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# Gut microbiota and malnutrition

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Malnutrition is the leading cause of death worldwide in children under the age of five, and is the focus of the first World Health Organization (WHO) Millennium Development Goal. Breastfeeding, food and water security are major protective factors against malnutrition and critical factors in the maturation of healthy gut microbiota, characterized by a transient bifidobacterial bloom before a global rise in an-aerobes. Early depletion in gut *Bifidobacterium longum*, a typical maternal probiotic, known to inhibit pathogens, represents the first step in gut microbiota alteration associated with severe acute malnutrition (SAM). Later, the absence of the Healthy Mature Anaerobic Gut Microbiota (HMAGM) leads to deficient energy harvest, vitamin biosynthesis and immune protection, and is associated with diarrhea, malabsorption and systemic invasion by microbial pathogens. A therapeutic diet and infection treatment may be unable to restore bifidobacteria and HMAGM. Besides refeeding and antibiotics, future trials including non-toxic missing microbes and nutrients necessary to restore bifidobacteria and HMAGM, including prebiotics and antioxidants, are warranted in children with severe or refractory disease.

## 1. Malnutrition

### 1.1. Definition of malnutrition

Malnutrition is the leading cause of death worldwide in children under the age of five, and accounts for the deaths of between one and six million children every year. It is also the focus of the first Millennium Development Goal [1]. Malnutrition can be defined as inadequate nutrition, ranging from under- to over-nutrition. Under-nutrition includes deficiency in macronutrients (protein, global energy) or micronutrients (metals such as zinc, selenium, or vitamins) [1]. Although stunting rates are dropping, 159 million children around the world continue to be affected by it [2], and wasting still threatens the lives of 50 million children across the globe [2].

### 1.2. Anthropometric definition

Childhood under-nutrition encompasses several clinical forms, including stunting (low height-for-age  $<-2$  Standard deviation (SD)), wasting (low weight-for-age  $<-2$  SD) and underweight (low weight-for-height  $<-2$  SD) [1]. Each of these terms can be considered as moderate (between  $-2$  and  $-3$  SD) and severe (under  $-3$ SD). While acute malnutrition corresponds to wasting (thinness), or nutritional edema, chronic malnutrition is associated with stunting (shortness/poor cognitive development), and acute and/or chronic malnutrition is associated with underweight [3]. While stunting is considered to be more common, wasting is associated with a higher mortality [4]. However, anthropometric markers are insufficiently sensitive to define childhood malnutrition in regards to edematous malnutrition [5,6].

### 1.3. Clinical definitions: Marasmus & Kwashiorkor

The importance of applying a mix of clinical and anthropometric methods to assess malnutrition has recently been emphasized [6]. Marasmus is a wasting syndrome without specific symptoms. Conversely, kwashiorkor is well-described and represents a form of severe malnutrition [4] in which anthropometric parameters are useless in relation to edema (Fig. 1). Kwashiorkor was first described in 1933 by an exceptional physician, Dr. Cicely D. Williams, and is the local term in the Gold Coast meaning 'the disease the deposed baby gets when the next one is born' [7,8]. Symptoms appear four to twelve months after the onset of defective feeding, while the patient develops normally. Irritability then begins, with attacks of diarrhea and swelling of the hands and feet. Seven to ten days later, changes to the skin begin to take place, including



Fig. 1. Children with kwashiorkor. Note the skin changes and edema.

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depigmentation, thickened black and crumpled patches, and raw areas where these patches have peeled off. A rash appears and spreads predominantly to the ankles, knees, elbows, wrists and buttocks, which are usual pressure points. If untreated, the child dies within a month after the onset of skin changes [7,8]. Post mortem, the only constant finding is an extreme fatty infiltration of the liver [7,8]. Stunting was noted in a few cases and may, accordingly, be considered part of the disease [7,9]. General puffiness of the face has been reported [7] often referred to as 'moon face'. Diarrhea occurs and becomes persistent in later stages with undigested food found in feces [7,8], suggesting that both human enzymes and gut microbiota are deficient for digesting food and harvesting energy.

#### 1.4. Definition of severe acute malnutrition (SAM)

Severe acute malnutrition (SAM) is defined as a weight-for-height z-score (WHZ) <−3 standard deviations, calculated according to WHO standards, a mid-upper-arm circumference (MUAC) less than 115 mm in children aged 6–59 months, or bilateral nutritional edema. The prevalence of this condition is estimated at 29 million children under the age of five [1]. SAM has been associated with high mortality (10–30%) and an increased risk of diarrhea, pneumonia and systemic infections, including bacteremia with *Staphylococcus aureus*, *Streptococcus*, *Salmonella*, *Klebsiella*, and *Escherichia coli*.

## 2. Previously known risk factors for malnutrition

### 2.1. Age and geography

Being under the age of five has been shown to be a risk factor for severe malnutrition. Geography is also associated with different risks of malnutrition: in 2014, Southern Asia ranked first for wasting [2] with 14% of children under five, Southeastern Asia and Africa ranked second, with percentages between 5 and 9%, and Central and South America and Central and Eastern Asia ranked third, with percentages ranging from 1.4 to 3.9% [2].

### 2.2. Abnormal breastfeeding and weaning

Abnormal breastfeeding and weaning are usually considered as risk factors and triggering factors in the onset of malnutrition [10], particularly for kwashiorkor disease (see section 2.4.). Golden [11] has reported the occurrence of kwashiorkor predominantly in newly-weaned children.

### 2.3. Impoverished diet

Along with deficient breastfeeding, food and water insecurity are major risk factors for malnutrition [12,13]. Diet of children with SAM is frequently restricted to cooked but contaminated starchy foods lacking meat, and non-starchy vegetables and fruits, which are natural sources of several micronutrients (type I and type II). Ready-to-use therapeutic food (RUTF), including vitamins and antioxidants (vitamins A, B1, B2, B3, B5, B6, B7, B9, B12, C, D, E, K and n-3, n-6 fatty acids) which have been controlled for contamination (aflatoxin, microorganism content, coliforms, *Clostridium perfringens*, yeasts, molds, pathogenic staphylococci, *Salmonella* and *Listeria* [14] – Table 1), has recently revolutionized the management and prognosis of SAM in outpatient settings for children over six months of age without complications [14].

**Table 1**

Microbial composition of ready-to-use therapeutic foods according to the WHO (WHO, 2007 [19]).

Maximum toxin levels	
Aflatoxin levels	5 ppb maximum
Microorganism content	10,000/g maximum
Coliform test	negative in 1 g
<i>Clostridium perfringens</i>	negative in 1 g
Yeast	maximum 10 in 1 g
Molds	maximum 50 in 1 g
Pathogenic staphylococci	negative in 1 g
<i>Salmonella</i>	negative in 125 g
<i>Listeria</i>	negative in 25 g

For detailed composition, including Fe limitation (max 14 mg/100 g), antioxidant therapy with both water (vitamins B1, B2, C, B6, B12) and fat-soluble agents (vitamins A, E), as well as Se, Mn, Zn, Cu and vitamin replacement therapy (vitamin D), with the avoidance of polyunsaturated fats (n-3 and n-6 fatty acids requested in RUTF), as suggested by Golden [12], see (WHO, 2007 [19]).

### 2.4. Specific diet associated with kwashiorkor

Kwashiorkor is a nutritional childhood disease associated with a maize diet [7]. According to Dr. Williams, all cases had an individual history of an abnormal diet and/or breastfeeding which contrasts with the regional and collective famine more commonly associated with marasmus or marasmic-kwashiorkor [7]. Breastfeeding was always abnormal: either the mother was dead and the patient had been breastfed by a grand-mother or an aunt, the mother became pregnant again while the patient was still young, or the mother had been ill or under-nourished, and the only supplementary food consisted of preparations of maize [7]. Dr. Williams noted that maize lacks carotene, tryptophan, lysine, and glycine, suggesting the malnutrition is qualitative rather than quantitative [7]. Cassava, yam, plantain, rice, maize, and cruciferae, frequently associated with kwashiorkor, have some of the lowest protein and higher carbohydrate content compared to other staples [9,11]. In addition, the lack of sufficient milk in the diet is a key factor, as an adequate supply of good milk could cure the disease [7,8]. In one fatal case, “the mother confessed that she had not fed her anything but [maize]. She had only once given the child any milk, and never again, as it made the child’s mouth [ . . . ] messy.” A kwashiorkor-like illness has been reproduced in baby baboons using low-protein, high-carbohydrate diets and with the addition of sucrose as a triggering factor [9]. The specific micronutrient deficiency associated with kwashiorkor has been confirmed very recently including, specifically, the antioxidant β-carotene [15], as already noted by Dr. Williams in 1933 [7].

### 2.5. Infections

Infections have been recognized for several years as risk factors for malnutrition [16]. Episodes of enteric infection and systemic infections have been reported to precede acute malnutrition [9]. Golden reported that kwashiorkor is precipitated by infection, particularly measles, tuberculosis, malaria and diarrhea [11].

## 3. Gut microbiota and health

### 3.1. Onset of gut microbiota

Recent discoveries suggest that maternal microbes colonize the baby’s gut before delivery [17,18]. At birth, common environmental exposures include the maternal vaginal, fecal, or skin microbiota [19]. Breastfeeding has been shown to be a milk-borne microbiota transplantation [20,21]. Colostrum and milk include several hundred species of bacteria distinct from other human niches, not

simply contaminants from the skin [20]. The human milk microbiome is shaped by maternal weight and mode of delivery [20]. Analyzing the effect of maternal BMI on breast-milk microbiota composition demonstrated that *Staphylococcus* and *Lactobacillus* were higher and *Bifidobacterium* was lower in obese compared with normal-weight women [20]. Strikingly, both *Lactobacillus* and *Bifidobacterium* concentration in the feces have been linked to weight regulation in adults [22]. All these results suggest that the nutritional status of the mother and the quality of breastfeeding are critical in both the onset of gut microbiota in the newborn and the pathogenesis of kwashiorkor [7,8].

### 3.2. Gut microbiota maturation: an increase in anaerobes and related bacteriophages

Gut microbiota maturation occurs mainly during the first three years of life [23] and is associated with increased diversity. *Bifidobacterium longum*, which is increased in mother's gut microbiota at delivery [24], is known to be viable in human milk [25] and predominant in infants' guts [24,26]. A significant decline in *B. longum* has been reported in proportion with increasing age in three different geographical areas (Amazonas State in Venezuela, rural Malawi and US metropolitan areas) [23]. This suggests a specific transient probiotic role for *B. longum* in the vertical mother-to-child gut microbial transmission associated with breastfeeding (see section 4.3.5). More recent metagenomics analyses are in line with seminal culture studies which showed that anaerobic bacteria increase with gut microbiota maturation and persist throughout the life-span of the animal constituting its autochthonous "flora" [27]. Aerotolerant microbiota, including lactic acid bacteria (*Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Enterococcus*) and *Proteobacteria* predominates at birth, with a gradual increase in healthy anaerobic gut microbiota mainly represented by *Firmicutes*, *Bacteroidetes* and archaeal members. Fungi and eukaryotes also increase with gut maturation [23]. From a viral point of view, the change in bacteriophages over time reflects alterations in their bacterial hosts [28].

### 3.3. Factors influencing gut microbiota

#### 3.3.1. Maternal gut microbiota

Many recent studies suggest a mother-to-infant vertical transmission of gut microbes, and particularly bifidobacteria and lactobacilli [21,26]. While the mother's microbiota is altered during pregnancy, *Bifidobacterium* and, specifically, *B. longum* are enriched in the mother's gut microbiota at delivery [24]. Strikingly, *B. longum* is also the predominant bifidobacterial species in the gut of infants from Italy, Spain and Ireland [26]. Several pieces of data suggest that the mother's gut microbes can reach the infant's gut via one or multiple routes, including *in utero* colonization and breastfeeding, but also vaginal delivery [29]. Each route may, however, transmit specific microbes, with the mother's milk being particularly associated with bifidobacteria [29–31]. This vertical transmission suggests a transgenerational alteration of gut microbiota.

#### 3.3.2. Breastfeeding: maternal milk, the first natural synbiotic

Breastfeeding induces gut microbiota rich in *Bifidobacterium* [30,31] and depleted in *E. coli*, *Clostridium difficile*, *Bacteroides*, and lactobacilli [32,33]. The mother's milk is critical shaping the baby's gut microbiota as it is a synbiotic food that supplies *B. longum* and its prebiotics (galactooligosaccharides) to the baby's gut, promoting its very-broad-spectrum lantibiotic [34]. This prevents the overgrowth of *Enterobacteriaceae*, linked to malnutrition-associated enteropathy [35]. Secretory IgA class (SIgA) antibodies in breast milk also promote long-term intestinal homeostasis by regulating

gut microbiota [36]. Infant formula more closely resembling human milk (with the addition of *B. longum*) was found to be more bifidogenic than the control formula without probiotic, and led to a microbiota profile similar to that for breastfed infants (decreased *Enterobacteria* and *Clostridia*) [31]. In developed countries most, if not all, infant formulae are supplemented with lactic acid bacteria probiotics, previously shown to be associated with better growth [37].

#### 3.3.3. Diet

For a long time, it has been demonstrated that food with or without probiotics is able to shape the gut microbiota [38]. In obese people, a restricted diet correlated with a global change in the Bacteroidetes/Firmicutes ratio [38]. Haro recently showed that a long-term consumption of a Mediterranean diet or a low-fat, high-complex carbohydrate diet has a protective effect on the development of Type 2 diabetes by introducing different specific changes to the gut microbiota, increasing the abundance of the *Roseburia* genus and *Faecalibacterium prausnitzii*, respectively [39]. We have been unable to find a human study reporting the alteration of gut microbiota following the maize and sucrose-restricted diet associated with kwashiorkor before the onset of the disease, so it is difficult to clarify whether changes to gut microbiota is a consequence of the diet, the disease, or both.

The diets of undernourished children are commonly depleted in iron, zinc and meat. Krebs et al. studied the effects of three different complementary feeding diets (commercially available pureed meats, iron- and zinc-fortified infant cereals, iron-only fortified infant cereals) upon enteric microbiota in exclusively breastfed infants [40]. The most significant difference between the diets was the butyrate-producing clostridia group XIVa increase in the meat group [40]. This cluster is of foremost importance, since it included several bacteria previously associated with gut microbiota maturation, notably in the *Lachnospiraceae* family, accounting for almost 60% of the mucin-adhered microbiota [41]. Of the many acetate and/or lactate-converting butyrate producers within this cluster, *Roseburia intestinalis* and *Eubacterium rectale* most specifically colonized mucins, and are two major representatives of gut microbiota maturation [23,24]. In African children, iron fortification produced a potentially more pathogenic gut microbiota profile, with increased *Enterobacteria* and *Clostridium* and decreased lactobacilli, and this profile was associated with increased gut inflammation [42], consistent with limited iron intake in RUTF (<14 mg/100 mg, Table 1) [14].

#### 3.3.4. Host genetics

Genome-wide associations have recently been reported, linking, for example, *Akkermansia* and a variant near PLD1, a gene previously associated with body mass index [43]. This confirmed previous culture studies linking the genetic constitution of animals with the composition of flora characteristic of the mouse colony [27].

#### 3.3.5. Host immunity: immunoglobulin A

Immunoglobulin A represent a carefully regulated link between host immunity and a fraction of the gut microbiota (the IgA + fraction) [35]. IgA-deficient mice have dramatically altered gut microbiota [44,45] and maturation of gut microbiota was shown to be, in part, regulated by induction of a  $\gamma$ -Proteobacteria-specific IgA response [46].

#### 3.3.6. Infections

Enteric infections are undoubtedly a critical factor in disrupting the overall gut microbiota, and this has been well described with *C. difficile* [47]. In addition, a global depletion of digestive bacteria has been described in enteric salmonellosis [48].

### 3.3.7. Seasonality

Several studies have reported seasonal variations in gut microbiota [49].

## 4. Gut microbiota and malnutrition

The main studies linking gut microbiota and malnutrition are summarized in Table 2.

### 4.1. Evidence suggesting a link between gut microbiota and malnutrition

#### 4.1.1. Non-dietary factors

Diet alone doesn't explain the full spectrum of the disease. Golden [11] noted that a relatively small proportion of children consuming a 'kwashiorkor-associated diet' actually develop the disease (0.5–2%), suggesting other contributing factors. Coward

**Table 2**  
Main studies analyzing the link between Gut Microbiota and Malnutrition.

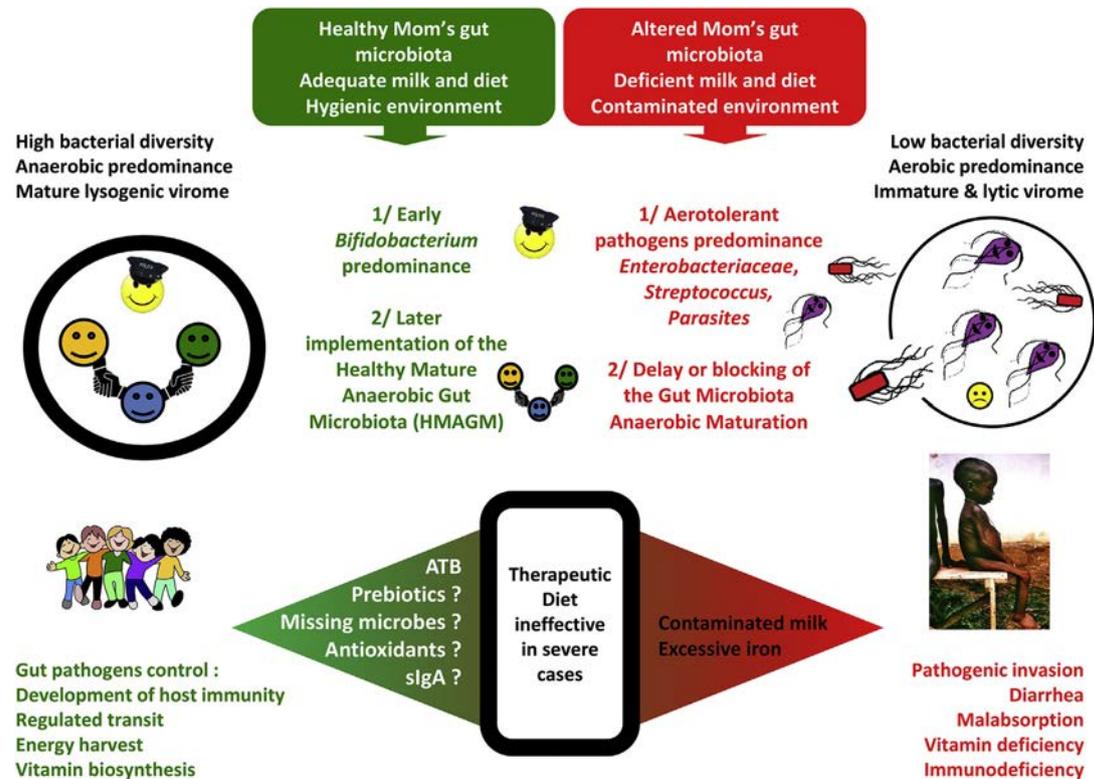
Reference	Components of the study	Methods used for gut microbiota/ microbiome analysis	Findings
Smythe, Lancet, 1958 [50]	South Africa, 33 kwashiorkor consecutive cases, before-after study, treatment associated chloramphenicol, neomycin, nystatin, ( $\pm$ penicillin/streptomycin), milk, yogurt with <i>Lactobacillus delbrueckii</i> subsp. <i>Bulgaricus</i>	Culture (gastric juice, rectal swab)	First work reporting a change in bacterial flora and role of infection in kwashiorkor Dramatic improvement of outcome with antibiotics and synbiotics (milk, <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> yogurt)
Mata, Am J Clin Nutr, 1972 [51]	Guatemala, 13 children with acute protein-calorie malnutrition/ four controls	Intubation technique (stomach, duodenum, upper jejunum) Culture	Anaerobic depletion associated with SAM partially corrected by nutritional therapy <i>Bifidobacterium</i> depletion Pathogenic <i>Enterobacteriaceae</i> ( <i>Edwardsiella</i> , <i>Shigella flexneri</i> , <i>Salmonella</i> ), <i>Proteus</i> , <i>Pseudomonas</i> and other not typically indigenous Gram negative bacilli proliferation Intestinal parasites in 11/13 cases ( <i>Giardia</i> , <i>Strongyloides</i> , hookworm) vs 1/4 controls (Barnard unilateral test, $p < 0.05$ )
Gupta, Gut Pathogen, 2011 [69]	India, one malnourished child/one apparently healthy child	16S DNA large scale sequencing	Overabundance of enteric pathogens including <i>Campylobacteraceae</i> and <i>Helicobacteraceae</i> Depletion in <i>Archeae</i> ( <i>Euryarchaeota</i> )
Monira, Front Microbiol, 2011 [70]	Bangladesh, seven malnourished children and seven controls	16S DNA large scale sequencing	Decreased bacterial diversity Analysis at the genus level Increase in <i>Proteobacteria</i> notably <i>Klebsiella</i> and <i>Escherichia</i> , but also <i>Streptococcus</i> and <i>Fusobacterium</i> Decrease in <i>Bacteroidetes</i> ( <i>Prevotella</i> ) and several anaerobic <i>Firmicutes</i> ( <i>Subdoligranulum</i> , <i>Faecalibacterium</i> , <i>Megasphaera</i> , <i>Acetivibrio</i> , <i>Blautia</i> ) Decrease in <i>Lactobacillus</i> and <i>Bifidobacterium</i>
Smith, Science, 2013 [67]	Malawi, 317 twin pairs but only 13 same gender twin pairs who became discordant for kwashiorkor were analyzed for gut microbiota	16S DNA large scale sequencing	Blockade of microbiome maturation, no taxonomic signature (probable overmatching bias <sup>a</sup> ) Transplant experiment transmit phenotype in animals
Ghosh, PlosOne, 2014 [71]	India, 20 children with varying nutritional statuses	16S DNA large scale sequencing	Increase in <i>Proteobacteria</i> ( <i>Escherichia</i> , <i>Shigella</i> , <i>Enterobacter</i> ), <i>Veillonella</i> and <i>Streptococcus</i> Decrease in several anaerobic <i>Firmicutes</i> ( <i>Roseburia</i> , <i>Faecalibacterium</i> , <i>Butyrivibrio</i> )
Subramanian, Nature, 2014 [24]	64 cases compared to 50 controls <sup>b</sup> (used in a linear mixed model)	16S DNA large scale sequencing DNA virome	First study identifying a taxonomic signature at the species level and controlling for age by a linear mixed model. Persistent immaturity despite therapeutic diet.
Reyes, PNAS, 2015 [28] (same samples, DNA virome)			Identification of an accurate taxonomic signature: 220 taxa significantly altered in malnutrition after controlling for age <sup>c</sup> . Among OTUs assigned at the species level: Increase in <i>Escherichia coli</i> , <i>Enterococcus faecalis</i> , <i>Streptococcus gallolyticus</i> Decrease in several anaerobic <i>Firmicutes</i> ( <i>Faecalibacterium prausnitzii</i> ), anaerobic <i>Bacteroidetes</i> ( <i>Bacteroides galacturonicus</i> , <i>B. fragilis</i> ) and lactic acid bacteria ( <i>Bifidobacterium longum</i> , <i>B. bifidum</i> and <i>Lactobacillus ruminis</i> , <i>L. mucosae</i> ) DNA virome study: Change in bacteriophages over time represents alterations in their bacterial hosts.
Kau, Sci Transl Med, 2015 [35]	Experimental study analyzing human gut microbes according to their IgA response and the influence of both diet (M: Malawian associated with SAM, S: healthy diet) and gut microbiota (K: kwashiorkor microbiota, H: healthy microbiota) in a mouse model.	IgA fraction of the gut microbiota	Dissection of a culture collection of 11 IgA-targeted strains from an undernourished donor, sufficient to transmit these phenotypes, disclosed that <i>Enterobacteriaceae</i> interacted with other consortium members ( <i>Enterococcus</i> and/or <i>Bacteroides</i> ) to produce enteropathy. <i>Enterobacteriaceae</i> were significantly enriched in the IgA + fraction prepared from the fecal microbiota of KM mice while <i>Verrucomicrobiaceae</i> and <i>Bacteroidaceae</i> were significantly enriched in the IgA + fraction prepared from the fecal microbiota of HM mice <i>Lachnospiraceae</i> were significantly enriched in the IgA- fraction prepared from the fecal microbiota of KM, HS and HM mice

SAM: severe acute malnutrition. OTUs: operational taxonomic units.

<sup>a</sup> See section 4.3.1.

<sup>b</sup> The number of cases and controls for whom a fecal sample was analyzed was kindly communicated by the authors.

<sup>c</sup> See Table 15a of the article [24].



**Fig. 2.** Instrumental role of gut microbiota in malnutrition. HMAGM: healthy mature anaerobic gut microbiota, ATB: antibiotics. SIgA: secretory immunoglobulin A. The picture representing a child with kwashiorkor (right) is in the public domain (CDC/Dr. Lyle Conrad).

noted that a calorie-restricted diet containing only maize and sucrose reproduced several features of the disease but not diarrhea [9]. Moreover, Golden noted that kwashiorkor is extremely difficult to produce under controlled, hygienic conditions and usually responds to treatment of an infection, provision of a hygienic environment, and a properly prepared milk-based diet. This suggests a microbial cofactor is at play in severe acute malnutrition.

#### 4.1.2. Enteric septicemia

In severely undernourished children, enteric septicemia with *Salmonella*, *Shigella* and *S. aureus* is a frequent complication or cause of death [50]. Other systemic pathogens include mainly *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*, with gut proliferation of *P. aeruginosa* reported in such children [51]. Intestinal organisms can be grown in blood-culture, raising the possibility of increased susceptibility of the bowel wall to invasion by bacteria [50]. Very recently, highly virulent enteropathogens were isolated from the spleen of experimental animals that died after transplantation of a kwashiorkor-associated microbiota fed on a deficient-diet [35].

#### 4.1.3. Seasonality overlapping with the seasonality of gastroenteritis

Smythe [50] noted the “regularity with which kwashiorkor and the gastroenteritis season overlap,” suggesting a putative role for seasonal microbial epidemics. Golden [11] reported an association with wet seasons.

#### 4.1.4. Beneficial role of antibiotics

Antibiotics have been shown to be beneficial in the systematic treatment of complicated and uncomplicated malnutrition since the 1950s with cessation of diarrhea [50]. The beneficial role of

antibiotics on mortality and weight gain has been confirmed very recently in a randomized, double-blind, placebo-controlled trial, using cefdinir, amoxicillin, or a placebo [52]. In contrast, neomycin, an aminoglycoside antibiotic, has been shown to be associated with iatrogenic malabsorption [53].

#### 4.1.5. Probiotics in malnutrition

In his seminal study, Smythe dramatically improved the survival of children with a diet including *Lactobacillus bulgariensis*, later reclassified as *Lactobacillus delbrueckii* subsp. *bulgaricus* [50]. However, the role of the probiotic in this therapeutic diet which included antibiotics and vitamins is entirely uncertain. In a very recent double-blind randomized trial, probiotic lactic acid bacteria including *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, *Lactobacillus paracasei* and *L. plantarum* (but not *B. longum* (see section 4.3.5.)) were unable to improve nutritional outcome [54].

### 4.2. Enteric diseases frequently reported in malnutrition

#### 4.2.1. Undocumented diarrhea & enteropathy

Diarrhea, a typical symptom of kwashiorkor [7,8], is reported in 70–90% of severely undernourished children [50,51]. Edema of the bowel wall was found in two autopsied cases, defining enteropathy [50]. Diarrhea >14 days during the first year of life was shown to be an independent predictor for malnutrition at 12 months [55]. In another multi-country pooled analysis, a higher cumulative burden of diarrhea increased the risk of stunting. Following prolonged episodes of acute diarrhea (lasting between 7 and 13 days), height-for-age and weight-for-age significantly decreased [56]. This suggests that diarrhea is not only a symptom of malnutrition but could be one of the causal factors (Fig. 2).

#### 4.2.2. Small bowel bacterial overgrowth

Golden reported the almost universal presence of infection and overgrowth of (aerobic) bacteria (SIBO, Small Intestinal Bowel Overgrowth) in the small intestine in children with kwashiorkor [11], similar to tropical sprue, where coliforms invade the stomach, ileum and jejunum combined with fat and vitamin B12 malabsorption [57,58]. Significantly, very recent studies have actually linked vitamin B12 absorption (microbial secretion) and mature microbiota [23]. Indeed, the proportional representation of vitamin B12 metabolism in human fecal microbiomes correlated with age, gut microbiota maturation and representation of members of *Bacteroidetes*, *Firmicutes* and *Archaea* in the microbiota, regardless of geographical origin [23].

In patients with chronic tropical sprue and who were not responding to folate, tetracycline led to a rapid (48 h) and dramatic improvement in absorptive function. This was associated with an improvement in the location and concentration of coliforms [57]. A delayed response to antibiotics (>48 h) was associated with resistant microorganisms in the small bowel (*Klebsiella* sp. and *E. coli* resistant to tetracycline) [57]. All these results suggest that the association between coliform invasion of the upper intestine and vitamin B12 malabsorption may be explained by the depletion of the normal mature anaerobic gut microbiota, and this may play a role in childhood malnutrition. However, this normal mature anaerobic gut microbiota has been ignored until very recently, reflecting the difficulties in growing extremely oxygen sensitive (EOS) microbes with standard culture techniques.

#### 4.2.3. Pathogen specific diarrhea

Enteric infections have been shown to have an impact on cognitive development and weight gain, both of which are impaired as a result of malnutrition [59]. Brazilian children with enteroaggregative *E. coli* (EAEC) in their stools had significant growth impairment after a positive culture, regardless of the presence or absence of diarrhea [60]. In Bangladesh, children with *Entamoeba histolytica*-associated diarrheal illness were three times more likely to have moderate-to-severe wasting and five times more likely to be moderately to severely stunted [61]. Giardiasis was a predictor of childhood malnutrition in Orang Asli children in Malaysia [62]. In another study, symptomatic cryptosporidiosis retarded weight gain more than asymptomatic cryptosporidiosis, but the latter was twice as common [63]. The authors concluded that since asymptomatic cryptosporidiosis was more prevalent, it may have more of an overall adverse effect on child growth in the community than symptomatic cryptosporidiosis [63]. Finally, *Cryptosporidium* and *Shigella* infections were associated both with prolonged episodes of acute diarrhea and growth failure [56]. This suggests that bacterial and protozoan enteric pathogens, along with the alteration of gut microbiota, impair child growth regardless of the presence of diarrhea. As diarrhea and enteric infection favor malnutrition, and malnutrition favors diarrhea and enteric infections, Guerrant concluded that further interventional studies on malnutrition should aim at interrupting the vicious diarrhea-malnutrition cycle, so that children may develop their full potential [12,64].

### 4.3. Gut microbial alterations in malnutrition

#### 4.3.1. The twin paradox: overmatching bias

In case-control studies, overmatching causes information to be lost [65,66]. We found this to be particularly true when analyzing the link between gut microbiota alteration and malnutrition. Smith et al. [67] reported “the absence of a distinct and consistent taxonomic signature of kwashiorkor.” We believe that this sentence is incorrect, and that no distinct and consistent taxonomic signature

was found because of overmatching bias and erroneous ascertainment of controls (most controls were undernourished, with moderate to severe stunting). In their study, Smith et al. [67] reasoned that a healthy (well-nourished) co-twin in a discordant twin pair represented a very desirable control, given his/her genetic relatedness to the affected co-twin, and their similar exposure to diet and microbial reservoirs in their shared early environment. In fact, this represents an example of overmatching, which represents a statistical bias that causes information to be lost and decreases efficiency [65,66]. This bias occurs when controls become more similar to the cases with regard to exposure than the general population. Stratifying too finely will cause information to be lost (although stratifying too coarsely will not remove sufficient confounders). Stratifying by confounder (the immediate environment of the undernourished child) will also stratify by exposure, and the relation of exposure to the disease will be obscured. This is called ‘overmatching’, and leads to biased estimates of risk. Generally speaking, overmatching bias reduces efficiency [65,66,68].

Strikingly, while twin pairs were believed to be the best controls in clinical studies, as genetic variability is controlled, this is in fact not the case. We found that most of the controls in the study by Smith were undernourished and so not acceptable controls for malnutrition. These limitations have catastrophic consequences: 1/ the risk of not finding a gut microbial contribution to malnutrition when there is one, 2/the identification of specific features of severe acute malnutrition among several forms of severe malnutrition, and the consideration that this pattern is specific to malnutrition in general. For instance, the enriched microbes in (stunted) controls will be considered to be associated with health, while they may be likely to favor stunting! Controls are controls only if they are healthy, without any form of moderate to severe malnutrition.

This was recently confirmed by the same team, who found that the virome DNA is disturbed in both members of discordant twin pairs, even though only one member overtly manifests disease [28]. Moreover, the same team, using a different design with unrelated children, finally identified an accurate taxonomic signature associated with SAM [24].

#### 4.3.2. Case-control studies: microbial level alteration

While several articles have been published on malnutrition and associated gut microbiota, we found only six bacterial studies [24,51,67,69–71], two viral studies [28,51] and one eukaryotic [51] case-controlled gut microbiota study (Table 2). Gupta [69] analyzed one malnourished child and one apparently healthy child in an urban slum in Kolkata, India. Both were 16-month-old females. The first striking difference was that 10% of sequences were human genome sequences in the undernourished child versus 0.3% in the healthy one [69]. This remains unexplained, but could be associated with human tissue exfoliation or gut microbiota global depletion in malnutrition.

4.3.2.1. *Bacterial microbiota*. Smythe [50] was among the first to link kwashiorkor to changes in the intestinal bacterial microbiota from culture studies conducted in the 1950s (Table 2). In this study, both gastric juice and rectal swabs were studied, revealing a predominance of coliforms and the frequent presence of *S. aureus*. In some cases, the same bacterial category was found in gastric juice and rectal swab, including coliforms in 11 cases, *S. aureus* in six cases, *Salmonella* and *Enterobacteriaceae* (“paracolon”) each in one case. After three days of antibiotics, all cultivated bacteria decreased significantly, particularly *S. aureus*, coliforms and *Proteus morgani*, later reclassified as *Morganella morgani*. *P. aeruginosa* was the most common organism to persist despite antibiotic therapy. Unfortunately, no controls were included in this pioneering study. Mata [51] compared the gastric, duodenal, jejuna and

**Table 3**

Comparative analysis of gut taxa enriched in malnutrition and their direction of variation in gut microbiota maturation in humans.

Phylum	Family	Genus	Species	Reference
Bacteroidetes	Porphyromonadaceae	Parabacteroides <sup>↗GM</sup>		Monira, 2011 <sup>a</sup>
<b>Proteobacteria</b>				<b>Ghosh, 2014</b>
Proteobacteria	Enterobacteriaceae			Ghosh, 2014 <sup>b</sup>
Proteobacteria	Enterobacteriaceae	Escherichia/Shigella <sup>c,↘GM</sup>		Monira, 2011 <sup>d</sup>
				Ghosh, 2014 <sup>b</sup>
Proteobacteria	Enterobacteriaceae	Escherichia	coli <sup>↘GM</sup>	Subramanian, 2014 <sup>e</sup>
Proteobacteria	Enterobacteriaceae	Enterobacter <sup>↘GM</sup>		Ghosh, 2014 <sup>b</sup>
Proteobacteria	Enterobacteriaceae	Klebsiella <sup>↘GM</sup>		Monira, 2011 <sup>d</sup>
<b>Proteobacteria</b>	<b>Neisseriaceae</b>	<b>Neisseria</b>		<b>Monira, 2011</b>
Firmicutes	Enterococcaceae	Enterococcus		Monira, 2011 <sup>a</sup>
Firmicutes	Enterococcaceae	Enterococcus	faecalis	Subramanian, 2014 <sup>e</sup>
<b>Firmicutes</b>	<b>Lachnospiraceae</b>	<b>Parasporobacterium</b>		<b>Monira, 2011</b>
Firmicutes	Streptococaceae			Ghosh, 2014 <sup>b</sup>
Firmicutes	Streptococaceae	Streptococcus <sup>↘GM</sup>		Monira, 2011 <sup>a</sup>
				Ghosh, 2014 <sup>b</sup>
Firmicutes	Streptococaceae	Streptococcus	gallolyticus	Subramanian, 2014 <sup>e</sup>
<b>Firmicutes</b>	<b>Veillonellaceae</b>	<b>Veillonella<sup>↘GM</sup></b>		<b>Monira, 2011</b>
				<b>Ghosh, 2014</b>
Fusobacteria	Fusobacteriaceae	Fusobacterium		Monira, 2011 <sup>a</sup>

↗GM: taxa increasing with gut maturation. ↘GM: taxa decreasing with gut microbiota maturation [23,24].

<sup>a</sup> Inference based on the relative ratio (no statistical test) [72].

<sup>b</sup> Negatively correlated to nutritional index [73].

<sup>c</sup> *Escherichia* and *Shigella* cannot be reliably discriminated between by metagenomic studies.

<sup>d</sup> Significant difference ( $p < 0.001$ , uncorrected t-test on taxa with the biggest relative abundance difference) [72].

<sup>e</sup> Significantly associated with SAM relative to healthy controls at enrollment prior to the intervention phase in a multilinear mixed model controlling for age [67].

fecal microbiota of 13 undernourished and four control children using the intubation technique and found pathogenic *Enterobacteriaceae* only in cases including *Shigella flexneri*, *Edwardsiella* and *Salmonella*. The child with *Shigella* died shortly after admission [51].

Using metagenomics, Gupta [69] reported a 35- and 12-fold increase in *Campylobacteraceae* and *Helicobacteraceae*, respectively. Other results with lower size effect were more likely to be due to sampling bias (only one case and one control, preventing any statistical tests) but it should be noted that in the Archaea domain, *Euryarchaeota* and *Thermoprotei* were increased in the healthy gut microbiota [69]. Monira [70], comparing seven healthy and seven malnourished children, reported decreased bacterial diversity and an increase in *Proteobacteria*, including a 174-fold and nine-fold increase in *Klebsiella* and *Escherichia* respectively, in undernourished children (Tables 2 and 3). In contrast, the anaerobic *Bacteroidetes* phylum, previously associated with gut microbiota maturation (see section 3.2.) was significantly depleted. Smith [67] found that there was functional gene-level gut microbiome immaturity but no taxonomic signature, probably relating to overmatching bias and control selection as explained above (see section 4.3.1.). In 2014, Gosh [71] analyzed the gut microbiota of 20 Indian children with varying nutritional statuses ranging from mildly to severely malnourished children in India. *Escherichia*, *Shigella*, *Enterobacter*, *Streptococcus* and *Veillonella* were negatively correlated with the nutritional index (Table 3). In contrast, *Roseburia*, *Butyrivibrio*, *Faecalibacterium* and *Phascolarctobacterium* had significant positive correlations with the nutritional index (Table 4). Subramanian [24], in a well-designed longitudinal study, used a linear mixed model (controlling for age) to identify 16 operational taxonomy units significantly associated with SAM. Twelve were *Enterobacteriaceae* (including *E. coli* and another *Escherichia* species). *Streptococcus gallolyticus* was also significantly enriched in SAM. This bacterium is highly pathogenic (notably biotype 1) and is unambiguously associated with colon cancer, with a statistically significant increase in the likelihood of finding *S. gallolyticus* in the stool of individuals with colorectal neoplasia [72,73], and with endocarditis [74]. *Enterococcus faecalis*, also found to be increased

in malnutrition in this study, is also one of the most frequent bacteria associated with endocarditis. In contrast, a much larger number of bacteria (and Operational Taxonomic Units (OTU)), including several anaerobes, were decreased in cases of malnutrition (Table 4). Significantly, the *Lachnospiraceae* family was associated with healthy children and was previously associated with diets including meat (section 3.3.3.), gut microbiota maturation (see section 3.2.), mucosal associated microbiota [41], and the host's metabolic status, including being positively correlated with diabetes and obesity [75]. The only taxa which was decreased in SAM but did not correspond to the healthy mature anaerobic gut microbiota (HMAGM) was *Bifidobacterium*, particularly *B. longum* and *B. pseudolongum* [24], the lactating mother's probiotics (see Section 4.3.5).

**4.3.2.2. Viral microbiota.** Mata [51], in his original intubation study comparing 13 undernourished and four control children, found enterovirus in the duodenum and jejunum of two undernourished children respectively and adenovirus in the feces of one child. These results are difficult to interpret since one (1/4) control had enterovirus in the feces. More recently, Reyes found that phage plus members of the Anelloviridae and Circoviridae families of eukaryotic viruses discriminate between discordant and concordant healthy pairs [28]. However, it has not been demonstrated that these viruses have any pathogenic role in malnutrition [28].

**4.3.2.3. Eukaryotic microbiota.** Mata [51] found that 11/13 (85%) of undernourished children had intestinal parasites. Nine had *Giardia*, two had *Strongyloides*, and three had hookworms. Again, one (1/4) control child had a parasite, *Giardia*, in gastric and duodenal aspirates. Here, *a posteriori* statistical testing confirmed the significant connection between intestinal parasites and severe malnutrition (11/13 vs 1/4, Barnard bilateral test  $p = 0.02$ ). Given the very high prevalence of intestinal parasites in this population and this significant connection, eukaryotic microbiota analysis is mandatory when studying the link between gut microbiota and malnutrition.

**Table 4**

Comparative analysis of gut taxa depleted in malnutrition and their direction of variation in gut microbiota maturation in humans.

Phylum	Family	Genus	Species (phylotype)	Reference
Actinobacteria	Actinomycetaceae	Actinomyces	odontolyticus	Subramanian, 2014 <sup>a</sup>
Actinobacteria	Bifidobacteriaceae	Bifidobacterium	\GM	Monira, 2011
Actinobacteria	Bifidobacteriaceae	Bifidobacterium	bifidum \GM	Subramanian, 2014
Actinobacteria	Bifidobacteriaceae	Bifidobacterium	longum \GM	Subramanian, 2014
Actinobacteria	Coriobacteriaceae	Collinsella	aerofaciens	Subramanian, 2014 <sup>a</sup>
Actinobacteria	Coriobacteriaceae	Eggerthella	lenta	Subramanian, 2014 <sup>a</sup>
Actinobacteria	Coriobacteriaceae	Slackia	isoflavoniconvertens	Subramanian, 2014 <sup>a</sup>
Bacteroidetes	Prevotellaceae	Prevotella		Monira, 2011
Bacteroidetes	Prevotellaceae	Prevotella	copri \GM	Subramanian, 2014
Bacteroidetes	Bacteroidaceae	Bacteroides	fragilis \GM	Subramanian, 2014 <sup>a</sup>
Bacteroidetes	Bacteroidaceae	Bacteroides	galacturonicus	Subramanian, 2014 <sup>a</sup>
Firmicutes	Acidaminococcaceae	Phascolarctobacterium		Ghosh, 2014
Firmicutes	Carnobacteriaceae	Granulicatella	adiacens	Subramanian, 2014 <sup>a</sup>
Firmicutes	Clostridiaceae	Clostridium	\GM	Monira, 2011
Firmicutes	Clostridiaceae	Clostridium	bartlettii	Subramanian, 2014
Firmicutes	Clostridiaceae	Clostridium	disporicum	Subramanian, 2014
Firmicutes	Clostridiaceae	Clostridium	glycolicum \GM	Subramanian, 2014
Firmicutes	Clostridiaceae	Clostridium	sp_SS2_1	Subramanian, 2014
Firmicutes	Erysipelotrichaceae	Catenibacterium	mitsuokai \GM	Subramanian, 2014 <sup>a</sup>
Firmicutes	Erysipelotrichaceae	Holdemania	biformis	Subramanian, 2014 <sup>a</sup>
Firmicutes	Eubacteriaceae	Eubacterium	desmolans	Subramanian, 2014
Firmicutes	Eubacteriaceae	Eubacterium	hallii \GM	Subramanian, 2014
Firmicutes	Eubacteriaceae	Eubacterium	rectale \GM	Subramanian, 2014
Firmicutes	Eubacteriaceae	Eubacterium	Sp_cl_10_1_3	Subramanian, 2014
Firmicutes	Lachnospiraceae	Blautia \GM		Monira, 2011 <sup>b</sup>
Firmicutes	Lachnospiraceae	Blautia	Sp_M25	Subramanian, 2014 <sup>a</sup>
Firmicutes	Lachnospiraceae	Coprococcus	comes \GM	Subramanian, 2014 <sup>a</sup>
Firmicutes	Lachnospiraceae	Dorea	formicigenerans \GM	Subramanian, 2014 <sup>a</sup>
Firmicutes	Lachnospiraceae	Dorea	longicatena \GM	Subramanian, 2014 <sup>a</sup>
Firmicutes	Lachnospiraceae	Roseburia \GM		Ghosh, 2014 <sup>c</sup>
Firmicutes	Lachnospiraceae	Butyrivibrio \GM		Ghosh, 2014 <sup>c</sup>
Firmicutes	Lactobacillaceae	Lactobacillus \GM		Monira, 2011
Firmicutes	Lactobacillaceae	Lactobacillus	ruminis	Subramanian, 2014
Firmicutes	Lactobacillaceae	Lactobacillus	mucosae	Subramanian, 2014
Firmicutes	Ruminococcaceae	Acetivibrio		Monira, 2011 <sup>b</sup>
Firmicutes	Ruminococcaceae	Faecalibacterium \GM		Monira, 2011 <sup>b</sup>
Firmicutes	Ruminococcaceae	Faecalibacterium	prausnitzii \GM	Subramanian, 2014 <sup>a</sup>
Firmicutes	Ruminococcaceae	Ruminococcus	gnavus \GM	Subramanian, 2014 <sup>a</sup>
Firmicutes	Ruminococcaceae	Ruminococcus	obeum \GM	Subramanian, 2014 <sup>a</sup>
Firmicutes	Ruminococcaceae	Ruminococcus	Sp_5_1_39BFAA	Subramanian, 2014 <sup>a</sup>
Firmicutes	Ruminococcaceae	Ruminococcus	torques \GM	Subramanian, 2014 <sup>a</sup>
Firmicutes	Ruminococcaceae	Subdoligranulum \GM		Monira, 2011 <sup>b</sup>
Firmicutes	Veillonellaceae	Anaerostipes		Monira, 2011
Firmicutes	Veillonellaceae	Allisonella	histaminiformans	Subramanian, 2014
Firmicutes	Veillonellaceae	Veillonella	ratti	Subramanian, 2014
Firmicutes	Veillonellaceae	Megasphaera		Monira, 2011
Firmicutes	Veillonellaceae	Megasphaera	elsdenii	Subramanian, 2014
Proteobacteria	Pasteurellaceae	Haemophilus	parainfluenzae	Subramanian, 2014 <sup>a</sup>
Synergistetes				Ghosh, 2014

\GM: taxa increasing with gut maturation. \GM: taxa decreasing with gut microbiota maturation [23,24].

<sup>a</sup> Significantly associated with SAM relative to healthy controls at enrolment prior to the intervention phase in a multilinear mixed model controlling for age [67].

<sup>b</sup> Inference based on the relative ratio (no statistical test) [72].

<sup>c</sup> Negatively correlated to nutritional index [73].

#### 4.3.3. Abnormal inversion of the ratio of anaerobes to aerobes

Mata [51], analyzing the proximal gut (intubation for gastric, duodenal and jejuna aspirate) and fecal cultivable microbiota of 13 severely undernourished children and four controls, found mainly an abnormal inversion of the ratio of anaerobes to aerobes in undernourished children. This inversion was corrected under treatment, with an increase in total anaerobes [51]. However, most children retained qualitatively abnormal fecal flora after treatment, lacking *Bifidobacterium* and without an overt predominance of anaerobes [51]. These results confirmed the fact that healthy mature gut microbiota is mainly anaerobic [23], and that therapeutic diet alone is unable to restore “anaerobic” healthy mature gut microbiota [24,67].

#### 4.3.4. Immaturity of gut microbiota

Gut microbiota immaturity was shown to be associated with severe acute malnutrition and persisted despite therapeutic food interventions [24,67] (Table 2). Smith et al. [67] followed 317 twin pairs during the first three years of life in different villages in Malawi. The most significant finding was that RUTF produced a transient maturation of metabolic functions in kwashiorkor microbiomes which regressed when RUTF was stopped. A “blocking of maturation” of the gut microbiome was found in children with kwashiorkor, but not in healthy co-twins from discordant pairs [67]. This functional content immaturity was later confirmed at the bacterial [24] and viral [28] levels.

#### 4.3.5. *Bifidobacterium longum*, maternal probiotics lost in malnutrition

*B. longum* and *B. pseudolongum* are aerotolerant probiotic bacteria [76] which are critical for the onset and maturation of early healthy gut microbiota (see section 3.3.1 & 3.3.2). They represent a relevant exception to the gut immaturity reported in severe acute malnutrition (SAM) [24]; indeed, the fact that these species decrease with age and are also depleted in SAM [24] suggest that other critical factors unrelated to gut immaturity alter the gut microbiota of undernourished infants. Milk, the basic nutrient of healthy newborns worldwide, but not of severely undernourished children [7], is probably one of these neglected factors because it contains both the viable bacteria (*B. longum* [25]) and its related prebiotics (galactooligosaccharides [77]). These maternal probiotics are critical in shaping early infant gut microbiota, probably through broad spectrum lantibiotics [34], optimal growth in a milk-based environment, high resistance to oxygen [76] before rapid decrease along with the rise of obligate anaerobes [24]. This confirmed the key pathogenic role of deficient breastfeeding and the absence of adequate milk in the diet of severely undernourished children, as already pointed out by Dr. Williams as early as 1933 [7].

#### 4.4. Predictive role of gut microbiota in therapeutic failure

Metagenomic studies have revealed that specific gut microbiota alterations predict therapeutic failure and that pervasive gut microbiota alterations were linked to the resilience of severe malnutrition, despite therapeutic feeding [24,67].

#### 4.5. Instrumental role of the gut microbiota: transplant experiments

Several studies involving transplantation of the malnutrition-associated human gut microbiota to germ-free animals transmitted the phenotype, suggesting an instrumental role [24,35,67]. To identify which of the commensal bacteria are able to transmit the phenotype in transplant experiments, it was hypothesized that bacteria associated with a high IgA response were more likely to be potentially pathogenic and able to induce a malnutrition associated enteropathy [35]. IgA represents the interaction between certain bacteria of the gastrointestinal tract and host immunity. Indeed, not all gut bacteria cause the same IgA response. In a healthy host, the IgA response, stimulated notably by  $\gamma$ -Proteobacteria and *Bacteroidales*, is correlated with microbiota maturity and the reduction of  $\gamma$ -Proteobacteria [46]. On the other hand, bacteria associated with a high IgA response contain many potential pathogens. Indeed, transplantation of the IgA + fraction of the kwashiorkor microbiota has been associated with the development of enteropathy associated with malnutrition [35].

Kau [35] (Table 2) recently reported that IgA responses to several bacterial taxa, including *Enterobacteriaceae*, correlated with anthropometric measurements of nutritional status in longitudinal studies on children with kwashiorkor. Gnotobiotic mouse recipients of an IgA + bacterial consortium (*Enterobacteriaceae*) purified from the gut microbiota of undernourished children exhibited a diet-dependent enteropathy, characterized by rapid disruption of the small intestinal and colonic epithelial barrier, weight loss and sepsis which could be prevented by administering two "healthy" IgA-targeted bacterial species (*Akkermansia muciniphila*, *Clostridium scindens*) from healthy microbiota. A consortium of eleven IgA-targeted strains cultivated from an undernourished donor transmitted the undernourished phenotype, including three Proteobacteria (*E. coli*, *Klebsiella variicola*, *Citrobacter amalonaticus*), two Firmicutes (*Enterococcus hirae* and *casseliflavus*) and six Bacteroidetes (*Bacteroides acidifaciens*, *fragilis*, *intestinalis*,

*thetaiotaomicron*, *uniformis* and *Parabacteroides distasonis*). Significantly, all the genomes of the *E. coli* strains isolated from undernourished children included virulence factors and frequently corresponded to pathogenic strains (enteropathogenic (EPEC) and enteroaggregative (EAEC) *E. coli*). In this animal model, both the altered gut microbiota and the macro- and micronutrient deficient diet were necessary to develop a significant IgA response to *Enterobacteriaceae*. Conversely, *Enterobacteriaceae* was the only family-level IgA-targeted taxon significantly enriched in KM (Kwashiorkor flora, Malawian diet) mice compared to the three other groups of animals (KS, Kwashiorkor flora, Standard diet: HM, Healthy microbiota, Malawian diet: HS, Healthy microbiota, Standard diet). However, *Enterobacteriaceae* + *Enterococcus* or *Bacteroides* + *Enterococcus* had a very significantly lower deleterious effect than the consortium (*Enterobacteriaceae*, *Bacteroides* and *Enterococcus*), revealing that *Enterobacteriaceae* interacted with other consortium members, particularly *Bacteroides*, to produce enteropathy [35]. Finally, *Lachnospiraceae* and *Eubacteriaceae*, and more specifically *E. rectale* (previously associated with gut microbiota maturation, see section 3.2.), was found not to be responsible for IgA response while *A. muciniphila* was the most prominent IgA response in mice colonized with 'healthy' microbiota, regardless of diet (HM and HS groups) [35].

#### 4.6. Loss of the core gut microbiome in malnutrition

Ghosh [71] found that the biosynthesis of secondary metabolites, transport and catabolism, energy production and conversion, amino acid transport and metabolism and carbohydrate metabolism were positively correlated with nutritional index. The genetic content associated with virulence and bacterial pathogenesis was negatively correlated with nutritional index. Moreover, the number of virulence factor homologs was negatively correlated with nutritional index [71]. This suggests that the 'functional' healthy microbiota has been depleted or destroyed in combination with an invasion by virulent pathogenic microbial consortia. More recent studies also confirmed these findings [24,67].

## 5. Future perspectives

### 5.1. Missing gut microbes?

All the literature analyzed for this review suggests a deficient mother-to-child vertical transmission of health-associated microbes preceding deficient gut microbiota maturation. The causes may include deficient breastfeeding but, as rare kwashiorkor cases have been reported in breastfed infants and as *in utero* colonization has recently been suggested, an alteration of the mother's gut microbiota could also be a possible cause, particularly involving bifidobacterial populations. Sterile milk with adequate micronutrients, including galactooligosaccharides (GOS) and supplemented with strictly selected microbes such as *B. longum* (see Chapter 4.3.5.) producing lantibiotics efficient on *Enterobacteria* and enterococci [34] could represent a synbiotic therapeutic option.

In the very recent experimental study by Kau et al. [35], the correction of survival rates in Mix IgA + mice suggests that the disease is mostly due to a microbial deficiency and is not the sole consequence of highly pathogenic microbe(s). As in the famous saying: "All that is necessary for the triumph of evil [microbes] is that good men [microbes] do nothing [or are missing]." *A. muciniphila* and *C. scindens* were identified as IgA-targeted bacteria able to significantly improve mice survival, and so are candidates for these "missing microbes" [35].

Missing microbes need to be accurately identified, but most probably correspond to the anaerobes associated with gut

microbiota maturation (see section 3.2.). The most recent studies clearly show that both gut microbiota and diet are indispensable factors in the pathogenesis of severe acute malnutrition [35]. Further studies should test both the supplementation of missing microbes (including carefully selected species and strains, as *Lachnospiraceae* bacteria are associated with the absence of malnutrition but also with diabetes) and the supplementation of high concentration of missing nutrients (antioxidants and prebiotics) in the relevant diet necessary to ensure the increase of these health-associated missing microbes in the gut. We believe that such microbes define healthy mature anaerobic gut microbiota (HMAGM). A putative scenario is proposed in Fig. 2, emphasizing the fact that a therapeutic diet alone is unable to reverse gut microbiota alteration [24].

### 5.2. Critical importance of the proximal gut microbiota

Several elements suggest that the upper intestine microbiota is more critical in the vicious circle of malnutrition than colonic microbiota [78] and that aerobic *Proteobacteria* (“coliform”) invasion at this level could interfere with absorption [57]. Ongoing studies should test the role of the healthy mature anaerobic gut microbiota, specifically in the upper intestine, and in fat and vitamin B12 absorption. Indeed, the upper gut microbiota is microbiologically very different and possibly more important than the microbiota in the large bowel in terms of harvesting energy [78]. Significantly, although the anaerobic/aerobic bacteria ratio in the feces is much higher than it is in the small intestine, anaerobic colonization, including Archaea, has been demonstrated at this level. For instance, in experimental animals, the abundance of *Methanobrevibacter smithii* is higher in the proximal gastrointestinal tract than in the colon [79].

### 5.3. Four domain interactions in undernourished gut microbiota

The gut microbiota factor recently identified in malnutrition includes not only bacteria but also viruses [28] and eukaryotes [51]. Future studies should focus on the relationship between all four microbial domains, including even giant viruses [80].

## 6. Conclusion and future therapeutic options

Golden [11] concluded that Fe chelation, antioxidant therapy with both water and fat-soluble agents, possibly allopurinol, as well as Se, Mn, Zn, Cu and vitamin replacement therapy, with the avoidance of polyunsaturated fats, suppression of the (aerobic) flora invading the small bowel, and active treatment of infections in children with kwashiorkor should be tested. History has confirmed most of his hypotheses, as the composition of ready-to-use therapeutic foods (RUTF) largely follows his recommendations, including toxic microbial assessment ([14] – Table 1). Only very recent metagenomics studies have identified the key role of *B. longum* and *B. pseudolongum* in the early days and, later, the hitherto neglected role of the healthy mature anaerobic gut microbiota (HMAGM), whose irreversible alteration should be suspected in refractory cases. Future case-controlled studies are needed to better understand and define the missing microbes before performing trials testing microbial supplementation with adequate nutrients in the treatment of refractory or severe cases.

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