 - 13.36]	0.0001
FMI (n=234)	2.22	[1.06 - 2.80]	1.00	[–0.32 - 2.02]	1.56	[0.28 - 2.64]	0.0006

Abbreviations: FFM, fat-free mass; FFMI, fat-free mass index; FM, fat mass; FMI, fat mass index; PA, phase angle; R, resistance; SDS, standard deviation score; TBW, total body water; Xc, reactance; Z, impedance.

Values are mean ± SD or median [IQR] for normally distributed and skewed data, respectively.

The following variables were significantly different among anthropometric phenotypes: R-SDS, H²/Z-SDS and 1/Z-SDS, TBW, FFM, FM, FFMI and FMI; a marginally significant difference for Xc-SDS among groups was detected.

To identify between which two anthropometric phenotypes the significant differences exist, further pairwise comparisons were conducted, presented in **Table 23.**

Table 23. Pairwise comparisons

	WHZ-only vs. MUAC-only	WHZ-only vs. WHZ&MUAC	MUAC-only vs. WHZ&MUAC
R-SDS	0.1521	0.2575	0.0182
Xc-SDS	0.2811	0.8831	0.0231
H^2-SDS	0.0192	0.0067	0.7065
1/Z-SDS	0.0236	0.0443	0.8155
TBW	0.0001	0.0001	0.5979
FFM	0.0001	0.0001	0.5979
FM	0.0007	0.0000	0.0539
FFMI	0.0001	0.0007	0.0063
FMI	0.1227	0.0001	0.0287

Abbreviations: FFM, fat-free mass; FFMI, fat-free mass index; FM, fat mass; FMI, fat mass index; PA, phase angle; R, resistance; SDS, standard deviation score; TBW, total body water; Xc, reactance; Z, impedance.

Values are p-values from pairwise comparisons in Table 22.

- Mean R-SDS and Xc-SDS were significantly lower in MUAC-only patients compared to both-WHZ & MUAC patients.
- Mean H^2/Z-SDS and median 1/Z-SDS was significantly lower in MUAC-only patients, and in both-WHZ & MUAC patients, compared to WHZ-only patients.
- Median TBW, FFM and mean FM were all significantly lower in MUAC-only patients, and in both-WHZ & MUAC patients, compared to WHZ-only patients.
- Median FFMI was significantly lower in WHZ-only patients, and in both-WHZ&MUAC patients, compared to MUAC-only patients; FFMI was also significantly lower in WHZ-only patients compared to both-WHZ&MUAC patients.
- Median FMI was significantly higher in WHZ-only patients, and in MUAC-only patients, compared to both-WHZ&MUAC patients.

Figure 27 illustrates group means and 95% confidence ellipses for each anthropometric phenotype and specific control reference, separated by sex (left panel males, right panel females).

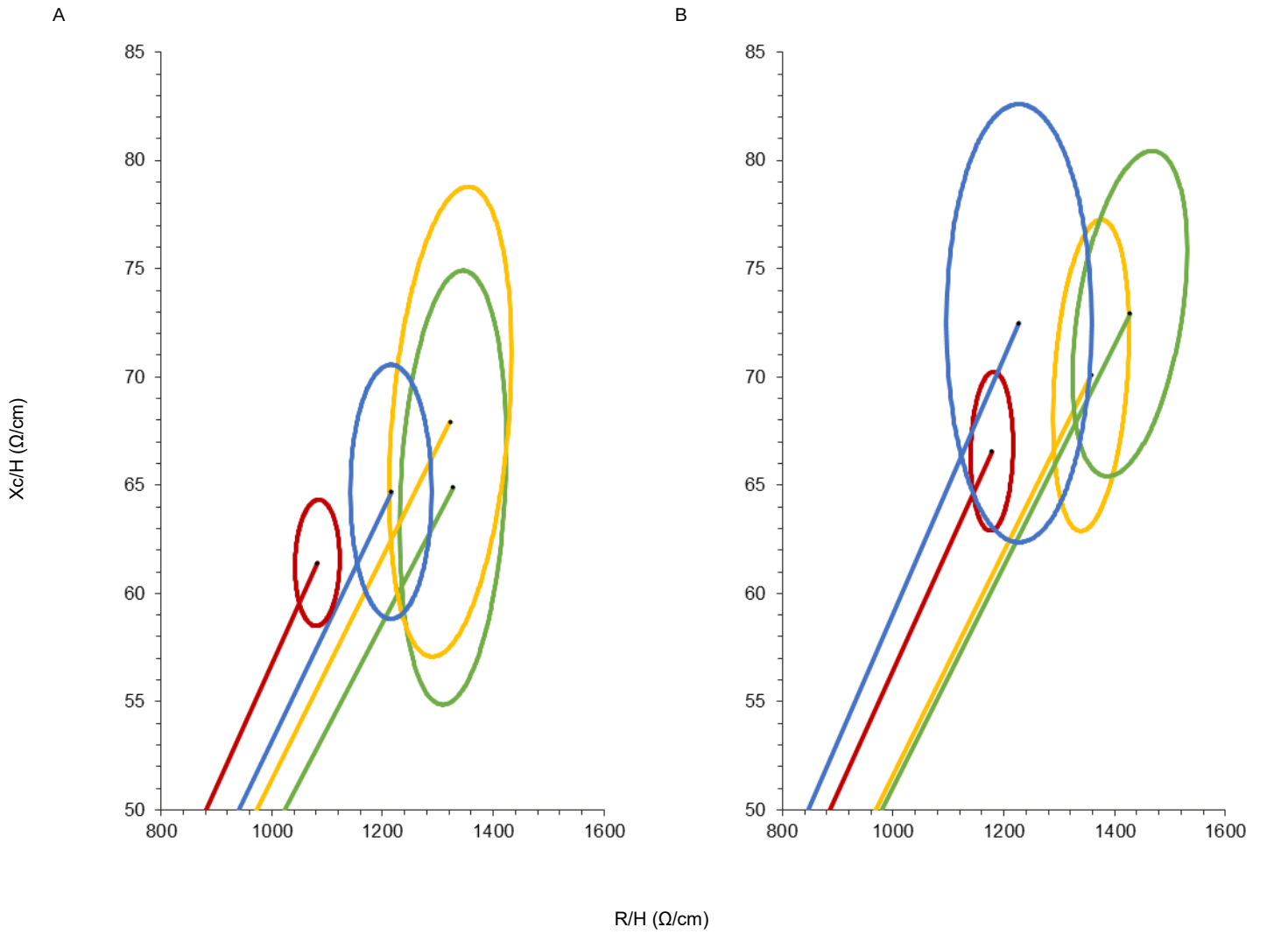

Figure 27: Confidence ellipses by anthropometric phenotype (left panel males, right panel females)
— reference; — MUAC-only; — both-WHZ&MUAC; — WHZ-only.

For both males and females, MUAC-only and both-WHZ & MUAC groups were broadly distributed in the same area, with significant overlap; WHZ-only groups were displaced slightly to the left, toward the reference group. No differences were observed between sex-separated anthropometric phenotypes, by Hotelling's t-test. For males, all anthropometric phenotypes were significantly different from the male reference group. For females, only MUAC-only and both-WHZ & MUAC diagnostic groups were significantly different from the female reference group.

Figure 28 and **Figure 29** illustrates the position of each SAM child within the 50%, 75% and 95% tolerance intervals, for males and females respectively.

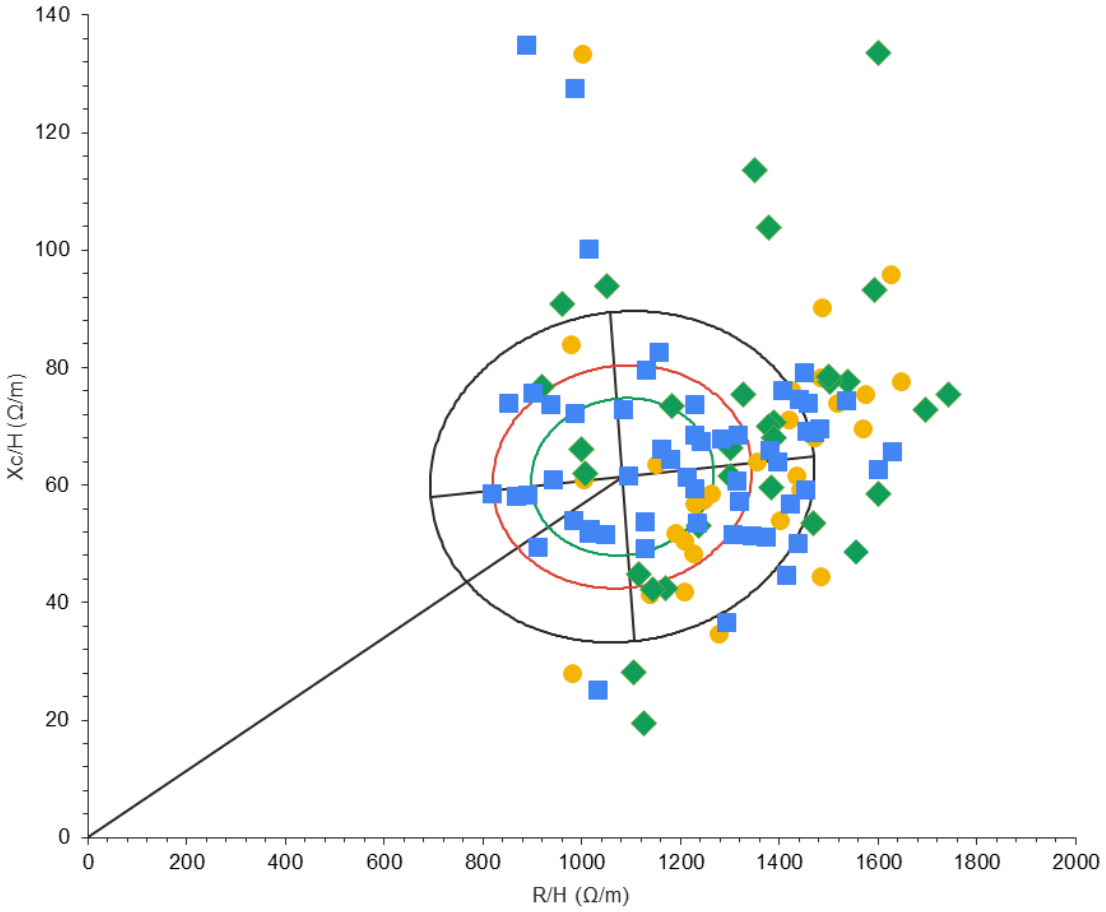

Figure 28: Tolerance ellipses for males

Abbreviations: H, height; R, resistance; Xc, reactance.

MUAC-only; both-WHZ&MUAC; WHZ-only.

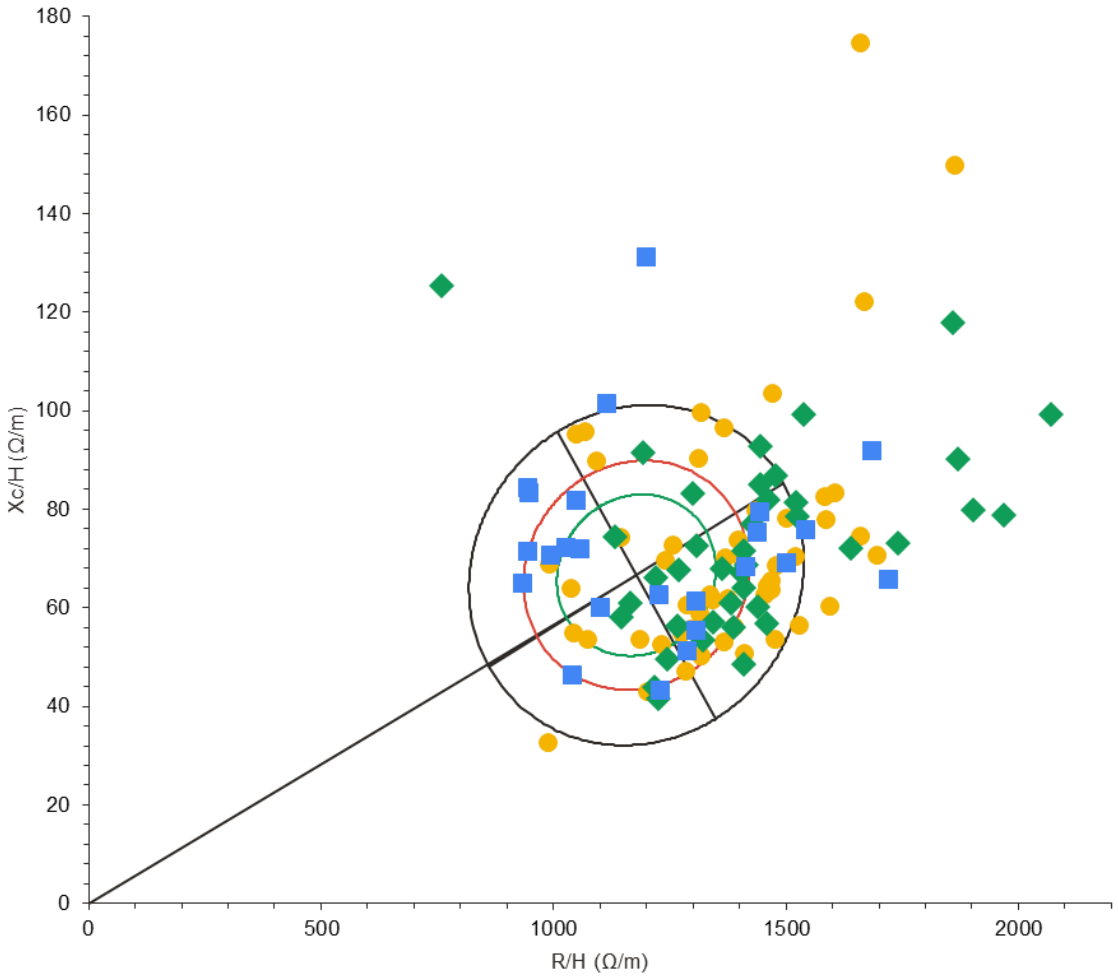

Figure 29: Tolerance ellipses for females

Abbreviations: H, height; R, resistance; Xc, reactance.

MUAC-only; MUAC&WHZ; WHZ-only.

Figure 28 and **Figure 29** show that, regardless of sex, SAM children are generally distributed in quadrants I and II. Moreover, the vast majority of patients fell inside the 95% tolerance interval, with some severe outliers. **Table 24** reports the bivariate chi-square analysis of ellipse/quadrant position and anthropometric category.

Table 24. Chi-square analysis of category by anthropometric phenotype

	MUAC-only		WHZ&MUAC		WHZ-only		*p*
>75% tolerance interval	60.2	(50)	64.8	(46)	52.4	(42)	0.296
>95% tolerance interval	30.1	(25)	40.9	(29)	25.0	(20)	0.105
Quadrant I: Lean	57.8	(48)	50.7	(36)	36.3	(29)	0.020
Quadrant II: Athletic	20.1	(25)	33.8	(24)	31.3	(25)	0.883
Quadrant III: Obese	2.4	(2)	7.0	(5)	17.5	(14)	0.003
Quadrant IV: Cachectic	9.6	(8)	8.5	(6)	15.0	(12)	0.384

Abbreviations: MUAC, mid-upper arm circumference; WHZ, weight-for-height *z*-score.

Values are % (*n*).

The only statistically significant differences detected among anthropometric categories was for quadrants I and III. To precisely identify significant differences, further pairwise comparisons between anthropometric groups confirmed the following:

- Compared to WHZ-only patients (36.3%), the proportion of MUAC-only patients (57.8%) in quadrant I was significantly higher (p=0.006); no significant difference was observed between WHZ-only and both-WHZ&MUAC patients (p=0.073)
- Compared to WHZ-only patients (17.5%), the proportion of MUAC-only patients (2.4%, p=0.001 from Fisher's exact test) in quadrant III was significantly higher; similar to quadrant I, no significant difference was observed between WHZ-only and both-WHZ&MUAC patients (p=0.053)

Discussion

In this study, we used bio-electric impedance to describe and compare body composition among anthropometric phenotypes in SAM. Because the interpretation of body-composition data requires adjustment for body size,[357] we first developed reference charts from age- and sex- matched community controls, and used them to generate adjusted BIA SDSs for SAM patients.

Our results can be segregated into those from the conventional BIA approach and those from the bio-electric vector analysis (BIVA) approach.

Quantitative Bioimpedance Analysis (BIA) results

Our conventional analyses indicate that mean H^2/Z-SDS, FM and FFM were significantly lower in MUAC-only and both-WHZ & MUAC patients, compared to WHZ-only patients. However, the aforementioned parameters serve to generate other parameters (TBW, FMI, and FMI, respectively) by way of conversion equations and indexation, and are not directly interpretable as is.

TBW was significantly higher in WHZ-only patients compared to MUAC-only and both-WHZ & MUAC groups. No statistical difference in TBW was detected between MUAC-only and both WHZ & MUAC groups. These results do not fully support our findings on clinical dehydration prevalence in **Article 1**. We find that children with both anthropometric deficits have less TBW and higher rates of clinical dehydration, compared to WHZ-only children. However, dehydration prevalence is significantly higher in both-WHZ&MUAC compared to MUAC-only children, whereas TBW was not significantly different between these groups. This contradiction is the first of multiple indications that using the Gambian conversion equation on our dataset has limitations, which we discuss further in the following paragraphs and in detail in the limitations section.

After indexation for height, FFMI reports that WHZ-only patients have the least muscle mass of the three phenotypes, followed by patients with both anthropometric deficits; MUAC-only patients therefore had the most muscle mass. Furthermore, FFMI was significantly different in each pairwise comparison between phenotype. This result is coherent with our preliminary findings in **Article 3**, which report that $\delta^{15}N$ values are negatively associated with MUAC <115 mm (but not with WHZ <–3), suggesting a link between MUAC deficit and the preservation of muscle mass. We hypothesize that this preservation of muscle mass still occurs in children with both anthropometric deficits, but

to a lesser extent, evidenced by a slightly higher FFMI, statistically midway 'between' that of WHZ-only and MUAC-only.

FMI suggests that WHZ&MUAC patients had significantly less fat mass compared to children with either deficit alone. This result is coherent with our findings in **Article 1**, which report that serum leptin was lowest in children with both anthropometric deficits. Interestingly, median FMI was not statistically different between WHZ-only patients and MUAC-only patients. Yet, **Article 1** also reports that leptin was significantly lower in WHZ-only children compared to MUAC-only children—a statistically significant effect that was not detected by FM. Failure to detect this difference again suggests the limits of the Gambian equation.

Qualitative Bioimpedance Vector Analysis (BIVA) results

Piccoli's *et al.* bioimpedance vector analysis technique provides an alternative, qualitative approach that does not rely on the use of conversion equations [360]. Based on these data, Piccoli's software constructs and analyzes two types of ellipses (confidence and tolerance) that provide important and distinct information.[360]

Because R and Xc components are significantly positively correlated in humans at all ages, and in health and disease,[360] they are not individually interpreted; instead combined changes (phase angle) are considered. Numerous studies suggest that PA is a valuable index of clinical status,[272–276] marking both cell mass and cellular health.[271] In our study, mean PA-SDS were not different among anthropometric phenotypes, suggesting a similar cellular health profile regardless of SAM type.

Piccoli's "confidence" software first establishes the mean impedance vector position for each subgroup (in our study MUAC-only, both-WHZ & MUAC, WHZ-only, and the sex-specific control reference) as well as the 95% confidence interval around each mean position. The 95% confidence interval models the variability around the mean for the subgroup. Generally, when the 95% confidence intervals between groups do not overlap, the group means are significantly different.[360] However, the reverse it not necessarily true. With this in mind, our data report that, in boys, mean impedance for all anthropometric phenotypes are significantly different from the male control reference. For girls, mean vector position for all anthropometric phenotypes are also significantly different from the control reference population, with the exception of WHZ-only girls. Note that the area of the confidence ellipse around the mean vector position measures the "elliptical probability region," and is directly proportional to the variability in the group, and the size of the sample.[360] Sex is a known determinant of anthropometric phenotype,[12,18] boys tend to be WHZ-only and girls tend to be MUAC-only, as is the case in our sample. Because of this, we had few WHZ-only girls (n=18) and the area confidence ellipse in this group is larger (**Figure 27**, right panel). It is impossible to speculate whether a much smaller confidence ellipse for WHZ-only girls would have overlapped with the reference population had we had a larger sample and better statistical power.

Piccoli's "tolerance" software evaluates soft tissues and hydration status for each individual based on his/her place within three concentric tolerance ellipses (50%, 75% 95%). Vectors falling outside the

75% tolerance ellipse are generally indicative of abnormal tissue impedance.[359] Moreover, relative displacement of patients along the major axis indicates changes in tissue hydration (dehydration with long vectors versus hyperhydration with short vectors); patient displacement along the minor axis indicates the quantity of cell mass contained in soft tissues (more soft tissue with vectors left of the major axis versus less soft tissue with vectors right of the major axis).[359] Based on this paradigm, Piccoli characterized the four quadrants created by the "tissue hydration" major axis and "soft tissue mass" minor axis[360] as follows: I=lean; II=athletic; III=obese; and, IV=cachectic. As mentioned previously, the names of these quadrants do not directly apply to the physical description of infants and young children; however, we use them as convention. Our data suggest that significant differences in the broad distribution of patients across these quadrants. First, the proportion of MUAC-only patients in the 'lean' quadrant I is significantly larger compared to WHZ-only patients. This result suggests a clustering of MUAC-only patients in this area, whereas WHZ-only patients appear to be more variably distributed across the quadrants. Second, the proportion of MUAC-only patients in the 'obese' quadrant I is significantly smaller compared to WHZ-only patients. Note that the number of observed values in the MUAC-only group (n =2) was less the minimum 5 required for Pearson's χ^2 test; we therefore used Fisher's exact test, but are hesitant to conclude on this effect.

Arguably, the most important result from our tolerance analyses is the lack of statistically significant differences in the proportions of children outside the 75%, and 95% ellipses. Indeed, Piccoli *et al.* suggest that vectors outside the 75% tolerance ellipse indicate abnormal tissue impedance.[360] Our data report alarmingly high proportions of abnormal (>75%) and very abnormal (>95%) tissue impedance across phenotype. These results are generally supported by those in Article 1, which report that, with the exception of some notable key differences, all SAM children presented with clinical/biochemical profiles of marked severity (*i.e.*, nutritional deprivation, micronutrient deficiencies, and morbidity).

Limitations

As previously alluded to, this study had some limitations. First, use of the Well's *et al.* Gambian equation to derive TBW, FM and FFM inevitably induces some error. Conventional BIA relies on population-specific equations[361–363] which are ubiquitous for western, adult populations, but scarce for children with SAM. The use of BIVA helps remedy some of BIA's pitfalls. Yet, Well's *et al.* have recently shown body composition outcomes failed to link with BIVA parameters expected based on Piccoli's model.[272]

Conclusions

In summary, bioimpeance impedance (vector) analyses appear to highlight differences in body composition between phenotypes in SAM, at admission. However, the clinical significance of these differences remains elusive to date. We are eager to complement these preliminary analyses with longitudinal data collected during nutritional rehabilitation, which we believe will greatly add to the interpretability of our results.

VIII General discussion

11. Discussion

Anthropometric tools have been widely and successfully used to assess nutrition status in children in the developing world,[364] and will continue to be used for the foreseeable future. The success of these tools is due in large part to their simplicity and practicality, ideal for field use, where the state of local infrastructure and poor technological capacity prohibits reliance on more sophisticated diagnostic methods. Yet, in recent years, actors involved in the management of acute malnutrition have pushed the envelope even further, simplifying diagnostic criteria to the sole use of MUAC for case finding and admission. Doing so leads to an enormous restriction in the humanitarian target population, effectively disqualifying nearly half of children with SAM from access to nutritional rehabilitation.[13] This paradigm shift has continued despite a dearth of robust scientific evidence, leaving policy makers and nutritional actors poorly equipped to make an informed decision on targeting those children most in need of treatment.

The links between pathophysiology, risk and diagnostic phenotype are still poorly understood. Lest we forget that anthropometric measures are proxies for an underlying clinical condition related to health and nutrition that cannot be adequately explored in most resource-poor field settings; abnormal anthropometry does not, in and of itself, provide any etiological information on nutritional status or disease.

The aim of this dissertation was to better characterize the health and nutrition status linked to diagnostic phenotype in SAM. In doing so, we attempted to begin to fill the dauntingly wide research gap with new, robust scientific evidence so policy makers can make an informed decision on which children to target for treatment. Most importantly, we hope that this work, in synergy with ongoing and future research in the same vein, will help policy makers decide if the abandonment of WHZ is justified.

11.1. Summary of key findings

The key findings of **Article 1** suggest that children with low WHZ and/or low MUAC present with clinical evidence of severe nutritional deprivation and micronutrient deficiency—but with notable biochemical and clinical heterogeneities. Indeed, low WHZ-only children presented with higher severity on a range of indicators (*e.g.*, hypoleptinemia, iron deficiency, dehydration, caretaker-reported health and nutritional deterioration) suggesting that these children are at higher risk of acute and long-term morbidity and mortality, compared to children with low MUAC-only. Similarly,

children with both anthropometric deficits presented with augmented severity (*e.g.*, hypoleptineima, bilirubinuria, visible severe wasting, and dehydration) suggesting that these children, too, are at higher risk of morbidity and mortality compared to those with low MUAC alone. Finally, a comparison between children who would be eligible for treatment in a MUAC-only program versus those who would be excluded highlighted no significant difference in serum leptin; however, children who would be excluded presented with higher proportions of iron deficiency and caretaker-reported health deterioration alone or with concomitant weight loss and reduced food intake. Together these results challenge the clinical and ethical justification of MUAC-only programming.

The key findings of **Article 2** report that stable isotopic ratios of $\delta^{15}N$ and $\delta^{13}C$ decrease with age in a representative population of children from aged 6-59 months (on the order of the loss of one tropic step) due to dietary transition from exclusive breastfeeding to weaning completion. This study did not identify an isotopic signature associated with acute malnutrition, but did evidence lower $\delta^{15}N$ and $\delta^{13}C$ in severely stunted children compared to non-stunted children. We hypothesize that these results likely reflect chronic exposure to diets low in animal source foods in stunted children.

Article 3's preliminary analyses of $\delta^{15}N$ and $\delta^{13}C$ stable isotopic ratios in children with SAM confirm the negative associations with age reported in **Article 2**. Moreover, adjusted analysis of $\delta^{15}N$ and phenotype reported a negative association with severe MUAC deficit, but not with severe WHZ deficit.

The preliminary findings of **Article 4** suggest some heterogeneity in body composition parameters among anthropometric phenotypes. Conventional BIA results report significantly lower FFMIs in WHZ-only and both-WHZ&MUAC patients compared to MUAC-only patients. Furthermore, FMI was significantly higher in WHZ-only patients, and in MUAC-only patients, compared both WHZ & MUAC patients.

Further BIVA analyses, however, do not indicate significant differences in cellular health (*i.e.*, PA) or in prevalence of abnormal tissue impedance (*i.e.*, >75% tolerance) among anthropometric phenotypes.

11.2. Interpretations

While the key findings of **Article 1** evidence particular phenotypic heterogeneities, suggesting divergent nutritional needs and/or morbi-mortality risks, our results also highlight broad impairment in nutrition and health status across anthropometric groups. Ample biochemical evidence of immunological stress and vitamin A deficiency, combined with ample clinical evidence of acute upper respiratory infections, diarrhea, fever and vomiting, together paint a bleak picture the SAM child, regardless the anthropometric category to which he or she belongs.

With **Article 2**, **Article 3**, and **Article 4** we sought to push the envelope even further, to better characterize the health and nutritional perturbations uncovered in **Article 1**. Indeed, while the biochemical and clinical indicators measured in **Article 1** provide novel, valuable information, the conclusions drawn from them were limited, inciting further exploration.

Article 2 and **Article 3** aimed to provide an objective, dietary and metabolic assessment using stable isotopic ratios in hair; first, in a representative sample of children, and then in children with SAM. Stable isotope analysis is a novel, promising tool for dietary assessment.[221] The scientific literature suggests that, at least in pregnant and/or anorexic women, stable isotopic ratios of nitrogen (and to a lesser extent carbon) provide clear evidence of metabolic (and specifically catabolic) orientation.[244–247] We hypothesized that SAM is associated with acute protein wasting which, like Fuller, Hatch and Mekota *et al.* illustrate, can be isotopically detected in hair by an increase in $\delta^{15}N$ values.

Results from the cross-sectional assessment in **Article 2** confirm the utility of stable isotope analysis in dietary assessment. We report a clear isotopic signature linked to diet, with the loss of one trophic step during the transition from exclusive breastfeeding to weaning completion. However, we failed to identify a specific isotopic effect linked to acute malnutrition. This is likely because the study was not originally intended to do so, and severely underpowered (n=18 for SAM). A secondary explanation is that dietary and metabolic drivers had opposite, competing effects on isotopic values: any increase in $\delta^{15}N$ due to muscle wasting may have been blurred by a decrease in $\delta^{15}N$ brought on by weaning. Indeed, children with SAM in our study were generally between 6-24 months, a period of rapid decline in $\delta^{15}N$ with diversification.

Unexpectedly, we found that $\delta^{15}N$ and $\delta^{13}C$ were significantly lower in severely stunted children compared to non-stunted children. Discussed in detail in **Article 2**, we hypothesize that this stunting-specific isotopic signature is due to: (1) a post-weaning diet low in animal source foods and essential nutrients for growth (*i.e.*, of low isotopic value) consumed by both children and/or their mothers (who breastfed them); and, (2) early origin of stunting beginning with low dietary diversity during pregnancy, possible intrauterine growth retardation, and finally stunting in early infancy. The two aforementioned hypotheses are not mutually exclusive. Low $\delta^{15}N$ and $\delta^{13}C$ in stunting may reflect metabolic adaptations to this context, with increased metabolic efficiency resulting in a preservation of lean body mass at the cost of linear growth in a limiting nutritional context.

The preliminary results in **Article 3** report a negative association between $\delta^{15}N$ and MUAC deficit, which is absent in children with WHZ deficit. Children with SAM by MUAC were more likely to be

both stunted and underweight. As mentioned in the **General Introduction**, underweight (*i.e.*, weight-for-age deficit) indexes both chronic and acute malnutrition.[52] Here again we hypothesize that these stunted (and incidentally wasted and underweight) children, by the nature of their multiple anthropometric deficits, undergo a metabolic adaptation limiting muscle wasting at the cost of linear and ponderal growth. This adaptation is detected isotopically, translating to a lower $\delta^{15}N$ value when compared to SAM children with WHZ deficit. These findings align well with those in **Article 2**, which suggest a particular metabolic adaptation in stunting leading to lower $\delta^{15}N$ values.

Unfortunately, the cross-sectional data we have analyzed to date do not allow for a dietary and metabolic characterization over time. However, we are eagerly working to exploit our longitudinal data, which will allow us to retrace the isotopic history before admission, and also track response to treatment during nutritional rehabilitation. We are optimistic that these additional analyses will bring depth to our understanding of the preliminary differences among phenotypes detected thus far.

We followed the same approach for **Article 4** that we did for **Article 2** and **Article 3**. Namely, we aimed to provide an objective characterization of body composition using bio-electric impedance (both BIA and BIVA)—first in a representative sample of children, and then in children with SAM. As is the case for SIA, BI(V)A are well-established tools for body composition assessment and adapted to field use. The scientific literature, albeit scarce, reports differences in FM and FFM between MUAC and WHZ deficit, purporting differences between these tools in their ability to index growth *vs.* nutritional status.[70] While these findings draw from a characterization of body composition not based on bio-electric impedance analysis, we still hypothesized that similar results would be detected using BI(V)A.

Our preliminary results from **Article 4** do suggest differences in body composition parameters between anthropometric categories. First, children with both anthropometric deficits had significantly less fat mass (FMI) compared to MUAC-only and WHZ-only children. This finding aligns well with findings from **Article 1**, which report that serum leptin was significantly lowest in this group. Surprisingly, **Article 1** also reports a significantly lower serum leptin level in WHZ-only children compared to MUAC-only children, which was not detected by BIA, potentially indicating a limit to the conventional BIA approach. Second, WHZ-only children had less muscle mass (FFMI) compared to MUAC-only children. This finding aligns well with our preliminary findings in **Article 3**, which report a negative association between $\delta^{15}N$ and MUAC deficit (that was not detected in WHZ deficit; this finding suggests isotopic evidence of muscle mass preservation in children with MUAC deficit, but not in children with WHZ deficit.

Since our body composition data are based on BIA, and not air-displacement plethysmography (like in [70]) deriving FMI and FFMI from them requires the use of conversion equations. This is a limit to the conventional BIA approach because the equations must be population-specific [361–363]. Unlike western, adult populations, for which robust BIA equations have been developed from large datasets, few equations exist for children with SAM. We choose Well's *et al.* equation, which is based on a cohort of rural Gambian infants [354]. We assume relative homogeneity in morphology for West African

children (*i.e.*, among Gambian, Burkinabè and Liberian children). However, we detected slight differences in bio-impedance parameters in the Bangladeshi cohort (compared to the African cohorts combined). Specifically, Bangladeshi children stood out has having higher PA, lower R/H and higher Xc/H values. We speculate that these differences indicate that Bangladeshi children have less fat, and therefore more fat-free mass, for a given weight. These differences might make the Wells equation less adapted to our Bangladeshi data. To our knowledge, a better-adapted equation for this population does not exist.

In an effort to overcome these limitations, we complemented the conventional BIA approach with the vector-based BIVA approach. Detailed in **Article 3**, BIVA is a qualitative technique proposed to indicate hydration, cell mass and cellular health.[271,272] The strength of this technique is that it allows for direct comparison between cases (*i.e.*, SAM children) and control data (*i.e.*, age- and sex- matched community controls). Arguably, the most interesting result from our preliminary BIVA analyses is the impressively large proportion of children (~50-60%) with abnormal and/or highly abnormal tissue impedance, without statistically significant differences between anthropometric groups. This finding also aligns well with those of **Article 1**, which generally conclude that (with the exception of some key indicators) all SAM children presented with clinical/biochemical profiles of marked severity.

We are eager to complement these preliminary analyses with longitudinal data collected during nutritional rehabilitation, which we believe will greatly add to the interpretability of our results.

11.3. Implications

The findings of this dissertation highlight complexity and particular heterogeneity in the clinical, biochemical and metabolic profiles in children with SAM. As previously highlighted, anthropometric deficit is a useful indicator to quickly and efficiently identify nutritional deprivation in children in the field setting. However, anthropometric tools are limited in that they do not provide etiological information regarding the complex and often multi-factorial causes that underpin severe acute malnutrition. While our work highlights clinical and biochemical particularities specific to certain anthropometric profiles, most importantly our results reveal that all children with SAM present with profiles characteristic of severe nutritional deprivation—these children unequivocally require nutritional rehabilitation. Our work is supported by recent evidence on mortality risk associated with anthropometric deficit in SAM, which has shown an elevated and similar risk between SAM children with WHZ deficit and MUAC deficit, and augmented risk in those with both anthropometric deficits.[1,2,27,28]

Our results challenge the widespread operational and advocacy strategy of MUAC-only programming because children with SAM who would be excluded from treatment in such programs are at high risk of acute and long-term morbidity and mortality.

Compared to MUAC, WHZ is without doubt more cumbersome to measure, especially in the community. Fortunately, the beginning of the 21st century has seen impressive strides in technological development. The ground today has never been more fertile for a step change towards novel diagnostic solutions to these age-old problems. Very promising endeavors to move SAM diagnosis away from traditional anthropometric techniques and towards more sophisticated methods are already underway. Indeed, both the Child Growth Monitor and SAM Photo Diagnosis projects are working to develop geometric morphometric mobile scanning technology for use in the diagnosis of acute malnutrition. Geometric morphometrics (GM) uses statistical analyses to compare shape, size and position of parts of the human body.[365] A thorough description of these methods is beyond the purview of this dissertation; but, in summary, GM use landmark coordinates to identify shape differences between children with SAM and reference data from children without anthropometric deficit.[324] Coordinate data come from various sources and can be either two- or three-dimensional.[365] They are usually captured using a tablet or specialized volumetric or surface scanners.[365] The data can be processed directly on a mobile application contained within the device, for immediate diagnosis.

I strongly believe that innovative technological solutions like GM are critical to democratize treatment to all SAM children in need. The enthusiastic use of MUAC has helped agencies achieve impressive results in terms of screening and outpatient treatment for ~55% of malnourished children globally.[26] The time has come to focus our efforts on the other ~45%—those with SAM by WHZ alone. These children are not identified in the community, and can only be screened in health facilities equipped with the tools required for measuring weight and height.[26] I am optimistic that the technological development of today's day and age can help target these neglected children.

11.4. Limitations

The OptiDiag project faced chronic resource limitations, both human and financial. No doubt sophisticated medical methods would have improved our characterization of vulnerability in SAM. Non-targeted metabolomics to describe a broad array of hormones, cytokines, growth factors, and metabolites[3] combined with microbial culturomics/metagenomics would have provided a vastly more nuanced understanding to the research question we sought to answer. But alas these methods were cost prohibitive, and entirely unavailable in the OptiDiag study sites.

A limitation in the generalizability of our results stems from the fact that we recruited uncomplicated SAM patients only. By strict definition, uncomplicated SAM refers to patients without fever, infection, or complicated disease according to the Integrated Management of Childhood Illness guidelines, and with an appetite sufficient to consume ready-to-use therapeutic food (RUTF); uncomplicated patients represent about 80% of all SAM children.[323] Our sample included patients with fever and infection, but did not include patients with IMCI danger signs (such as inability to drink and/or breastfeed, uncontrolled vomiting, convulsions, lethargy and/or unconsciousness) or anorexia. We excluded these patients because we assumed that the superimposition of life-threatening illnesses in complicated SAM would obfuscate the interpretation of biochemical data. A simultaneous parallel OptiDiag study run only on SAM patients with medical complications could provide valuable information on vulnerability linked to anthropometric deficit in complicated SAM. Ethical approval for the OptiDiag study was granted on the condition of noninvasive biosample collection and procedures that do not greatly increase opportunity costs for caretakers and their malnourished children. The ethical justification for conducting time-consuming and heavy medical procedures on children with complicated SAM (with their caretakers) would have been very hard to defend. Finally our clinical/biochemical results in **Article 1** would have been greatly strengthen had we had a control group for comparison. Without this group, we can only conclude on relative severity among anthropometric phenotypes, but not absolute severity based on an age- and sex-matched community references. Again, biosample collection on a representative population of healthy children would have been ethically difficult to justify.

11.5. Recommendations

This dissertation is the first step down a long path towards a better understanding of the health and nutrition status of children with SAM, by anthropometric phenotype. A research priority should be to harness technological capacity to develop innovative diagnostic methods to identify low WHZ-only children in the community. Until then, both WHZ and MUAC should be retained as independent diagnostic criteria for SAM, in line with repeated WHO recommendations.

11.6. Perspectives

A truly impressive amount of robust, quality data were collected during the OptiDiag implementation phase—the fruit of which will come to bear for years. We deliberately chose to prioritize analysis of admission data, before nutritional rehabilitation begins, to directly characterize severity in SAM at

its assumed worst. Yet, (mostly) all indicators collected at admission were also collected at key time points throughout nutritional rehabilitation (after 2 weeks, and again after 8 weeks nearing discharge).

Our next step is to tackle these follow-up data, to characterize their flux during rehabilitation, and to compare differences in response between anthropometric groups. Furthermore, we expect that our preliminary BI(V)A and SIA results (presented in **Article 3** and **Article 4**) will be reinforced when combined with these longitudinal analyses. The future perspectives for the OptiDiag project are therefore to first explore these data, and then to use them to strengthen the messaging of our preliminary results.

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Title: Biomedical Investigations for the Optimized Diagnosis and Monitoring of Severe Acute Malnutrition: The OptiDiag Study

Keywords: severe acute malnutrition (SAM); mid-upper arm circumference (MUAC); weight-for-height *z*-score (WHZ).

Abstract: Current WHO diagnostic recommendations segregate non-edematous children with severe acute malnutrition (SAM) into one of three anthropometric phenotypes, those with: (1) low mid-upper arm circumference (MUAC) only; (2) low weight-for-height *z*-score (WHZ) only; or (3) both low MUAC and low WHZ—all of which are eligible for nutritional rehabilitation according to WHO guidelines.

But, based on both ease of use and reports purporting higher mortality in SAM identified by MUAC, many agencies and some national governments use only MUAC as the sole diagnostic criterion for admission to therapeutic refeeding programs—disqualifying low WHZ only children from access to treatment. This diagnostic paradigm shift is premature because the links between anthropometric phenotype and functional severity have not yet been clearly delineated. In fact, recent secondary analyses of historic databases have shown that children with SAM that are excluded from treatment within the framework of a MUAC-only program (*i.e.*, low WHZ only) have a similar risk of death as those who are included; moreover, children with both anthropometric deficits (*i.e.*, low MUAC and low WHZ) have a higher risk of death.

This dissertation aims to describe and compare the pathophysiology and functional severity associated with the anthropometric phenotypes of children with SAM today. Building on existing comparative work on vulnerability in SAM, it asks: how does the vulnerability profile of children with SAM who are excluded from treatment within the framework of a MUAC-only program (*i.e.*, low WHZ only) compare to the profiles of those children who are included (*i.e.*, low MUAC only and/or both low MUAC and low WHZ)?

A multi-centric cohort study was conducted in uncomplicated, non-edematous children with SAM in Bangladesh, Burkina Faso and Liberia. Participants were recruited equally into each of the three anthropometric phenotypes. A wide range of clinical and biochemical indicators of health and nutritional status were collected at admission to, and at key time points throughout, therapeutic refeeding. We assessed emerging biomarkers of pathophysiology and viability in addition to traditional indicators of health status and nutritional deprivation. These included: serum leptin, a robust biochemical predictor of mortality in children with SAM; natural isotopic abundances of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) in hair, promising archives of metabolic status; bio-electric impedance, a portable, non-invasive technique for assessing body composition in the field-setting; and combined biochemical assessment of micronutrient deficiencies (vitamin A and iron) and inflammation (acute phase proteins).

Analysis of these indicators demonstrated that all children with SAM (*i.e.*, low WHZ and/or low MUAC) presents with clinical evidence of nutritional deprivation and micronutrient deficiencies, with significant heterogeneities on key criteria. Children with low WHZ only have biochemical and clinical deficits that are more severe than those in children with low MUAC only. These results also indicate that children with both anthropometric deficits have the highest risk of acute and post-discharge death and morbidity. On this basis, low WHZ must be retained as an independent diagnostic criterion, in line with WHO recommendations. Further research is urgently needed to develop innovative diagnostic solutions to identify low WHZ children in the community.

Titre : Investigations biomédicales pour l'optimisation du diagnostic et du suivi de la malnutrition aiguë sévère : l'étude OptiDiag

Mots-clés : malnutrition aiguë sévère (MAS) ; périmètre brachiale (PB) ; indice poids-taille (IPT).

Résumé : Les recommandations actuelles de l'OMS séparent les enfants atteints de malnutrition aiguë sévère (MAS) non-œdémateux en trois phénotypes anthropométriques : (1) faible périmètre brachiale (PB) seul ; faible indice poids-taille (IPT) seul ; ou faible PB et ITP. Tous sont éligibles à une réhabilitation nutritionnelle selon les lignes directrices de l'OMS.

Cependant, pour des raisons de facilité d'utilisation, et sur la base de données suggérant une surmortalité chez les enfants atteints de MAS diagnostiquée par le seul PB, plusieurs agences et gouvernements considèrent ce dernier critère comme nécessaire et suffisant à l'éligibilité pour une prise en charge de la MAS, excluant de fait les enfants présentant uniquement un faible IPT. Ce changement de paradigme semble prématuré : les liens entre phénotype anthropométrique, retentissement fonctionnelle et risque associés n'ont pas encore été élucidés. En effet, plusieurs ré-analyse de données anciennes montrent que les enfants atteints de MAS inéligibles à une prise en charge sur la base du seul PB (c'est-à-dire les enfants avec uniquement un faible IPT) présentent un risque de décès similaire à ceux éligibles. De plus, ces analyses suggèrent que les enfants présentant simultanément les deux déficits anthropométriques ont un risque de mortalité encore supérieur.

Cette thèse vise à décrire et comparer physiopathologie et de la gravité fonctionnelle associée aux phénotypes anthropométriques d'enfants atteints de MAS. En se basant sur l'état actuel des connaissances sur la vulnérabilité chez ces derniers, ces travaux cherchent à répondre à la question suivante : comment les profils de vulnérabilité des enfants atteints de MAS inéligibles à une prise en charge dans le cadre d'un programme basé sur le PB seul (*i.e.*, faible IPT-seul) se comparent-ils aux profils de ceux qui seraient éligibles?

Une étude de cohorte multicentrique a été réalisé chez des enfants atteints de MAS sans complications ni œdèmes au Bangladesh, au Burkina Faso, et au Libéria. Les patients ont été inclus de façon équilibré parmi les trois phénotypes anthropométriques. Un large panel de paramètres cliniques et biochimiques indicateurs du statut nutritionnel et de l'état de santé a été recueilli à l'admission à – et à des points clés pendant – la réhabilitation nutritionnelle. Nous avons évalué de nouveaux biomarqueurs de la physiopathologie et de la survie en plus des biomarqueurs conventionnels de l'état de santé et de la dénutrition : la leptine sérique, un paramètre prédictif robuste de mortalité chez les enfants atteints de MAS ; l'abondance isotopique naturelle du carbone et de l'azote ($\delta^{13}C$ et $\delta^{15}N$) dans le cheveux, offrant un intéressant historique nutritionnel et métabolique ; la bio-impédancemétrie, une technique mobile et non-invasive pour l'évaluation de la composition corporelle adaptée à une utilisation sur le terrain ; et la mesure de paramètres biochimiques d'inflammation (protéines de la phase aiguë) et de déficience en micronutriments (vitamine A et fer).

L'analyse de ces indicateurs ont monté que tous les enfants atteints de MAS, quel que soit leur phénotype anthropométrique, présentent des signes cliniques de dénutrition et des preuves biologiques de déficience en micronutriments—mais avec une hétérogénéité significative sur certains paramètres clés. Les enfants avec un faible IPT seul présentent des anomalies cliniques et biologiques plus sévères que les enfants avec un faible PB seul. Ces résultats montrent également que les enfants avec les deux déficits anthropométriques présentent le risque de morbi-mortalité le plus élevé sur le court terme, et après prise en charge. Ainsi, nos résultats plaident en faveur du maintien de l'IPT comme critère diagnostique indépendant, en accord avec les recommandations de l'OMS. Le développement de méthodes diagnostiques innovantes permettant d'identifier, directement dans la communauté, les enfants présentant un faible IPT doit être une priorité de recherche.