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Nutrition meets the microbiome: micronutrients and the microbiota

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There is increasing evidence that food is an important factor that influences and shapes the composition and configuration of the gut microbiota. Most studies have focused on macronutrients (fat, carbohydrate, protein) in particular and their effects on the gut microbiota. Although the microbiota can synthesize different water-soluble vitamins, the effects of vitamins synthesized within the microbiota on systemic vitamin status are unclear. Few studies exist on the shuttling of vitamins between the microbiota and intestine and the impact of luminal vitamins on the microbiota. Studying the interactions between vitamins and the microbiota may help to understand the effects of vitamins on the barrier function and immune system of the intestinal tract. Furthermore, understanding the impact of malnutrition, particularly low micronutrient supply, on microbiota development, composition, and metabolism may help in implementing new strategies to overcome the deleterious effects of malnutrition on child development. This article reviews data on the synthesis of different micronutrients and their effects on the human microbiota, and further discusses the consequences of malnutrition on microbiota composition.

Keywords: microbiome; micronutrients; bioavailability; malnutrition

Introduction

Micronutrients influence human metabolism and organ function through either a direct effect following absorbance and transfer to target cells or organs, or an indirect effect mediated by the microbiota in the intestinal tract. With respect to human nutrition, it is important to discriminate between energy-delivering macronutrients and essential nonenergy-delivering micronutrients (vitamins, minerals, trace elements). Micronutrients are of critical importance for energy metabolism, cellular growth and differentiation, and organ and immune function. While the interaction of macronutrients with the microbiota, particularly with the immune system and intestinal barrier function, has been well studied, few studies exist on the effects of micronutrients within the host–microbe–metabolic axis and their impact on health. This article summarizes existing data on the synthesis of different micronutrients and their effects on the human microbiota, as well as the consequences of malnutrition on the composition of the microbiota.

Water-soluble vitamins

Water-soluble vitamins are critically involved in regular energy metabolism and enzymatic functions important for gene expression (e.g., via methylation processes). Deficiency of one or more water-soluble vitamins, which could occur at systemic, tissue-specific, or cellular levels, results in different specific and nonspecific symptoms and contributes to various diseases and dysfunctions. An adequate supply of vitamins obtained by dietary intake seems necessary to ensure sufficient vitamin status. In addition to dietary sources of water-soluble vitamins, which are mainly absorbed in the small intestine, the large intestine microbiota can also produce these vitamins. Whereas understanding of the absorption and function of most vitamins is based on a large body of scientific data, studies on vitamins synthesized

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Table 1. Water-soluble vitamins synthesized by the gut microbiota

Vitamin	Function	Absorption	Transport
Thiamine	Energy metabolism (sugar) and ATP formation	Absorption in the large intestine via sodium-dependent pH-sensitive carrier.	The human thiamine transporters (hTHTR-1 and -2) are expressed in human colonocyte. Thiamine can enter the colonocytes in the free and phosphorylated forms and thus can contribute to the colonic energy metabolism. Whether thiamin is delivered to the systemic circulation is unknown.
Riboflavin	Potential redox system. Cofactor for folate and vitamin B_6 activation	Uptake is adaptively regulated by the concentration of riboflavin in the diet.[5]	Riboflavin transporters (RFVT-1, -2, -3) are present in the colonocytes and mediate the uptake of riboflavin in colonocytes and intestinal cells. No data are available regarding influence on systemic status.
Niacin	Energy metabolism, redox system	Uptake into colonocytes is regulated by extracellular substrate availability.	High-affinity transporter; important for niacin concentration in colonocytes. No data are available regarding influence on systemic status.
Biotin	Immune function, substrate metabolism	Colonocytes absorb biotin via a carrier-mediated process.	Transport via sodium-dependent multivitamin transporter (SMVT). No data are available regarding influence on systemic status.
Pantothenic acid	Energy metabolism	Colonocytes absorb pantothenic acid via a carrier-mediated process.	Transport via SMVT.
Folate	Methyl donor, nucleotide synthesis	Colonocytes absorb folate via a carrier-mediated process.	Transport via reduced folate carrier and proton-coupled folate carrier. Evidence for basolateral absorption and contribution to systemic status.[6,7]

within the microbiota and their effects on systemic vitamin status are scarce.

The microbiota of the large intestine can synthesize several types of water-soluble vitamins, which are absorbed into the colonic mucosa. Most of these vitamins are involved in energy metabolism and genetic adaptation to different conditions (Table 1[1–3]).

Thiamine

Thiamine (vitamin B_1) exists, in most cases, in a phosphorylated form and serves as an important cofactor for different enzymes within the citrate cycle as well as the formation of adenosine triphosphate and energy metabolism. Poor thiamine status results in inadequate energy supply from mitochondria, oxidative stress, and, finally, in nervous lesions and edema (beriberi).[4]

The colonic microbiota synthesizes thiamine in considerable amounts, and the colonic mucosa absorbs the free (unphosphorylated) thiamine and the phosphorylated form (thiamine pyrophosphate) via a specific carrier mechanism.[5,6] It has been shown that bacterial-derived thiamine can be absorbed into human colonocytes, which may contribute to colonic thiamine status and may also be potentially important for systemic status. An increase in thiamine has also been described as a result of soy fermentation with *Streptococcus thermophilus* and *Lactobacillus helveticus*.[7]

Vitamin B_2

Vitamin B_2 (riboflavin) acts as a cofactor or prosthetic group of different enzymes. Its active compound, flavin mononucleotide or adenine dinucleotide, functions as an oxidoreductase, delivering

electrons in biological oxidation–reduction reactions. This contributes to the frequently observed antioxidant activity of the vitamin. In cases of inadequate supply, depending on the severity, degeneration of nervous tissue and endocrine dysfunction can occur.

Both the free absorbable and protein-bound forms of vitamin B_2 are synthesized in the human microbiota. However, a specific strain has not yet been determined. In dairy products, the concentration of vitamin B_2 can vary depending on the microorganisms used in the fermentation process.[8] Early studies using human colonocytes documented that free riboflavin can be absorbed into these cells,[9] which seems to be mediated by a specific B_2 transporter (RFVT-1, RFVT-2) expressed in the large intestine. The uptake of vitamin B_2 into colonocytes is concentration dependent—the higher the luminal concentration, the lower the uptake, and vice versa;[10] an effect on systemic vitamin B_2 status has not been studied.

Vitamin B_6

Vitamin B_6 is a cofactor for over 100 enzymes, mostly in amino acid metabolism. The human microbiota (in particular, *Eubacterium rectale* and *Porphyromonas gingivalis*) contains enzymes that depend on vitamin B_6.[11] The vitamin B_6 needed for aminotransferase metabolism in bacteria might be delivered from either other bacteria or the host. Even though pyridoxal phosphate–dependent enzymes account for approximately 1.5% of most prokaryotic genomes, little is known about the interactions of diet-derived vitamin B_6 and the microbiota. Recently, it was reported that the virulence and motility of *Helicobacter pylori* depend on the presence of functional enzymes important for bacterial *de novo* vitamin B_6 synthesis.[12] Whether vitamin B_6 contributes to the virulence and colonization of pathogenic bacteria remains to be elucidated.

Folate

Folate is essential for the synthesis of precursors of DNA and RNA, cellular methylation reactions that contribute to epigenetic effects, and amino acid metabolism. An inadequate supply of folate results in megaloblastic anemia, growth retardation, and neural tube defects.

Folate can be synthesized by the large intestine microbiota in different amounts. For example, *Bifidobacterium bifidum* and *Bifidobacterium longum* produce folate in high concentrations, whereas other bifidobacteria produce only low levels of folate.[13] It is questionable whether higher levels result in higher absorption because the administration of high-producing strains results in higher fecal folate concentrations.[14] Nevertheless, it has been shown that supply of a radiolabeled folate precursor (*p*-aminobenzoic acid) into the large intestines of rats resulted in the appearance of radiolabeled folate in different tissues.[15] It is unknown whether microbiota-derived folate substantially affects general folate status.

Vitamin B_{12}

Vitamin B_{12} (cobalamin) and its analogues (corrinoids) play an important role in the gut microbiota. Human gut microbes express transporters to capture corrinoids and use vitamin B_{12} as a cofactor for metabolic pathways. The vitamin B_{12}–dependent methylmalonyl coenzyme A mutase receives carbon from the catabolism of odd-chain fatty acids, cholesterol, propionic acid, and branched-chain amino acids, resulting in the formation of succinyl-CoA as an important substrate for the tricarboxylic acid cycle generation of energy and recycling of carbon atoms. Another important metabolic process that requires vitamin B_{12} is the methionine and folic acid cycle. The vitamin B_{12}–dependent enzyme methionine synthase converts homocysteine to methionine; inadequate vitamin B_{12} or folate supply results in increased formation of homocysteine. However, there is no evidence that increased homocysteine, despite its critical role in endothelial function, has an effect on the microbiota.

Vitamin B_{12} is synthesized by different bacteria, for example, *Propionibacterium freudenreichii*, *Salmonella enterica*, *Listeria innocua*, and *Lactobacillus reuteri*. In the latter bacterium, genomic analysis has revealed 30 genes for the *de novo* synthesis of vitamin B_{12}. Vitamin B_{12} is not synthesized in plants but exclusively by bacteria and archaea from corrinoids, which are used by a couple of types of bacteria (80% of the sequenced human gut bacteria) for methyl transfer reactions. In a study surveying over 300 sequenced microbiota-derived bacterial strains, 83% of the sequenced strains were shown to have enzymes that need vitamin B_{12} as a cofactor.[16] The authors of this study further showed that many strains have multiple genes that encode corrinoid transporters; for example, *Bacteroides thetaiotaomicron* encodes three vitamin B_{12} acquisition systems,

and each corrinoid transporter is differentially competent in vitamin B_{12} transport. Folate and vitamin B_{12} also serve as regulators of gene expression in human gut bacteria, and might control genomic interactions between the microbiota and host.

Over 80% of sequenced human gut microbial species (260/313) encode vitamin B_{12}–dependent genes; most of the 260 species lack the genes required to synthesize vitamin B_{12} *de novo* and rely on transport to meet their vitamin B_{12} requirements.[17] Indeed, the great majority of human gut microbial species seem to require vitamin B_{12}, and, consequently, they compete with the host for dietary vitamin B_{12} in the small intestine. Whether substantial uptake of vitamin B_{12} in gut microbes contributes to vitamin B_{12} deficiency has not been elucidated. In contrast, humans who ingest high doses of vitamin B_{12} (1–2 mg daily), for example, for compensation of intrinsic factor deficiency, show higher levels of vitamin B_{12} in feces but higher levels of different corrinoids as well. It is argued that the increase in total corrinoid levels is indicative of corrinoid remodeling, in which the lower ligand of a corrinoid is removed and another is attached.[18] Numerous genes encoding different corrinoid enzymes present in the gut microbiota are not present in human cells, and these enzymes contribute to different metabolic steps in gut microbes. Therefore, dietary vitamin B_{12} is an important source for some metabolic reactions in gut microbes.

It is frequently argued that vitamin B_{12} synthesized by gut bacteria is a source of vitamin B_{12} for humans. However, there are some experimental data showing that this is not the case. Vitamin B_{12} formed in the colon is not bioavailable, owing to missing receptors in this area. In contrast, it appears that human gut microbes compete with their host for cobalamin, because they need exogenous corrinoids obtained through the diet.[17] Over 80% of the nonabsorbed dietary vitamin B_{12} is converted to corrinoids.[19]

Impact on the innate immune system

Water-soluble vitamins that are either synthesized or delivered via nutrition may have strong effects on the gut immune system. However, there is no clear evidence for whether vitamins involved in the immune system of the gut act via the blood supply or via luminal interaction with the microbiota or by uptake into colonocytes. There are some data showing an interaction with the microbiota and the gut immune system. For example, insects' host mutualistic bacteria that supply them with B vitamins can act as a defense barrier against pathogens. In a recent study examining the mechanism underlying the stable maintenance and control of mutualistic bacteria,[20] elimination of the gut symbionts resulted in up- and downregulation of antimicrobial defensins, which may also be involved in lysis of symbiont bacteria (downregulation of c-type lysozyme) to harvest the symbiont B vitamins. B vitamins (vitamin B_2 and folate) can serve as precursors to activate special immune T cells, called mucosa-associated invariant T (MAIT) cells.[21] Kjer-Nielsen *et al.*[21] documented that during bacterial metabolism of B vitamins, molecules are formed that can activate MAIT cells. The authors further showed that the degradation products of the B vitamins, attached to the surface of infected cells, may act as antigens that activate T and B cells of the immune system. Recently, it has been documented that *B. thetaiotaomicron* synthesizes vitamin B_{12}, which reduces the activity of the main virulence factor, the Shiga toxin 2 of enterohemorrhagic *Escherichia coli* (EHEC).[22] These data indicate that the synthesis of different water-soluble vitamins may help to affect the outcome of infections.

Interestingly, inflammatory diseases of the gut are frequently associated with deficiencies of certain water-soluble vitamins. For example, 90% of patients with pellagra, who have vitamin B_6 deficiency, suffer from colitis.[23] Biotin regulates activity of natural killer cells, which is reduced, along with systemic biotin levels, in patients with Crohn's disease.[24–26] This association between inflammatory gut diseases and vitamin deficiencies may be explained by the importance of these vitamins and other micronutrients for colonocyte function with respect to the mucosal barrier and the local immune system.

Fat-soluble vitamins

There is now strong evidence that different water-soluble vitamins are synthesized by the microbiota and contribute to interactions with the host. Microbiota-derived folate and vitamin B_{12} and the corrinoids could affect host DNA methylation patterns, and consequently exert or modify epigenetic effects. Fat-soluble vitamins A, D, K, and E are involved in the regulation of the immune response,

particularly T cell responses. There is some evidence that documents the importance of these vitamins for intestinal immune responses and barrier function. However, data related to the effects of the microbiota on these vitamins are limited.

Vitamin K

Of the two forms of vitamin K—phylloquinone (vitamin K_1) and menaquinone (vitamin K_2)—vitamin K_1 is primarily found in green leafy vegetables, while vitamin K_2 is found in meat, dairy products, and fermented food products. Most aerobic Gram-positive bacteria and the majority of anaerobic bacteria produced by the gut use menaquinones as electron carriers in the cytoplasmatic membrane. Intestinal bacteria produce vitamin K_2 in different forms. The predominant vitamin K congeners contain different numbers (6–13) of isoprenoid units. Depending on the bacterial strain, different congeners are synthesized: *Bacteroides* sp. synthesizes menaquinone-10 (MK-10) and MK-11, *Enterobacter* sp. synthesizes MK-8 and MK-9, *Veillonella* sp. synthesizes MK-7, and *Eubacterium lentum* synthesizes MK-6. The question of whether menaquinones contribute to vitamin K status remains open. Vitamin K_2 shows interesting effects on human health (protection against coronary heart disease and osteoporosis).[27] However, clear bioavailability of vitamin K_2 from bacteria has not been shown. Absorption of all vitamin K forms takes place in the small intestine through a process requiring bile salts and pancreatic enzymes, which are both absent in the colon. Some vitamin K_2 might be absorbed from the large intestine, as indicated by substantial amounts of vitamin K_2 in the human liver, which, however, does not exclude that the majority of vitamin K_2 enters the systemic circulation though the diet (e.g., from fermented food).

Vitamin A

Vitamin A is needed for proliferation, differentiation, and functional integrity of many cells and tissues, particularly rapid proliferating cells of mucous membranes and their barrier function.[28] Part of the vitamin A absorbed in the upper intestine remains within the cells as retinyl ester, and the majority is incorporated into chylomicrons and transported to the intestinal liver. There are two different ways to supply the cells of the upper and lower intestine with vitamin A, either by uptake of retinol delivered from the liver and bound to the retinol-binding protein into the intestinal cells, or from cellular retinyl ester stores within intestinal cells, which deliver retinol after hydrolysis of the retinylester.[29] Cellular retinol dehydrogenases oxidize retinol to the aldehyde retinal, and retinal is then irreversibly oxidized to retinoic acid by one of three cytosolic retinal dehydrogenase (RALDH) enzymes.[30]

Vitamin A is important for the intestinal immune response to pathogens and tolerance to food-derived antigens. Gut-associated dendritic cells (DCs) can produce retinoic acid from retinal, which could be produced by either oxidation of retinol or cleavage of β-carotene. Diet-derived β-carotene is absorbed in the small intestine (5–50%), depending on the food matrix. Part of dietary β-carotene as a fat-soluble compound can be adsorbed to fibers, consequently circumventing absorption in the upper intestine.[31] The bacterial digestion of fibers may liberate β-carotene that can then be absorbed into colonocytes. Indeed, we found different carotenoids, including β-carotene, in the human colonic mucosa, which were highest in the colon ascendens and lower in the colon descendens.[32] Mucosal DCs of the small intestine can cleave β-carotene to form retinal and, subsequently, retinol, the precursor for the active metabolite retinoic acid.[33]

A second pathway that might result in the formation of retinoic acid involves the microbiota. In a metabolomics study, an enzyme was identified in the human gut microbiome that encoded a putative enzyme with homology to the β-carotene monooxygenase 1.[34] The expression of this protein in *E. coli* results in accumulation of β-carotene, but there is no evidence to date that the enzyme cleaves β-carotene to form retinal.

It has been documented that *Bifidobacterium infantis*, which has anti-inflammatory activity, is sampled by mucosal DCs, resulting in increased expression of RALDH.[35] Feeding *B. infantis* to mice has been shown to result in an increased number of $CD103^+$ $RALDH^+$ DCs, which can metabolize vitamin A into retinoic acid. Indeed, the anti-inflammatory effect of *B. infantis* depends on the presence of retinoic acid because addition of citral, which blocks RALDH, increases the severity of gut inflammation in the mouse model. Whether retinal comes from the oxidation of retinol or cleavage of β-carotene remains uncertain. If, however, provitamin A is a source for retinol and, subsequently, retinoic

acid for colonocytes, this might explain the preventive effect of vitamin A against colon cancer.[36] This potential preventive effect of vitamin A requires the activity of another vitamin that is thought to prevent colon cancer—vitamin D.

Vitamin D

The vitamin D receptor (VDR) is absent in prokaryotic cells. Therefore, any effects of vitamin D on the microbiota must be through indirect effects of the host that alter the microbiome.

Vitamin D is of great importance for the intestinal immune system and mucosal barrier function, exerting its effects, in most cases, together with vitamin A through nuclear receptor heterodimerization. Consequently, the formation of retinoic acid in colonocytes from the provitamin β-carotene may be modulated by the microbiota and by the supply of vitamin D to colonocytes from the systemic circulation. The active form of vitamin D, $1,25(OH)_2D$, can be formed in colonocytes from its precursor, 25(OH)D, regulated by growth hormones, sex hormones (estrogen), and epigenetic factors.[37] Decreased intestinal synthesis owing to low calcium intake or low concentration of vitamin D stemming from frequently occurring vitamin D deficiency is thought to contribute to colon cancer and inflammatory bowel disease.[38]

In inflammatory bowel disease, T helper (T_H) 17 cell functions seem to be unregulated and associated with disease pathology. Large number of T_H1 and T_H17 cells have been shown to contribute to increased inflammation through production of interleukin-17 and interferon-γ in vitamin D receptor–deficient mice.[39] The observation that defensins are produced within human macrophages and DCs under the control of vitamin D can be taken as an example of a vitamin D–induced host response to bacteria.[40] Indeed, components of the gut microbiota have the ability to enhance the expression of the VDR in intestinal epithelial cells.[41] Short-chain fatty acids (SCFAs) as products of bacterial fermentation from undigested dietary fibers enhance the vitamin D–controlled formation of cathelicidin in colonocytes and consequently contribute to the bacterial–host defense barrier.[42] In human colonic cell lines (HT29), litcholic acid (from bile) and butyrate (from food) act synergistically to induce human cyclic adenosine monophosphate gene expression via the VDR, present in colonocytes.[43] Both vitamins A and D are involved in the induction of antimicrobial gene expression (for a review, see Ref. 44). Primary bile acids induce cathelicidin gene expression via heterodimerization of the farnesoid X receptor and the retinoid X receptor (RXR, with 9-cis retinoic acid as ligand) in biliary epithelial cells. Secondary bile acids occurring after bacterial fermentation of primary bile acids in the intestine induce cathelicidin expression via heterodimerization of the VDR and RXR. The subsequent synthesis of defensins is related to the local immune reaction. Consequently, the presence of vitamins D and A, as well as their nuclear receptors, is important for intestinal immune and barrier function. The critical impact of malnutrition on the intestinal microbiota and immune function has emerged as a new field of interest, and a challenge for the treatment of micronutrient malnutrition–related enteropathy.

Malnutrition and the microbiota

Micronutrients

A diet low in micronutrients, but not necessarily low in energy, is frequent in populations of low-income countries, but may also be present in poverty-affected settings in middle- and high-income countries.[45] It is estimated that more than three billion people worldwide suffer from various types of micronutrient deficiencies (predominantly vitamin A, iron, and zinc), with the majority being women and children. According to the Food and Agriculture Organization/World Health Organization, two billion people worldwide have an inadequate iron supply, one billion have inadequate zinc and vitamin D supply, and one-half billion have inadequate vitamin A.[45] Poor vitamin D status is a global problem not only in the Northern Hemisphere because of low sun exposure and subsequent low synthesis of vitamin D in the skin, but also in countries with high sun exposure.[46] Inadequate supply of vitamins A and D, as well as other micronutrients, might interfere with microbiota function and explain the relationship between dysbiosis and malnutrition.

Vitamin A can modulate the immune response of the intestine by direct interactions with immune cells or indirect modulation of the microbiota. Retinoic acid potentiates transforming growth factor-β–dependent regulatory T (T_{reg}) cell induction and inhibits proinflammatory T_H17 differentiation.[47] Indeed, it has been shown that vitamin A

deficiency results in a significant reduction of segmented filamentous bacteria because of morphological alterations of the intestinal cells and a nearly complete loss of T_H17 cells in the small intestine.[48] Vitamin A enhances the induction of T_{reg} cells and imprints the homing of leucocytes to the gut, documenting the important role of vitamin A for mucosal tolerance. Even mild vitamin A deficiency is often associated with infectious diseases of the respiratory and intestinal tracts, particularly in children. Supplementation of vitamin A has been shown to reduce childhood mortality by up to 40%.[49] *Citrobacter rodentium* infection of vitamin A deficient–mice was found to be lethal in 40% of the mice, whereas vitamin A–sufficient mice survived.[50] Other important effects of vitamin A on the intestinal immune system have recently been described in detail elsewhere.[33,47]

Vitamin A deficiency results in a (reversible) terminal differentiation of mucosal epithelial cells, leading to a loss of barrier function.[51,52] It seems likely that retinoic acid is responsible for an adequate immune response and barrier function of the intestinal mucosa. The impaired mucosal response stemming from vitamin A deficiency, with decreased mucin and defensin 6 expression, may allow easier penetration of pathogenic bacteria through the intestinal barrier.[53] This process includes the actions of intestinal Paneth cells, which produce defensins, lysocyme C, and other factors involved in host defense; Paneth cells, located at the base of the crypts, may control the bacterial population density at the mucosal surface through the secretion of defensins.[54] During moderate (subclinical) vitamin A deficiency in rats, we have detected crystalloid inclusions with lysozyme immunoreactivity in intestinal Paneth cells.[55] Paneth cells can limit bacterial translocation by controlling the numbers of mucosa-associated bacteria. In particular, the antibacterial factors secreted from Paneth cells may be retained in the mucus layer and subsequently control bacteria transfer from the lumen to the intestinal cells.[56] An important defensin, the cathelicidin (LL-37), is synthesized in Paneth cells and in colonocytes under the control of vitamins A and D.[57] These findings highlight the importance of adequate supply of both vitamins A and D to ensure normal mucosal barrier function.

It is well known that a deficiency in vitamins A or D significantly affects the immune system, particularly T cell responses, lymphocyte activation, proliferation, and regulation of the immune response.[58] Vitamins A and D act through heterodimerization of their nuclear receptors (VDR/RXR or VDR/retinoic acid receptor) to affect gene expression related to the immune system and to growth and proliferation of cells, particularly mucosal epithelial cells. Consequently, both vitamins exert effects that are related to host defense via the immune system and the intestinal barrier.

Vitamins A and D work in concert to maintain intestinal barrier function, which explains why many interactions of vitamin A with the intestinal barrier are also documented in studies on vitamin D and intestinal barrier function.[59] In addition to the effects on intestinal barrier function, vitamin D induces the expression of several antimicrobial peptides (β-defensin and cathelicidin) in DCs and is important in maintaining adequate tight junction formation.[60]

In the case of the micronutrient iron, lactobacilli seem to depend on iron; and it has been recently shown that iron-deficiency anemia is related to a depletion of lactobacilli.[61] Iron transporters are present in the cecum and right colon. In the presence of easily fermented carbohydrates that promote the growth of bacteria that produce propionate, iron absorption increases.[62] The fact that, in fermentation systems, lactobacilli convert lactate to propionate may explain the reduction of lactobacilli and iron deficiency.[63]

The effects of the abovementioned micronutrients on intestinal immune regulation and barrier function may explain the recently discovered effect of malnutrition on the gut microbiota and on health and development.

Microbiota and malnutrition

While a couple of studies have evaluated the effect of dietary habits on the microbiota, few studies have examined the effect of malnutrition on human gut microbes. To understand the impact of inadequate nutrition on the microbiota, two different types of inadequate nutrition need to be discriminated: a diet low in energy, which results in undernourishment and visibly low body weight, and a diet low in micronutrients (with or without inadequate energy), which is frequently overlooked because of a lack of detectable symptoms. To aid in the discrimination of different types of inadequate nutrition, the term "hidden hunger" has been introduced, which

describes a condition where one or more micronutrients are not present in the diet. Before clinical signs of a severe deficiency are visible, hidden hunger has an intensive impact on health, with the typical sign being stunting, characterized by low height for age (2 SD below normal). A total of 160 million children worldwide are stunted, but many cases are unrecognized because, in stunted families, a short stature is considered normal. Fifty million children suffer from very low body weight. While low body weight or wasting due to starvation is immediately visible and draws attention, stunting and, consequently, poor dietary quality, remain hidden and without intervention in many cases.

Recurrent intestinal infections and episodes of diarrhea as a consequence of hidden hunger (frequent in children with zinc deficiency) predispose to further micronutrient deficiencies, as well as to impaired barrier function. Supplementation of zinc in animal models has been shown to increase the presence of Gram-negative facultative anaerobic bacterial groups, the colonic concentration of SCFAs, as well as overall species richness and diversity.[64,65] A leading symptom of zinc deficiency is chronic diarrhea.[66] Up to two billion people are affected, predominantly children under 5 years of age, with a global prevalence of 31% and 80% in Asia.[67] To treat diarrhea in children, intervention with zinc supplementation has been successful, with the intensity and severity of diarrhea being reduced up to 30%.[68] Zinc supplementation affects the local and systemic immune response[69] and the integrity of the mucous membranes,[70] and reduces pathogenic infections and diarrheal episodes.[71] Supplementation with high levels of zinc has been shown to result in an increase of *Lactobacillus* in the gut microbiota in weaned pigs[72] and results in favorable effects on the development and metabolic activity of the intestinal microbiota.[73] Using chicks as a model, one study recently showed that zinc deficiency results in a remarkable change of the microbiota, with metabolic changes, reduced output of SCFAs, and a subsequent reduction of zinc bioavailability.[74]

Environmental enteropathy (EE), characterized by damaged gut architecture, deregulated mucosal permeability, inflammation, and increasing malabsorption of macro- and micronutrients, is a consequence of malnutrition and an important contributor to the high maternal and child mortality in low-income settings. Hidden hunger experienced during the critical 1000-day window from conception until the end of the second year of life has a negative impact on physical and cognitive development. During the first 1000 days of human life the most important developmental steps occur, which ultimately affect long-term health. The nutritional status of the mother at the time point of conception is critical with respect to embryonic development, and poor nutrition during pregnancy results in poor development of the fetus. The consequent intrauterine growth retardation (IUGR) results in low birth weight of the newborn; however, low birth weight is only an external sign of poor development. IUGR affects all organs and is thought to contribute to diseases later in life (e.g., metabolic syndrome, cardiovascular disease).[75]

Major determinants of low birth weight, particularly in developing countries, are poor nutritional status of the mother and subsequent low nutrient flow to the developing child. Newborns with low birth weight (<2.5 g) are four times more likely to die during their first 28 days of life than those weighing 2.5–2.999 g and are 10 times more likely to die than newborns weighing 3.0–3.499 grams.[76] Low birth weight is, however, a phenotype of intrauterine stunting and, consequently, a visible marker for potentially impaired organ and brain development. Restricted growth for age, defined as stunting (−2 SD below 95% percentile), is a consequence of malnutrition during pregnancy and early childhood.

Infections and chronic diarrhea occurring frequently between 6 and 11 months of age further deplete micronutrient body stores and promote malnutrition, which negatively affects the immune system and further promotes infections. In addition to the abovementioned dietary factors, inadequate provision of care and living conditions may also affect the development of stunting. Stunting, however, is not an isolated sign of growth retardation but a biomarker for impaired development of the brain.[77]

Impact of malnutrition on the developing microbiota

During the critical 1000-day window, the intestinal microbiota develops and attains a similar composition as in adulthood.[78] The maternal microbiota colonizes the fetal gut before delivery. At delivery, a child is exposed to different types of microbiota,

and during breastfeeding, milk-associated bacteria are transferred to the breastfeeding baby. Taken together, maternal nutrition may control the composition of the maternal microbiota and, consequently, of the newborn and breastfed child.[79]

Micronutrient deficiencies during the 1000-day window might critically influence the maturation of the microbiota and its interaction with the host, with consequences for adolescence and adulthood. Epigenetic processes may have an important influence on these microbiota–host interactions. Folate is a major one-carbon source and an important methyl group donor involved in methylation reactions related to epigenetic modifications.[80] Supplementation of methyl donors (folate, vitamin B_{12}) has been shown to increase the one-carbon pool and change the methylation at selected loci in mice[81] and humans.[82] In contrast, famine resulted in hypomethylation of insulin-like growth factor 2.[83] Supplementation of pregnant mice with methyl donors (betaine, choline, folate, vitamin B_{12}) resulted in significant differences in the postnatal microbiota composition, compared to that in unsupplemented mice.[84] Whether microbiota-related folate synthesis plays a role in the epigenomic regulation of host–microbiota interactions has not yet been evaluated.

During the 1000-day window, inadequate dietary intake of micronutrients (vitamins A and D, iron, zinc) together with a low dietary supply of one-carbon sources (folate, vitamin B_{12}), which occurs frequently in low- and high-income countries, might have consequences for the maturation of the microbiota.

How far will micronutrient deficiency contribute to EE?

In low-income countries with poor hygiene and malnutrition, small children in particular are at risk of developing EE, with changes in the intestinal tract including impaired barrier function, reduced height of small intestinal villi, and increased formation of intraepithelial lymphocytes and T_H1 cells. Similar changes have been also described as a consequence of deficiencies in vitamins A[85] and D.[86]

Together with an immature microbiota resulting from malnutrition, EE is a highly critical condition, particularly for small children, not only increasing early childhood mortality but also having additional negative effects on growth and development. Zinc deficiency is thought to be a major contributor to the development of EE, and supplementation studies with zinc have resulted in a shorter duration of diarrhea. However, because of inconclusive results and missing primary prevention strategies, it has been recommended to use zinc supplements together with multivitamins for primary prevention.[87] Whether this might affect the maturation of the microbiota remains to be evaluated.

Different studies have used distinct intervention strategies to evaluate the impact on the microbiota and EE. In most cases, the children studied were severely malnourished and showed typical signs of undernutrition (marasmus and kwashiorkor). However, this kind of severe malnutrition is a combination of low food quantity and quality, and it is therefore not possible to discriminate between a poor energy–related effect and one of poor micronutrient supply. Subramanian *et al.* considered this issue in a study of the gut microbiota in children with severe acute malnutrition (SAM), which affects approximately 4% of children in low-income countries, and moderate acute malnutrition (MAM), affecting 19% of children in low-income countries. In Bangladesh, at least 40% of children below 5 years of age show stunting as a sign of MAM.[88] The microbiota of children aged 6–20 months with either SAM or MAM was compared with that of healthy children, using anthropometric scores (weight for age and height for age). In children with SAM, the effect of ready-to-use food (RUTF) and a locally produced, lower-cost food combination (plus micronutrients and iron) on the microbiota was evaluated. SAM was associated with significant microbiota immaturity, which was only transiently improved following RUTF and the local diet. Children with MAM showed significantly lower microbiota immaturity than healthy children. These findings suggest that microbiota immaturity in children with MAM may increase malnutrition and explain the children's high risk of developing SAM. Further studies with severely malnourished children have documented the immaturity of the microbiota and the lack of a long-term effect of RUTF.[89]

Conclusion

To understand the interactions of hidden hunger, the microbiota, and impaired immune system and barrier function, research on the interaction

of micronutrients (e.g., zinc, vitamins D and A) is needed. Malnutrition results in persistent impaired maturation of the intestinal microbiota in children,[88] which results in continuous and at least severe malnutrition despite dietary interventions with ready-to-use food, a composition of macro- and micronutrients.[89] The persistent alteration of the microbiota, which occurs as a consequence of under- and malnutrition, is far less studied than the interaction of the microbiota and obesity. Studies need to explore the interactions of diet, dietary components, and the microbiota in the context of hidden hunger and undernutrition. This may save more lives than interventions with gut microbiota to reduce obesity. Indeed, even in obesity, it is increasingly realized that malnutrition can be associated, a combination that is defined as a dual burden.[90] Studies focusing on the microbiota and its impact on obesity should also consider the influence of micronutrient deficiencies. Furthermore, in approaches using microbiota transplantation as a treatment option for intestinal diseases, there should be consideration of the possibility of moderate malnutrition. Advancing the understanding of the impact of intestinal micronutrient synthesis, and the action of micronutrients on the composition and activity of the microbiota, is indeed a scientific challenge.

Conflicts of interest

The author declares no conflicts of interest.

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