



**HAL**  
open science

## Bacteria from the gut influence the host micronutrient status

Dorgeles Dje-Kouadio, Frank Wieringa, Valérie Greffeuille, Christèle Humblot

► **To cite this version:**

Dorgeles Dje-Kouadio, Frank Wieringa, Valérie Greffeuille, Christèle Humblot. Bacteria from the gut influence the host micronutrient status. 2021. ird-03427472

**HAL Id: ird-03427472**

**<https://ird.hal.science/ird-03427472v1>**

Preprint submitted on 14 Nov 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## “Bacteria from the gut influence the host micronutrient status”

Dorgeles Dje-Kouadio<sup>1,2</sup>, Frank Wieringa<sup>1,2</sup>, Valérie Greffeuille<sup>1,2</sup>, Christèle Humblot<sup>1,2\*</sup>

1 QualiSud, University of Montpellier, Avignon University, CIRAD, Institut Agro, IRD, University of La Réunion, Montpellier, France.

2 Institut of Research for Développement (IRD), France

\* Corresponding author: [christèle.humblot@ird.fr](mailto:christèle.humblot@ird.fr) - 0033 4 67 41 64 13

### **Abstract**

Micronutrient malnutrition remains a serious public health problem in most low- and middle income countries, with severe consequences for child physical and psychomotor development. Traditional methods of treatment and prevention, such as supplementation and fortification, have not always proven to be effective and may have undesirable side-effects, including increased growth and virulence of pathogenic gut bacteria, resulting in diarrhea and gut inflammation. Commensal bacteria in the gut have demonstrated the ability to increase the bioavailability of certain micronutrients, notably by removing anti-nutritional compounds, such as phytates and polyphenols, or by the synthesis of vitamins or their derivatives. The intestinal microbiota is also, in symbiosis with the gastrointestinal mucosa, the first line of protection of the internal environment against the external environment. It thus contributes to the reinforcement of the integrity of the intestinal epithelium and to a better absorption of micronutrients. They represent a save alternative to prevent micronutrients deficiencies. However, the metabolic characterization of the intestinal microbiota is not yet complete and its role in micronutrient malnutrition is still poorly understood. The bacterial metabolism is also dependent of micronutrients acquired from the gut environment and resident bacteria may compete or collaborate to maintain micronutrient homeostasis. Gut microbiota composition can therefore be modulated by micronutrient availability. This review brings together current knowledge on this two-

way relationship between micronutrients and gut microbiota bacteria, with a focus on iron, zinc, vitamin A and folate (vitamin B9), which are respectively minerals and vitamins of interest in a global context.

## **key words**

bacteria – gut microbiota – human – micronutrients – deficiency - prevention

## **1. Introduction**

Micronutrients, including minerals and vitamins, are nutrients needed only in small quantities on a daily basis, but who are vital to human metabolic processes (1,2). Vitamin and mineral deficiencies, also called ‘Hidden Hunger’ to highlight the lack of visibility, is a serious public health problem, encountered in all social strata of the world, but especially affecting vulnerable groups in low- and middle income countries (3). Micronutrient deficiencies are widespread around the world, estimated to affect over 2 billion people, and are implicated in a wide range of adverse health outcomes such as increased prevalence of morbidity and mortality, anaemia, stunting and delayed cognitive development. In a global context, the diets of two-thirds of women and children, mainly from resource-poor households, are deficient in at least one micronutrient (4). The most prevalent micronutrient deficiencies worldwide include iron, zinc, vitamin A and folate (vitamin B9) (4,5).

Several strategies to improve micronutrient status of individuals or populations exist, with fortification of staple foods being regarded as the most cost-effective intervention to improve micronutrient status of populations (6). Nevertheless, strategies to fight micronutrients deficiencies are not always efficient. Absorption of micronutrients occurs mainly in the small intestine, through

different mechanisms depending on the micronutrient. For example, zinc and iron are mainly absorbed in the duodenum and jejunum through specific transport carriers (7,8), whereas folate is not only absorbed in the small intestine, but also in the colon (9).

In the digestive tract, there is a complex community of microorganisms with a concentration and diversity that increases from mouth to colon (10). Although other microorganisms (viruses, fungi) share this niche, most studies have dealt with bacteria, the phyla Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Verrucomicrobia being the most represented (10). The role of the intestinal microbiota in human health is undeniable today (11). Indeed, the composition of the gut microbiota have been associated with diseases such as inflammatory bowel disease (Gonzalez and al. 2011), or obesity (Turnbaugh and al. 2007). And the intestinal microbiome plays an important role in the aetiology of severe acute malnutrition (14,15). Faecal bacterial composition strongly vary among individuals, which was found to be dictated by the host's genetics, but also by many environmental factors including diet (16,17).

For long, most research on the effect of food on the intestinal microbiota has focused on macronutrients (fats, carbohydrates, proteins) (18,19). However, bacteria need micronutrients for their survival. For example, bacteria require iron from external sources, and some of them even have developed specific mechanisms such as transferrin transporters to compete for iron sources (20). In contrast, many bacteria can directly synthesise vitamins such as folate (vitamin B9), thus, not necessarily requiring an external source.

Even though the absorption of most micronutrients by the host is now well described, the role of the intestinal microbiota on micronutrient absorption and/or availability is less clear. Considering the amount and the diversity of the bacteria along the digestive tract, there is a need to sum up the actual knowledge and to underline the gaps and research needed in this field. Absorption of micronutrients

generally occurs in the upper part of the digestive tract, usually in the duodenum or jejunum through specific transport mechanisms (21). However, typically only 5%-50% of micronutrients are absorbed, meaning that the most part of the ingested micronutrients reach the colon (22).

Gut microbiota have different modes of action on the micronutrients, which can result in a better or a worse bioavailability for the host. These actions can be direct, targeting directly the micronutrients of interest. For example, bacteria can take up iron and zinc, but in case of excess efflux of these metals is possible. These mechanisms allow pathogenic bacteria to scavenge minerals efficiently and escaped nutritional immunity and develop in presence of commensal bacteria from the gut (Celis 2020). Similar mechanisms in commensal bacteria are not completely understood, even if it some authors underline that they should be less aggressive than the ones used by pathogens (Celis 2020). Actions can also be indirect, for example through production of short chain fatty acids (SCFA), which will result in a better health of the gut epithelium, resulting in a better absorption of the micronutrient by the host.

This review aims to summarise the current knowledge on the bilateral relationship that exists between the intestinal microbiota and micronutrients and discuss emerging themes. A recurrent theme will be the bi-directional character of the relationship, with micronutrient status of the host modifying the microbial composition of the gastrointestinal tract (Tian and al. 2018; Zimmermann and al. 2010), but with also the intestinal microbiota modulating the bioavailability of micronutrients and thereby the micronutrient status of the host (25). We have restricted the scope of this review to four micronutrients important for global public health: iron, zinc, vitamin A and vitamin B9.

## 2. Micronutrients and host health

Micronutrients play a central role in metabolism and the maintenance of tissue functions that govern human health (21). However, despite efforts to diversify diets to better meet micronutrients needs (26), the prevalence of micronutrient deficiencies remains very high in all parts of the world, including in industrialised countries (27). Children and women are the most affected, due to higher requirements to sustain growth and higher losses (e.g. menses). The clinical signs of single micronutrient deficiencies are varied and often micronutrient-specific (28).

Iron deficiency is considered the most common form of micronutrient malnutrition, with more than 2 billion people estimated to be iron-deficient (29). Iron deficiency anemia is a common disease associated to insufficient iron intake and iron stores (30). Factors contributing to iron deficiency include (i) a low intake of haem iron, which has a higher bioavailability than non-haem iron, (ii) diets with a high content of phytates or phenolic compounds responsible for a lower bioavailability (31) and (iii) chronic inflammation, leading to higher levels of hepcidin, downregulating iron absorption (32).

Zinc deficiency is not only associated with growth retardation in children, leading to stunting, but also diminished immunocompetence, thereby increase the susceptibility of children to diarrhoeal disease, pneumonia and perhaps malaria (33). Moreover, it seems that zinc deficiency can predispose to other micronutrient deficiencies (Biesalski 2016). Zinc deficiency is estimated to affect more than 1 billion people worldwide (34).

Similarly, vitamin A deficiency leads to decreased immunocompetence, as well as reduced integrity of intestinal epithelial mucosa (35). Half-yearly high dose vitamin A supplements are given to children less than 5 years of age in many low- and middle income countries in a drive to decrease child mortality (36), but impact on vitamin A status is questionable.

Insufficient folate intake during pregnancy is associated with neural tube defects during the first weeks of embryonic life (37). In the general population, folate deficiency also leads to megaloblastic anaemia, and neurological symptoms that partly overlap with those found in vitamin B12 deficiency (28). It is estimated that more than 20% of women of reproductive age in low- and middle income countries are folate deficient (38).

### 3. The complexity of host-bacteria interaction: the case of iron.

For a complete overview on iron, bacteria and host interplay we would like to refer to a recent review (39). Here we have summarized the elements which describe the complexity of this relationship (39).

As human are not able to actively excrete iron, its absorption is tightly regulated in the duodenum (Figure 1) (29). Heme iron and non-heme iron are absorbed through two distinct pathways (40), with heme iron absorbed by a heme carrier protein 1 (HCP1) at the border of the duodenal brush membrane. Non-heme iron, usually in its ferric form, is not bioavailable and must be reduced to the ferrous form by duodenal cytochrome b (DCYTB), before being transported over the duodenal brush membrane by the divalent metal transporter (DMT1) (41). Iron is also absorbed in the ileum and colon since the presence of DMT1 has been described (42). But the role of these absorption sites on the host iron status remains to be estimated (43). Iron homeostasis is regulated by hepcidin, a hormone that circulates in the plasma and inhibits the release of iron into the plasma through different mechanisms (44). Hepcidin is upregulated during systemic inflammation, thereby reducing iron bioavailability. But pathogenic bacteria could induce systemic inflammation, thereby reducing the intestinal bioavailability of iron (Nicolas and al. 2002).

Iron is also essential for many bacteria, requiring it for their growth (46). Bacteria can acquire iron through different mechanisms such as using ferric-specific chelators called siderophores, absorbing ferrous iron after reducing ferric iron or using host iron compounds such as heme or transferrin. Despite its bacteriostatic function, lactoferrin, which is found in milk, can also be used by bacteria with specific receptors (47).

Siderophores are a wide family comprising hundreds of forms produced and secreted by many-microorganisms (48). The resulting ferric-siderophores complex are internalised via specific outer membrane proteins. Bacteria can also use siderophores produced by other bacteria, through the production of outer membrane receptors with different ligand-binding sites. Siderophores are expressed in case of bacterial iron deficiency, thus not in iron-sufficient environments (49).

In anaerobic conditions and at low pH, iron is mainly in the form of ferrous iron, which can be transported directly by bacteria, mainly through the dedicated transport system *feo* (50). Transportation of heme-iron, transferrin, lactoferrin is through specific receptors or direct absorption (39). Regulation of iron uptake is highly important in bacteria since iron is toxic at high doses. The Fur regulation system represses transcription of genes involved in iron uptake when iron concentration is too high (51).



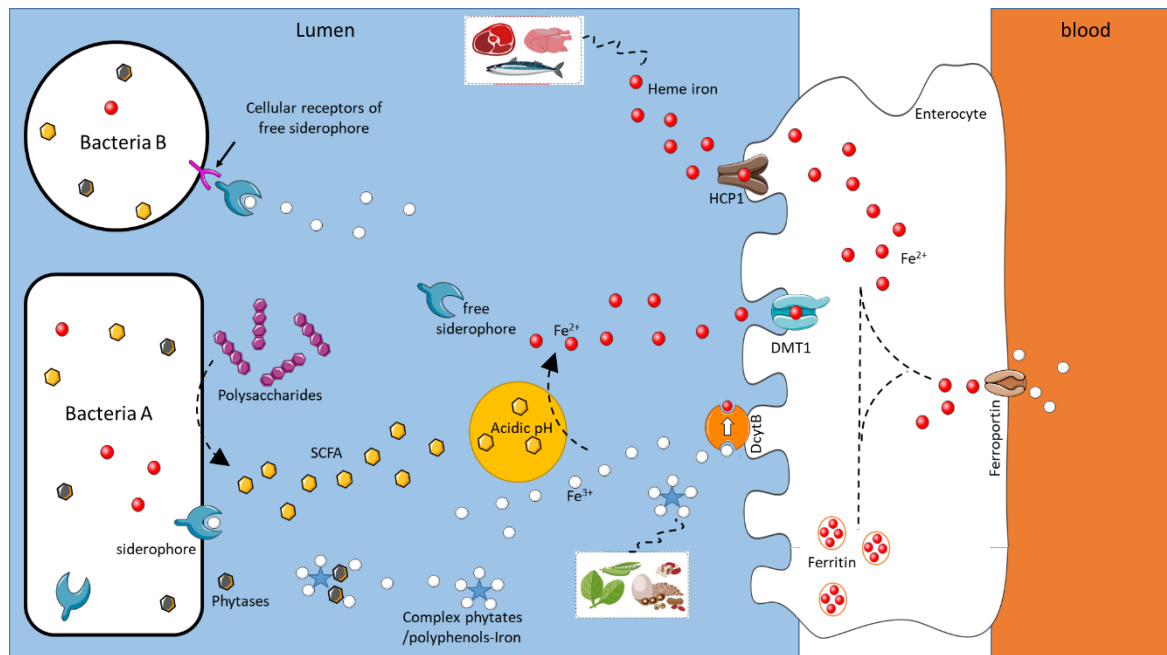


Figure 1. Schematic representation of the gut microbiota role in the intestinal absorption of iron. SCFA : short-chain fatty acids ; HCP1 : haem carrier protein 1 ; DMT1 : Divalent metal-ion transporter-1 ; DctyB : duodenal cytochrome b

It is important to note that most of our knowledge of bacterial iron absorption comes from research done on pathogenic bacteria. It has been considered for a long time that a few beneficial bacteria, such as members of *Lactobacillus* genus, do not require iron for their growth (52). Nevertheless, specific systems have been identified in *L. plantarum* and *L. sakei* (53,54). Most probably, other systems may exist, and studying these bacteria may reveal new iron absorption pathways. At the main site of iron absorption, in the duodenum, there are roughly  $10^3$  bacteria/g, which is less than in the jejunum ( $10^4$  bacteria/g) and far less than in the colon where up to  $10^{12}$  bacteria/g of content can be found (55). Also, members of *Lactobacillus* genus are numerous in the small intestine where iron absorption is maximum, thus the role of these bacteria on iron absorption by the host should receive more attention, to determine whether they play or not a role in bioavailability of iron for the host.

It has been calculated that iron concentration in the colon should be high, which is by far more than the minimum requirement for bacterial growth (56). But the bioavailability of iron for bacteria depends also on a range of factors (form of iron, iron speciation, pH, oxygen levels). For bacteria and human, non-heme iron is the most affected by the composition of the food matrix and the physico-chemical composition of the lumen. Indeed, non-heme iron is linked in many food matrices to inhibitors, such as polyphenols, fibres or phytates, which can be degraded by dedicated bacterial enzymes, thus resulting in a better iron absorption (57). This property is usually utilised in fermentation, where bacteria reduce the concentration of inhibitors in the food, resulting in higher iron bioavailability. But the presence of phytases has been demonstrated also for a number of bacteria found in the gut (including the ones in the small intestine). It was suggested that whatever the phytates content of the food, all iron in the food matrix should be liberated since bacteria from the gut exhibit phytase activity (58). However, this appears to be in conflict with real-life observations, where phytate-high diets are linked to iron deficiency.

Also, some promoters of iron bioavailability, such as lactic acid, are produced by a number of bacteria, present all along the digestive tract. The acidified intestinal environment could also degrade complexes chelating micronutrients and facilitate absorption of iron (59). Bacteria can also produce short chain fatty acids (SCFA) by fermentation of non-digestible carbohydrates from the diet, which will lower the luminal pH, and thus reduce ferric to ferrous iron, thereby improving its absorption by bacteria and host (60).

It is obvious from above that gut bacteria play an important role in modulating the bioavailability of iron for the host. However, host factors can modulate iron uptake by bacteria, for example, in the case of inflammation, through the synthesis of lipocalin, which will bind to the siderophores and thus counter the trapping of iron by the intestinal bacteria (61). Recently, it has been suggested that rather than a battle for minerals, commensal bacteria use different mechanisms not only to liberate and capture iron, but also to share iron among themselves and with their host (62,63).

Overall, it appears that depending on the model used (*in vitro*, animals or humans) results are dramatically different, making any generalization difficult. One of the few consistent results is the decrease in the proportion of bacteria of the phylum Actinobacteria in case of iron-deficiency anemia (64). In the case of iron supplementation or fortification, the chemical form of iron used (ferrous sulfate, ferrous fumarate, NaFeEDTA, ferric sodium ethylenediaminetetraacetate) seems to affect bacterial composition differently (65–67). Surprisingly, no general trends could be identified, even in the same model and using the same method with iron supplementation (39). For example, in human models, iron supplementation was associated with an increase (68), a decrease (69) and had no effect (70) on the population of bacteria of the phylum Firmicutes. The only consistent results were for Lactobacillaceae family and the phylum Actinobacteria. Indeed, Lactobacillaceae always decreased during iron supplementation, whatever the model used (68,71,72). Also, against all expectations, the effect of iron supplementation was not directly opposite to that of iron deficiency (39).

#### 4. Role of bacteria in zinc bioavailability

Zinc is involved in vital processes such as DNA synthesis, transcription, and translation (73). Zinc absorption occurs in the duodenum and involve the transmembrane zinc transporter protein Zip4 (figure 2). Zinc is then shuttled to the metalloprotein synthesis sites by the zinc-regulated proteins ZnT2-10 or exported to the circulation by Zinc transporters ZnT1 (74). Contrarily to iron, zinc can also be actively excreted in case of excess. It will go from the circulation to the enterocytes by the protein Zip5 at the basolateral side of these cells and an efflux from the enterocyte to the lumen via ZnT5 (75).

In food, zinc can be complexed with antinutrient factors such as phytates or polyphenols, thus reducing its absorption (76). Zinc that is not absorbed in the small intestine will reach the colon and

its possible absorption by colonocytes where the zinc transporters are also expressed (77). As for iron, the phytase activity found in many members of the commensal bacteria as well as their ability to metabolise polyphenols could increase the bioavailability of zinc for the host.

In bacteria, zinc is rapidly bound to small molecules such as metallothionein or is transported to metalloprotein synthesis sites by zinc chaperones (78). Zinc uptake by bacteria is regulated by the zinc uptake repressor Zur and the zinc efflux repressor CzcA (78). As in the case for iron, most studies were done on pathogens, rather than on commensal bacteria. Some pathogenic bacteria can overcome zinc limited conditions (nutritional immunity produced by host through sequestration of essential micronutrients such as zinc by secreting calprotectin) by expressing a high affinity transporter (ZnuABC) (79). Zinc can also be actively exported in case of excess (80).

Zinc deficiency has been repetitively associated with chronic diarrhoea (59). The effect of zinc supplementation on the intestinal microbiota has been mainly studied in animal models. It has been used in poultry and pig industry at pharmaceutical dose to reduce gastrointestinal infections and diarrhoea (81). Zinc has antimicrobial activity and different studies underlined the changes of bacterial composition of the different parts of the gut (from stomach to colon) induced by such high doses of zinc (82–85). Depending on the studies, there was a reduction or an increase of Enterobacteriaceae and lactic acid bacteria (82–85). One study showed that *Ruminococcus* genus was predicting host zinc adequacy (86). Bacterial compositional changes were accompanied by changes in the metabolism of the bacteria as shown the increase colonic concentration of short chain fatty acids (SCFA) (83).

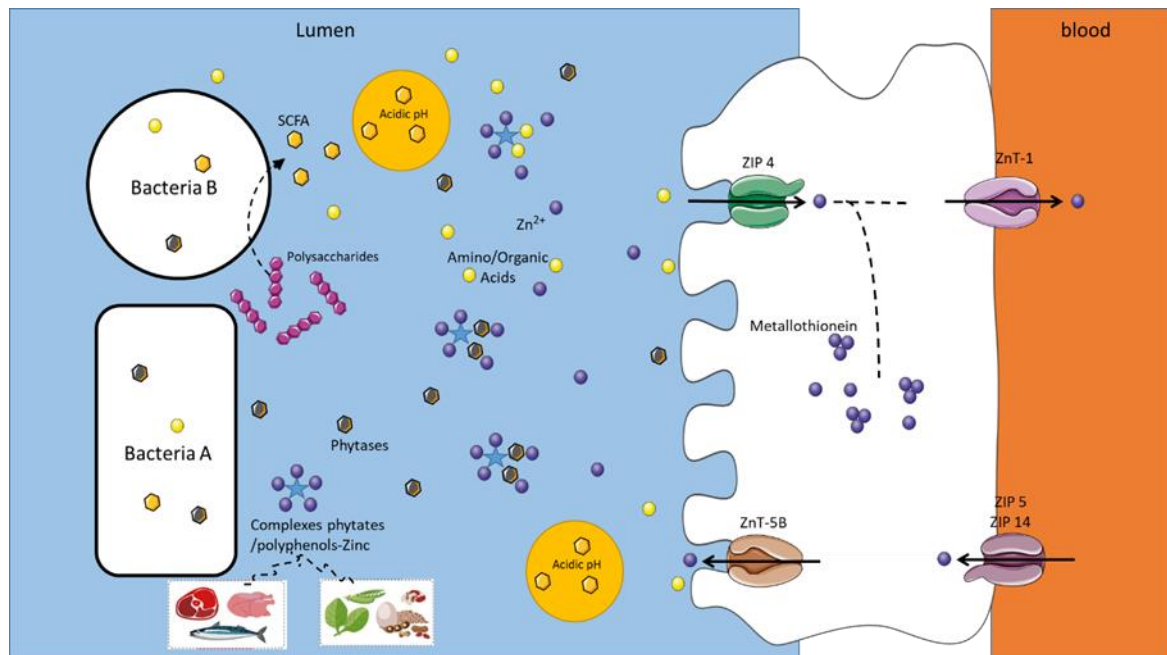


Figure 2. Schematic representation of the gut microbiota role in the intestinal absorption of zinc. SCFA: short-chain fatty acids ; ZIP : Zrt-/Irt-like proteins ; ZnT : Zinc transporters.

An original study using initially germ-free mice inoculated with different bacterial species from the human gut microbiota shown almost no effect on zinc depletion or repletion on the bacterial composition of faecal samples in non pathogenic conditions (87).

As for iron, studies on zinc status and host microbiota mainly focused on pathogenic conditions, and little is known about the role of bacteria in a normal situation.

### 5. Acquisition of folates by host and bacteria

Several commensal bacteria have been shown to synthesize certain vitamins. The role of intestinal bacteria in the production of vitamin K has been known for several decades, but bacteria can also be a source of folates for the host, even if the extent of the overall contribution is still unknown (88). Folate or vitamin B9 is necessary for DNA methylation and replication, as well as for cell division,

which is characteristic of better growth (89). Folates from the diet exist in mono and poly-glutamate forms, which are deconjugated to the monoglutamate form and then absorbed in the ileum via specific transporter FFLR1,2 (proton-coupled folate transporter (PCFT)). In the epithelium, folate monoglutamate is converted into an active form, tetrahydrofolate (THF). Then, it is transported to the blood (90,91). In the colon, a version of the same receptors has been identified (92).

Bacteria require folate for their growth, with some bacteria being prototrophic and can synthesize folate from precursors present in the environment (figure 3). The others, auxotrophic, must acquire folate from the environment. All of them have transporters for folates or folates precursors (93,94). The ability to synthesize folate is found in different taxonomy communities (95). Some bacteria also have uncomplete synthesis pathways in their genome (93). A body of data are in favour of a significant contribution of bacterial folates synthesis to host status. Early studies reported that human folate concentration in faecal samples where 300-500 µg/day, while dietary intakes were below 100 µg/day, suggesting a synthesis of folate by the gut bacteria (96,97). Modification of gut microbiota composition due to fibers consumption may result in increased folate concentration in colonic content or in circulation (98,99). Also, despite that folate absorption rate in the large intestine is 100 time slower than in the small intestine, labelled folates specifically encapsulated to reach and disintegrate in the colon, were incorporated in host tissue (100,101).

Direct ingestion of folate producing bacteria can also improve folate status of rodents on folic acid deficient diets (102–104). Another study showed that, in a model of *Caenorabditis Elegans*, bacteria can convert folic acid into tetrahydrofolates, which are better absorbed by the host (105).

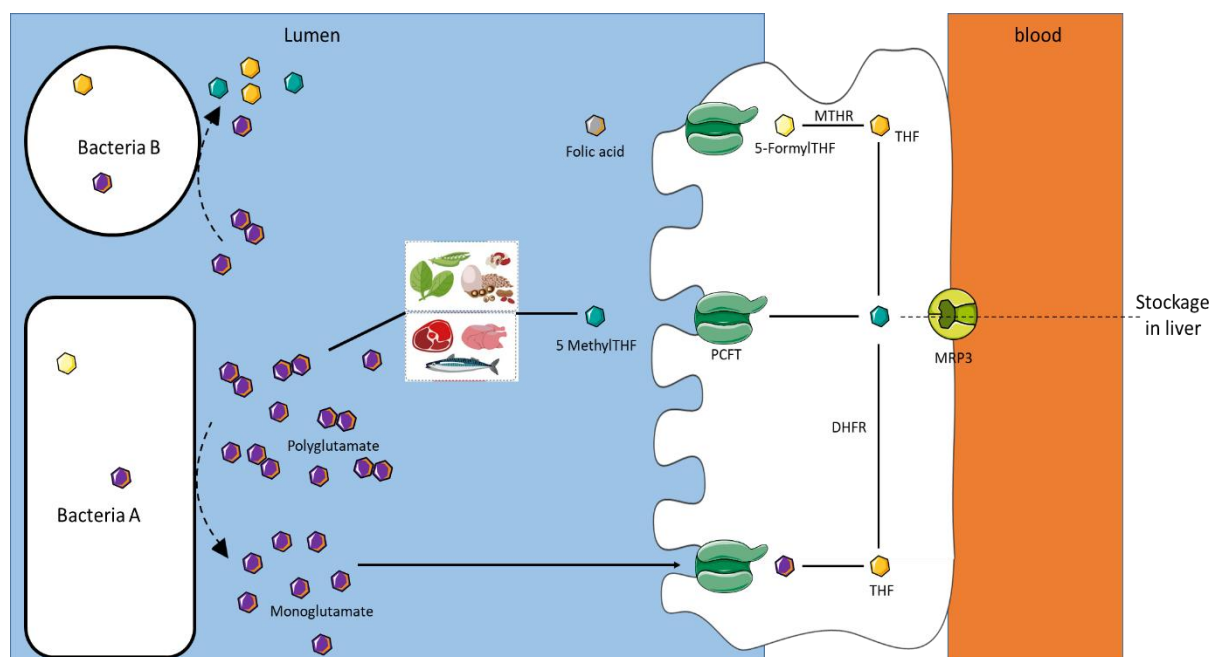


Figure 3. Schematic representation of the gut microbiota role in the intestinal absorption of vitamin B9. PFCT : proton coupled folate transporter ; THF : tetrahydrofolate ; MRP : multidrug resistance protein ; DHFR : dihydrofolate reductase ; MTHFR : methylenetetrahydrofolate reductase

The metabolic capacity of the gut microbiota depends on its composition but also on the physiological characteristics of the host. For example, genes coding enzymes involved in the folate biosynthesis, are found more frequently in gut microbiota of babies young children as compared to adults (106). Interestingly, the microbiome of children suffering from malnutrition showed a significant lower abundance of genes in multiple path-ways involved in B-vitamin metabolism (Gehrig and al. 2019).

Contrarily to irons and zinc, it has been shown that the dietary folate deficiency doesn't affect the gut bacterial composition (108). Perhaps because of the presence of phototrophic bacteria, producing folates in periods of deficiency, fluctuations in folate intake have less impact on gut flora. (95,109).

## 6. Role of gut microbiota in vitamin A absorption and metabolism

Dietary vitamin A is found in the form of carotenoids and retinyl esters, respectively present in vegetables and animal products (110). The uptake of carotenoids and retinol occurs in the upper half of small intestine (110,111).

Vitamin A directly contributes to the maintenance of the gut barrier by regulating proliferation and differentiation of immune cells in the intestinal epithelium and plays a crucial role in the resistance to pathogens invasion in the gut (35,112,113). The epithelial and mucosal cells, in symbiosis with commensal bacteria, constitute a first physical barrier against external bacteria and contribute to the regulation of the gut microbiota composition (114,115). Disruption of the integrity of the intestinal barrier can lead to poor absorption of nutrients, and various diseases in the host, including inflammatory bowel disorders (116,117)

Vitamin A and carotenoids are dietary fat-soluble components that require solubilization by micelles prior to absorption in enterocytes (118). An emulsification phase into droplets occurs in the stomach and duodenum, with vitamin A being incorporated into micelles formed with bile salts (119,120).

Carotenoids can be absorbed by passive diffusion, while retinoid are absorbed through carrier-dependent proteins (111). Only free forms of retinoids can be absorbed so that retinyl-esters must be hydrolyzed to retinol either by pancreatic enzymes or by a retinyl esters hydrolase (REH) present in the mucus (121,122). After absorption retinol is reesterified with long-chain fatty acids by the enzyme lecithin-retinol acyltransferase (LRAT) or by retinol dehydrogenases (RDHs) into retinal (figure 4).

In the enterocyte, carotenoids are directly converted into all-trans retinal by carotene-15,15'-dioxygenase [BCO(D)M] (123). Retinaldehyde can be irreversibly converted with retinaldehydrogenases (RALDH) into bioactive retinoic acid molecules or converted into retinol ester by LRAT, to be stored in the liver (124). The secretion of secondary or tertiary bile salts produced by bacteria belonging to *Lactobacillus*, *Bifidobacterium*, *Bacteroides* or *Clostridium* genus could favour



the solubilisation of vitamin A and improve its absorption (125) since bile salts are essential for the micellarisation process (126).

One role of bacteria is a direct synthesise of carotenoids or an indirect effect on their bioavailability. Indeed, the genome of bacteria from the gut has analogues of different enzymes, found in animal and plant products, which allow the synthesis of carotenoids and retinoids from Acetyl-CoA through the mevalonate pathway (MVA) (127,128). In addition, bacteria from the genus *Bacteroides*, *Enterococcus* and *Streptococcus* possess the genes *brp/blh* that are similar to the host BCM(D)O genes, and hence could convert  $\beta$ -carotene into all-trans retinal (129).

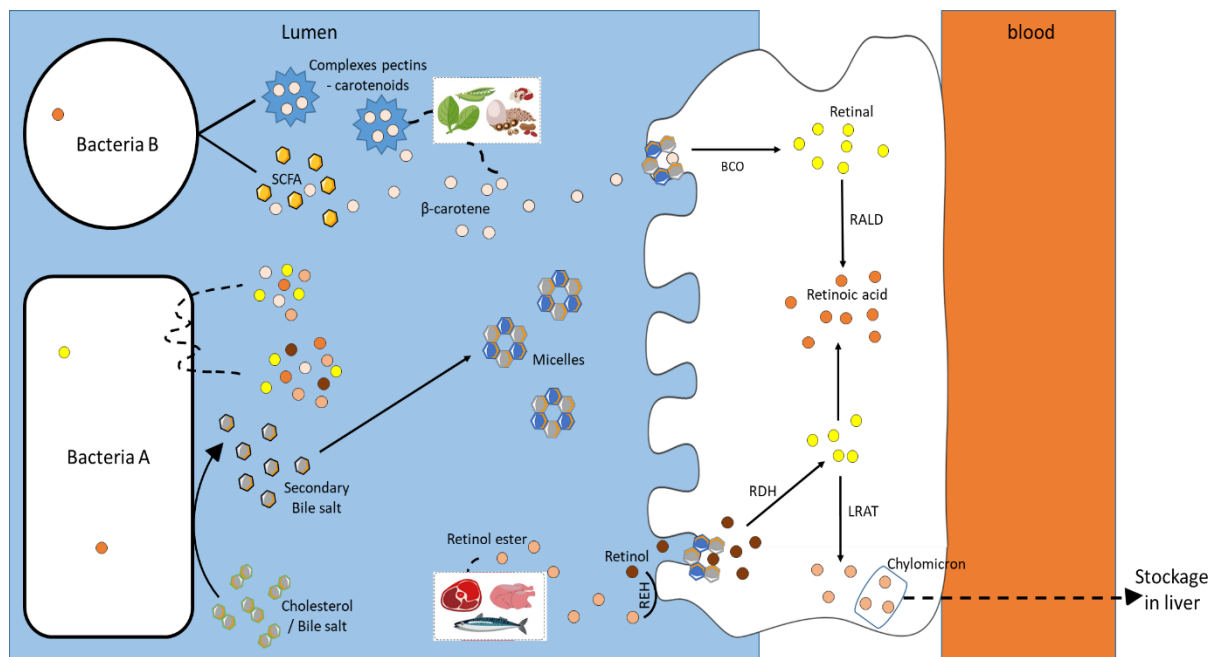


Figure 4. Schematic representation of the the gut microbiota in the intestinal absorption of vitamin A. REH : retinyl ester hydrolase ; RDH : retinol dehydrogenase ; BCO : Beta-carotene 15,15'-mono ou dioxigenase ; RALDH : Retinal dehydrogenase; LRAT : Lecithin retinol acyltransferase.

The bioavailability of carotenoids can be affected by various food components among which the cell wall structure and composition of vegetables in the food matrix which form a physical barrier, thus limiting the action of digestive enzymes (130). Pectins and some polyphenols such as naringenin have been shown to decrease the carotenoid bioavailability *in vitro* by binding cholesterol and bile salts in the lumen, and thus inhibiting the micelles formation (131). In contrast, pectins can be degraded by numerous commensal bacteria (132) and this degradation in the gut may increase the bioavailability of carotenoids. Also, the gut microbiota synthesizes enzymes or complement proteins, such as bacterial lipocalins, which could contribute to the transport of retinoid in enterocyte (133,134). Similarly, the intestinal microbiota can digest dietary fibers and thus release  $\beta$ -carotene or other entangled micronutrients (59).

Additionally, gut bacterial population can also regulate the host metabolism of retinol modulating the production of retinoic acid (RA) which drives the immune responses. Grizotte-Lake showed that bacteria of *Clostridium* class can regulate RA concentration in the gut epithelium by suppressing the gene *Rdh7* expression, which pilot the transformation of retinol into retinoic acid (135). Moreover, this study showed that commensal bacteria communities lower RA production which is balanced by an increase in the liver storage form and favour the RE pathways; whereas potentially pathogenic bacteria promoted RA production.

The availability of retinoids in the gut environment can modulate the microbial composition. For example, the gut microbial composition of vitamin A-deficient mice had lower abundance of bacteria of genus *Enterococcus*, *Lactobacillus* and of the species *Clostridium difficile* from the phylum Firmicutes, as well as species from the genus *Escherichia* from the Proteobacteria phylum compared to non-deficient mice (136). The effect of vitamin A deficiency on a model of initially germ-free mice inoculated with a community of bacterial species found in human gut, resulted in a strong increase of *Bacteroides vulgatus* proportion (87). In this last study, it was suggested that the modulation of gut

microbiota composition can be induced by the altered bile acid metabolism associated with acute vitamin A deficiency.

In mice, the proportion of Bacteroides members was lower in vitamin A deficient mice, and several class family and genus proportion were different between vitamin A deficient and vitamin A sufficient groups (23). In children with autism, vitamin A supplementation increased the proportion of bacteria from the order Bacteroidales and reduced the one of the genus *Bifidobacterium* (137). The modulation of gut microbiota composition by vitamin A can also serve as indirect pathway to modulate immune responses at the intestinal level. For example, according to experiments conducted in a mouse model, vitamin A can modulate the abundance of Segmented Filamentous Bacteria (SFB), a group of bacteria able to induce the differentiation of Th17 Cells (136,138).

## Conclusion

In summary, there is a lack of data on the effect of micronutrients (vitamin A, vitamin B9, iron and zinc) on gut microbial composition, but it seems likely that the interplay between micronutrients and the microbiome is an important factor in determining risk for vitamin and mineral deficiencies, and thus for global public health. Hence, policies designated to fight micronutrient deficiencies should start to take this compartment into consideration.

Given the huge differences among protocols, the outcomes measured and the interpretation of results, harmonization is urgently needed (106). Also, studies on micronutrient metabolism should be expanded to include the general gut microbial composition, not just pathogenic bacteria, as has long been done in the case of iron. Better cooperation between microbial scientists, food scientists, nutritional scientists, would create the opportunities to fight micronutrient deficiencies in an innovative way.

The effects of iron on the intestinal microbiota has been the most studied, as iron deficiency is likely the most widespread nutritional deficiency (29). But more studies into the effects of other micronutrients (vitamin A, zinc, B-vitamins) are needed, alone or in combination, to elucidate the interaction between micronutrients, the microbiome and the host.

## References

1. Gharibzahedi SMT, Jafari SM. The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. *Trends in Food Science & Technology*. 2017 Apr 1;62:119–32.
2. Shenkin A. Micronutrients in health and disease. *Postgraduate Medical Journal*. 2006 Sep 1;82(971):559–67.
3. Stein AJ, Qaim M. The Human and Economic Cost of Hidden Hunger. *Food Nutr Bull*. 2007 Jun 1;28(2):125–34.
4. Kennedy G, Nantel G, Shetty P. The scourge of “hidden hunger”: global dimensions of micronutrient deficiencies. 2003;
5. Bailey RL, West KP, Black RE. The epidemiology of global micronutrient deficiencies. *Ann Nutr Metab*. 2015;66 Suppl 2:22–33.
6. Bhutta ZA, Ahmed T, Black RE, Cousens S, Dewey K, Giugliani E, et al. What works? Interventions for maternal and child undernutrition and survival. *The Lancet*. 2008 Feb 2;371(9610):417–40.
7. Frazer DM, Anderson GJ. The regulation of iron transport. *BioFactors*. 2014;40(2):206–14.
8. Krebs NF. Overview of Zinc Absorption and Excretion in the Human Gastrointestinal Tract. *The Journal of Nutrition*. 2000 May 1;130(5):1374S-1377S.
9. Visentin M, Diop-Bove N, Zhao R, Goldman ID. The intestinal absorption of folates. *Annu Rev Physiol*. 2014;76:251–74.
10. Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. *Nature*. 2012 Sep;489(7415):242–9.

11. Clemente JC, Ursell LK, Parfrey LW, Knight R. The Impact of the Gut Microbiota on Human Health: An Integrative View. *Cell*. 2012 Mar 16;148(6):1258–70.
12. Gonzalez A, Clemente JC, Shade A, Metcalf JL, Song S, Prithiviraj B, et al. Our microbial selves: what ecology can teach us. *EMBO Rep*. 2011 Aug;12(8):775–84.
13. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The Human Microbiome Project. *Nature*. 2007 Oct 17;449:804–10.
14. Million M, Diallo A, Raoult D. Gut microbiota and malnutrition. *Microbial Pathogenesis*. 2017 May 1;106:127–38.
15. Subramanian S, Huq S, Yatsunenkov T, Haque R, Mahfuz M, Alam MA, et al. Persistent gut microbiota immaturity in malnourished Bangladeshi children. *Nature*. 2014 Jun 19;510(7505):417–21.
16. Subramanian S, Blanton LV, Frese SA, Charbonneau M, Mills DA, Gordon JI. Cultivating Healthy Growth and Nutrition through the Gut Microbiota. *Cell*. 2015 Mar;161(1):36–48.
17. Voreades N, Kozil A, Weir TL. Diet and the development of the human intestinal microbiome. *Front Microbiol* [Internet]. 2014 [cited 2019 Feb 1];5. Available from: <https://www.frontiersin.org/articles/10.3389/fmicb.2014.00494/full>
18. Conlon MA, Bird AR. The impact of diet and lifestyle on gut microbiota and human health. *Nutrients*. 2014 Dec 24;7(1):17–44.
19. Turnbaugh PJ, Gordon JI. The core gut microbiome, energy balance and obesity. *J Physiol (Lond)*. 2009 Sep 1;587(Pt 17):4153–8.
20. Andrews SC, Robinson AK, Rodríguez-Quinones F. Bacterial iron homeostasis. *FEMS Microbiol Rev*. 2003 Jun 1;27(2–3):215–37.

21. Basu TK, Donaldson D. Intestinal absorption in health and disease: micronutrients. *Best Practice & Research Clinical Gastroenterology*. 2003 Dec 1;17(6):957–79.
22. Welch RM, Graham RD. Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany*. 2004 Feb 1;55(396):353–64.
23. Tian Y, Nichols RG, Cai J, Patterson AD, Cantorna MT. Vitamin A deficiency in mice alters host and gut microbial metabolism leading to altered energy homeostasis. *The Journal of Nutritional Biochemistry*. 2018 Apr 1;54:28–34.
24. Zimmermann MB, Chassard C, Rohner F, N’goran EK, Nindjin C, Dostal A, et al. The effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Cote d’Ivoire. *Am J Clin Nutr*. 2010 Dec;92(6):1406–15.
25. Rossi M, Amaretti A, Raimondi S. Folate Production by Probiotic Bacteria. *Nutrients*. 2011 Jan;3(1):118–34.
26. Mallard SR, Houghton LA, Filteau S, Chisenga M, Siame J, Kasonka L, et al. Micronutrient Adequacy and Dietary Diversity Exert Positive and Distinct Effects on Linear Growth in Urban Zambian Infants. *J Nutr*. 2016 Oct;146(10):2093–101.
27. Biesalski HK. Hidden Hunger. In: Biesalski HK, editor. *Hidden Hunger* [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2013 [cited 2018 Dec 12]. p. 25–50. Available from: [https://doi.org/10.1007/978-3-642-33950-9\\_2](https://doi.org/10.1007/978-3-642-33950-9_2)
28. Allen L. *Guidelines on food fortification with micronutrients*. Geneva: World Health Organization [u.a.]; 2006.
29. Zimmermann MB, Hurrell RF. Nutritional iron deficiency. *The Lancet*. 2007 Aug 11;370(9586):511–20.

30. Miller JL. Iron deficiency anemia: a common and curable disease. *Cold Spring Harb Perspect Med.* 2013 Jul 1;3(7).
31. Petry N, Egli I, Zeder C, Walczyk T, Hurrell R. Polyphenols and phytic acid contribute to the low iron bioavailability from common beans in young women. *J Nutr.* 2010 Nov;140(11):1977–82.
32. Rivella S, Crielaard BJ. Disorders of Iron Metabolism: Iron Deficiency and Iron Overload and Anemia of Chronic Diseases. In: McManus LM, Mitchell RN, editors. *Pathobiology of Human Disease* [Internet]. San Diego: Academic Press; 2014 [cited 2021 Apr 27]. p. 1471–87. Available from: <https://www.sciencedirect.com/science/article/pii/B978012386456707903X>
33. Hambidge M. Human zinc deficiency. *J Nutr.* 2000;130(5S Suppl):1344S-9S.
34. Wessells KR, Brown KH. Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. *PLoS One.* 2012;7(11):e50568.
35. Hall JA, Cannons JL, Grainger JR, Dos Santos LM, Hand TW, Naik S, et al. Essential Role for Retinoic Acid in the Promotion of CD4+ T Cell Effector Responses via Retinoic Acid Receptor Alpha. *Immunity.* 2011 Mar 25;34(3):435–47.
36. Imdad A, Yakoob MY, Sudfeld C, Haider BA, Black RE, Bhutta ZA. Impact of vitamin A supplementation on infant and childhood mortality. *BMC Public Health.* 2011 Apr 13;11(Suppl 3):S20.
37. Bailey LB, Rampersaud GC, Kauwell GPA. Folic Acid Supplements and Fortification Affect the Risk for Neural Tube Defects, Vascular Disease and Cancer: Evolving Science. *J Nutr.* 2003 Jun 1;133(6):1961S-1968S.



38. Lm R, Am C, Cm P, Db H, Bl T, Lm D-R, et al. Global folate status in women of reproductive age: a systematic review with emphasis on methodological issues. *Ann N Y Acad Sci*. 2018 Sep 21;1431(1):35–57.
39. Seyoum Y, Baye K, Humblot C. Iron homeostasis in host and gut bacteria – a complex interrelationship. *Gut Microbes*. 2021 Jan 1;13(1):1–19.
40. Fuqua BK, Vulpe CD, Anderson GJ. Intestinal iron absorption. *Journal of Trace Elements in Medicine and Biology*. 2012 Jun 1;26(2):115–9.
41. Canonne-Hergaux F, Gruenheid S, Ponka P, Gros P. Cellular and subcellular localization of the Nramp2 iron transporter in the intestinal brush border and regulation by dietary iron. *Blood*. 1999 Jun 15;93(12):4406–17.
42. Bouglé D, Vaghefi-Vaezzadeh N, Roland N, Bouvard G, Arhan P, Bureau F, et al. Influence of Short-Chain Fatty Acids on Iron Absorption by Proximal Colon. *Scandinavian Journal of Gastroenterology*. 2002 Jan 1;37(9):1008–11.
43. Yeung CK, Glahn RE, Welch RM, Miller DD. Prebiotics and Iron Bioavailability—Is There a Connection? *Journal of Food Science*. 2005;70(5):R88–92.
44. Nemeth E, Ganz T. Regulation of iron metabolism by hepcidin. *Annu Rev Nutr*. 2006;26:323–42.
45. Nicolas G, Viatte L, Bennoun M, Beaumont C, Kahn A, Vaulont S. Hepcidin, A New Iron Regulatory Peptide. *Blood Cells, Molecules, and Diseases*. 2002 Nov 1;29(3):327–35.
46. Zaharik ML, Cullen VL, Fung AM, Libby SJ, Choy SLK, Coburn B, et al. The *Salmonella enterica* Serovar Typhimurium Divalent Cation Transport Systems MntH and SitABCD Are Essential for Virulence in an Nramp1G169 Murine Typhoid Model. *Infection and Immunity*. 2004 Sep 1;72(9):5522–5.

47. Vega-Bautista A, de la Garza M, Carrero JC, Campos-Rodríguez R, Godínez-Victoria M, Drago-Serrano ME. The Impact of Lactoferrin on the Growth of Intestinal Inhabitant Bacteria. *Int J Mol Sci*. 2019 Sep 23;20(19).
48. Chu BC, Garcia-Herrero A, Johanson TH, Krewulak KD, Lau CK, Peacock RS, et al. Siderophore uptake in bacteria and the battle for iron with the host; a bird's eye view. *Biometals*. 2010 Aug;23(4):601–11.
49. Troxell B, Hassan HM. Transcriptional regulation by Ferric Uptake Regulator (Fur) in pathogenic bacteria. *Front Cell Infect Microbiol*. 2013;3:59.
50. Lau CKY, Krewulak KD, Vogel HJ. Bacterial ferrous iron transport: the Feo system. *FEMS Microbiology Reviews*. 2016 Mar 1;40(2):273–98.
51. Banci L, Bertini I. Metallomics and the Cell: Some Definitions and General Comments. In: Banci L, editor. *Metallomics and the Cell* [Internet]. Dordrecht: Springer Netherlands; 2013 [cited 2021 May 13]. p. 1–13. (Metal Ions in Life Sciences). Available from: [https://doi.org/10.1007/978-94-007-5561-1\\_1](https://doi.org/10.1007/978-94-007-5561-1_1)
52. Archibald F. *Lactobacillus plantarum*, an organism not requiring Iron. *FEMS Microbiology Letters*. 2006 Mar 27;19:29–32.
53. Duhutrel P, Bordat C, Wu T-D, Zagorec M, Guerquin-Kern J-L, Champomier-Vergès M-C. Iron sources used by the nonpathogenic lactic acid bacterium *Lactobacillus sakei* as revealed by electron energy loss spectroscopy and secondary-ion mass spectrometry. *Appl Environ Microbiol*. 2010 Jan;76(2):560–5.
54. Verplaetse E, André-Leroux G, Duhutrel P, Coeuret G, Chaillou S, Nielsen-Leroux C, et al. Heme Uptake in *Lactobacillus sakei* Evidenced by a New Energy Coupling Factor (ECF)-Like

Transport System. *Appl Environ Microbiol* [Internet]. 2020 Sep 1 [cited 2021 May 13];86(18). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7480364/>

55. O'Hara AM, Shanahan F. The gut flora as a forgotten organ. *EMBO Rep*. 2006 Jul;7(7):688–93.
56. McCance RA, Widdowson EM. The absorption and excretion of iron following oral and intravenous administration. *J Physiol*. 1938 Oct 14;94(1):148–54.
57. Reddy NR, Sathé SK. *Food Phytates*. CRC Press; 2001. 282 p.
58. Stentz R, Osborne S, Horn N, Li AWH, Hautefort I, Bongaerts R, et al. A Bacterial Homolog of a Eukaryotic Inositol Phosphate Signaling Enzyme Mediates Cross-kingdom Dialog in the Mammalian Gut. *Cell Reports*. 2014 Feb 27;6(4):646–56.
59. Biesalski HK. Nutrition meets the microbiome: micronutrients and the microbiota. *Annals of the New York Academy of Sciences*. 2016;1372(1):53–64.
60. Alexander C, Swanson KS, Fahey GC Jr, Garleb KA. Perspective: Physiologic Importance of Short-Chain Fatty Acids from Nondigestible Carbohydrate Fermentation. *Advances in Nutrition*. 2019 Jul 1;10(4):576–89.
61. Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, et al. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestering iron. *Nature*. 2004 Dec 16;432(7019):917–21.
62. Kramer J, Özkaya Ö, Kümmerli R. Bacterial siderophores in community and host interactions. *Nature Reviews Microbiology*. 2020 Mar;18(3):152–63.
63. Lechardeur D, Cesselin B, Liebl U, Vos MH, Fernandez A, Brun C, et al. Discovery of Intracellular Heme-binding Protein HrtR, Which Controls Heme Efflux by the Conserved

- HrtB-HrtA Transporter in *Lactococcus lactis*\*. *Journal of Biological Chemistry*. 2012 Feb 1;287(7):4752–8.
64. Muleviciene A, D'Amico F, Turrone S, Candela M, Jankauskiene A. Iron deficiency anemia-related gut microbiota dysbiosis in infants and young children: A pilot study. *Acta Microbiologica et Immunologica Hungarica*. 2018 Dec;65(4):551–64.
65. Baldi AJ, Larson LM, Pasricha S-R. Balancing Safety and Potential for Impact in Universal Iron Interventions. *Global Landscape of Nutrition Challenges in Infants and Children*. 2020;93:51–62.
66. Latunde-Dada GO, Aslam MF, Ellis PR, Berry SE, Sharp PA. Chapter 39 - Wheat Flour Fortification to Prevent Iron-Deficiency Anemia. In: Preedy VR, Watson RR, editors. *Flour and Breads and their Fortification in Health and Disease Prevention (Second Edition)* [Internet]. Academic Press; 2019 [cited 2021 Apr 28]. p. 485–91. Available from: <https://www.sciencedirect.com/science/article/pii/B9780128146392000393>
67. Powell JJ, Bruggraber SFA, Faria N, Poots LK, Hondow N, Pennycook TJ, et al. A nano-disperse ferritin-core mimetic that efficiently corrects anemia without luminal iron redox activity. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2014 Oct 1;10(7):1529–38.
68. Jaeggi T, Kortman GAM, Moretti D, Chassard C, Holding P, Dostal A, et al. Iron fortification adversely affects the gut microbiome, increases pathogen abundance and induces intestinal inflammation in Kenyan infants. *Gut*. 2015 May 1;64(5):731–42.
69. Paganini D, Uyoga MA, Kortman GAM, Cercamondi CI, Moretti D, Barth-Jaeggi T, et al. Prebiotic galacto-oligosaccharides mitigate the adverse effects of iron fortification on the gut microbiome: a randomised controlled study in Kenyan infants. *Gut*. 2017 Nov 1;66(11):1956–67.

70. Dostal A, Baumgartner J, Riesen N, Chassard C, Smuts CM, Zimmermann MB, et al. Effects of iron supplementation on dominant bacterial groups in the gut, faecal SCFA and gut inflammation: a randomised, placebo-controlled intervention trial in South African children. *Br J Nutr*. 2014 Aug 28;112(4):547–56.
71. Kortman GAM, Mulder MLM, Richters TJW, Shanmugam NKN, Trebicka E, Boekhorst J, et al. Low dietary iron intake restrains the intestinal inflammatory response and pathology of enteric infection by food-borne bacterial pathogens. *Eur J Immunol*. 2015 Sep;45(9):2553–67.
72. Kortman GAM, Dutilh BE, Maathuis AJH, Engelke UF, Boekhorst J, Keegan KP, et al. Microbial Metabolism Shifts Towards an Adverse Profile with Supplementary Iron in the TIM-2 In vitro Model of the Human Colon. *Front Microbiol* [Internet]. 2016 [cited 2019 Nov 12];6. Available from: <https://www.frontiersin.org/articles/10.3389/fmicb.2015.01481/full>
73. Falchuk KH. The molecular basis for the role of zinc in developmental biology. In: Pierce GN, Izumi T, Rupp H, Grynberg A, editors. *Molecular and Cellular Effects of Nutrition on Disease Processes* [Internet]. Boston, MA: Springer US; 1998 [cited 2021 May 10]. p. 41–8. (Developments in Molecular and Cellular Biochemistry). Available from: [https://doi.org/10.1007/978-1-4615-5763-0\\_5](https://doi.org/10.1007/978-1-4615-5763-0_5)
74. Zhu W, Spiga L, Winter S. Transition metals and host-microbe interactions in the inflamed intestine. *Biometals*. 2019 Jun;32(3):369–84.
75. Maares M, Haase H. A Guide to Human Zinc Absorption: General Overview and Recent Advances of In Vitro Intestinal Models. *Nutrients* [Internet]. 2020 Mar 13 [cited 2021 May 13];12(3). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7146416/>
76. Kortman GAM, Raffatellu M, Swinkels DW, Tjalsma H. Nutritional iron turned inside out: intestinal stress from a gut microbial perspective. *FEMS Microbiol Rev*. 2014 Nov 1;38(6):1202–34.

77. Gopalsamy GL, Alpers DH, Binder HJ, Tran CD, Ramakrishna BS, Brown I, et al. The Relevance of the Colon to Zinc Nutrition. *Nutrients*. 2015 Jan;7(1):572–83.
78. Capdevila DA, Wang J, Giedroc DP. Bacterial Strategies to Maintain Zinc Metallostasis at the Host-Pathogen Interface\*. *Journal of Biological Chemistry*. 2016 Sep 1;291(40):20858–68.
79. Liu JZ, Jellbauer S, Poe AJ, Ton V, Pesciaroli M, Kehl-Fie TE, et al. Zinc Sequestration by the Neutrophil Protein Calprotectin Enhances Salmonella Growth in the Inflamed Gut. *Cell Host & Microbe*. 2012 Mar 15;11(3):227–39.
80. King JC, Shames DM, Woodhouse LR. Zinc Homeostasis in Humans. *The Journal of Nutrition*. 2000 May 1;130(5):1360S-1366S.
81. Souffriau J, Libert C. Mechanistic insights into the protective impact of zinc on sepsis. *Cytokine & Growth Factor Reviews*. 2018 Feb 1;39:92–101.
82. Hojberg O, Canibe N, Poulsen HD, Hedemann MS, Jensen BB. Influence of Dietary Zinc Oxide and Copper Sulfate on the Gastrointestinal Ecosystem in Newly Weaned Piglets. *Applied and Environmental Microbiology*. 2005 May 1;71(5):2267–77.
83. Pieper R, Vahjen W, Neumann K, Van Kessel AG, Zentek J. Dose-dependent effects of dietary zinc oxide on bacterial communities and metabolic profiles in the ileum of weaned pigs. *Journal of Animal Physiology and Animal Nutrition*. 2012 Oct 1;96(5):825–33.
84. Starke IC, Pieper R, Neumann K, Zentek J, Vahjen W. The impact of high dietary zinc oxide on the development of the intestinal microbiota in weaned piglets. *FEMS Microbiol Ecol*. 2014 Feb 1;87(2):416–27.
85. Vahjen W, Pieper R, Zentek J. Increased dietary zinc oxide changes the bacterial core and enterobacterial composition in the ileum of piglets. *J Anim Sci*. 2011 Aug 1;89(8):2430–9.

86. Reed S, Knez M, Uzan A, Stangoulis JCR, Glahn RP, Koren O, et al. Alterations in the Gut (Gallus gallus) Microbiota Following the Consumption of Zinc Biofortified Wheat (Triticum aestivum)-Based Diet. *J Agric Food Chem*. 2018 Jun 27;66(25):6291–9.
87. Hibberd MC, Wu M, Rodionov DA, Li X, Cheng J, Griffin NW, et al. The effects of micronutrient deficiencies on bacterial species from the human gut microbiota. *Science Translational Medicine*. 2017 May 17;9(390):eaal4069.
88. Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, et al. Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr*. 2018;57(1):1–24.
89. Duthie SJ, Narayanan S, Brand GM, Pirie L, Grant G. Impact of Folate Deficiency on DNA Stability. *The Journal of Nutrition*. 2002 Aug 1;132(8):2444S-2449S.
90. Hosomi K, Kunisawa J. The Specific Roles of Vitamins in the Regulation of Immunosurveillance and Maintenance of Immunologic Homeostasis in the Gut. *Immune Netw*. 2017 Feb;17(1):13–9.
91. Said HM. Intestinal absorption of water-soluble vitamins in health and disease. *Biochemical Journal*. 2011 Jul 13;437(3):357–72.
92. Maynard C, Cummins I, Green J, Weinkove D. A bacterial route for folic acid supplementation. *BMC Biol*. 2018 Jun 15;16(1):67.
93. Jaehme M, Slotboom DJ. Diversity of membrane transport proteins for vitamins in bacteria and archaea. *Biochimica et Biophysica Acta (BBA) - General Subjects*. 2015 Mar 1;1850(3):565–76.
94. Putnam EE, Goodman AL. B vitamin acquisition by gut commensal bacteria. *PLOS Pathogens*. 2020 Jan 23;16(1):e1008208.

95. Magnúsdóttir S, Ravcheev D, de Crécy-Lagard V, Thiele I. Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes. *Front Genet* [Internet]. 2015 [cited 2020 Nov 4];6. Available from: <https://www.frontiersin.org/articles/10.3389/fgene.2015.00148/full>
96. Denko CW, Grundy WE. The excretion of B-complex vitamins in the urine and feces of seven normal adults. *Arch Biochem*. 1946 May;10:33–40.
97. Girdwood RH. The intestinal content in pernicious anemia of factors for the growth of *Streptococcus faecalis* and *Lactobacillus leichmannii*. *Blood*. 1950 Nov;5(11):1009–16.
98. Aufreiter S, Kim JH, O'Connor DL. Dietary Oligosaccharides Increase Colonic Weight and the Amount but Not Concentration of Bacterially Synthesized Folate in the Colon of Piglets. *J Nutr*. 2011 Mar 1;141(3):366–72.
99. Sepehr E, Peace RW, Storey KB, Jee P, Lampi BJ, Brooks SPJ. Folate derived from cecal bacterial fermentation does not increase liver folate stores in 28-d folate-depleted male Sprague-Dawley rats. *J Nutr*. 2003 May;133(5):1347–54.
100. Lakoff A, Fazili Z, Aufreiter S, Pfeiffer CM, Connolly B, Gregory JF, et al. Folate is absorbed across the human colon: evidence by using enteric-coated caplets containing <sup>13</sup>C-labeled [6S]-5-formyltetrahydrofolate. *Am J Clin Nutr*. 2014 Nov;100(5):1278–86.
101. Wright AJA, Finglas PM, Dainty JR, Hart DJ, Wolfe CA, Southon S, et al. Single oral doses of <sup>13</sup>C forms of pteroylmonoglutamic acid and 5-formyltetrahydrofolic acid elicit differences in short-term kinetics of labelled and unlabelled folates in plasma: potential problems in interpretation of folate bioavailability studies. *British Journal of Nutrition*. 2003 Aug;90(2):363–71.



102. LeBlanc JG, Milani C, de Giori GS, Sesma F, van Sinderen D, Ventura M. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Current Opinion in Biotechnology*. 2013 Apr 1;24(2):160–8.
103. Strozzi GP, Mogna L. Quantification of Folic Acid in Human Feces After Administration of Bifidobacterium Probiotic Strains. *Journal of Clinical Gastroenterology*. 2008 Sep;42:S179.
104. Tamene A, Baye K, Kariluoto S, Edelmann M, Bationo F, Leconte N, et al. Lactobacillus plantarum P2R3FA Isolated from Traditional Cereal-Based Fermented Food Increase Folate Status in Deficient Rats. *Nutrients*. 2019 Nov;11(11):2819.
105. Kim TH, Yang J, Darling PB, O'Connor DL. A large pool of available folate exists in the large intestine of human infants and piglets. *J Nutr*. 2004 Jun;134(6):1389–94.
106. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. *Nature*. 2012 Jun;486(7402):222–7.
107. Gehrig JL, Venkatesh S, Chang H-W, Hibberd MC, Kung VL, Cheng J, et al. Effects of microbiota-directed foods in gnotobiotic animals and undernourished children. *Science*. 2019 12;365(6449).
108. Sharma V, Rodionov DA, Leyn SA, Tran D, Iablokov SN, Ding H, et al. B-Vitamin Sharing Promotes Stability of Gut Microbial Communities. *Front Microbiol* [Internet]. 2019 [cited 2021 May 12];10. Available from: <https://www.frontiersin.org/articles/10.3389/fmicb.2019.01485/full>
109. Rodionov DA, Arzamasov AA, Khoroshkin MS, Iablokov SN, Leyn SA, Peterson SN, et al. Micronutrient Requirements and Sharing Capabilities of the Human Gut Microbiome. *Front*

Microbiol [Internet]. 2019 [cited 2021 Apr 26];10. Available from: <https://www.frontiersin.org/articles/10.3389/fmicb.2019.01316/full>

110. Blomhoff R, Blomhoff HK. Overview of retinoid metabolism and function. *Journal of Neurobiology*. 2006;66(7):606–30.
111. Reboul E. Absorption of Vitamin A and Carotenoids by the Enterocyte: Focus on Transport Proteins. *Nutrients*. 2013 Sep 12;5(9):3563–81.
112. Czarnewski P, Das S, Parigi SM, Villablanca EJ. Retinoic Acid and Its Role in Modulating Intestinal Innate Immunity. *Nutrients*. 2017 Jan;9(1):68.
113. Coombes JL, Siddiqui KRR, Arancibia-Cárcamo CV, Hall J, Sun C-M, Belkaid Y, et al. A functionally specialized population of mucosal CD103<sup>+</sup> DCs induces Foxp3<sup>+</sup> regulatory T cells via a TGF- $\beta$ - and retinoic acid-dependent mechanism. *Journal of Experimental Medicine*. 2007 Jul 9;204(8):1757–64.
114. Artis D. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nat Rev Immunol*. 2008 Jun;8(6):411–20.
115. Yu Y, Sitaraman S, Gewirtz AT. Intestinal epithelial cell regulation of mucosal inflammation. *Immunol Res*. 2004 Jun 1;29(1):55–67.
116. Kunzelmann K, Mall M. Electrolyte Transport in the Mammalian Colon: Mechanisms and Implications for Disease. *Physiological Reviews*. 2002 Jan 1;82(1):245–89.
117. Rescigno M. The intestinal epithelial barrier in the control of homeostasis and immunity. *Trends in Immunology*. 2011 Jun 1;32(6):256–64.

118. Borel P. Factors affecting intestinal absorption of highly lipophilic food microconstituents (fat-soluble vitamins, carotenoids and phytosterols). *Clin Chem Lab Med*. 2003 Aug;41(8):979–94.
119. Reboul E, Berton A, Moussa M, Kreuzer C, Crenon I, Borel P. Pancreatic lipase and pancreatic lipase-related protein 2, but not pancreatic lipase-related protein 1, hydrolyze retinyl palmitate in physiological conditions. *Biochim Biophys Acta*. 2006 Jan;1761(1):4–10.
120. Tyssandier V, Reboul E, Dumas J-F, Bouteloup-Demange C, Armand M, Marcand J, et al. Processing of vegetable-borne carotenoids in the human stomach and duodenum. *Am J Physiol Gastrointest Liver Physiol*. 2003 Jun;284(6):G913-923.
121. Rigtrup KM, Kakkad B, Ong DE. Purification and partial characterization of a retinyl ester hydrolase from the brush border of rat small intestine mucosa: probable identity with brush border phospholipase B. *Biochemistry*. 1994 Mar 8;33(9):2661–6.
122. Dhuique-Mayer C, Borel P, Reboul E, Caporiccio B, Besancon P, Amiot M-J. Beta-cryptoxanthin from citrus juices: assessment of bioaccessibility using an in vitro digestion/Caco-2 cell culture model. *Br J Nutr*. 2007 May;97(5):883–90.
123. Lobo GP, Amengual J, Palczewski G, Babino D, von Lintig J. Carotenoid-oxygenases: Key Players for Carotenoid Function and Homeostasis in Mammalian Biology. *Biochim Biophys Acta*. 2012 Jan;1821(1):78–87.
124. Harrison EH. Mechanisms of digestion and absorption of dietary vitamin A. *Annu Rev Nutr*. 2005 Aug 21;25(1):87–103.
125. Gérard P. Metabolism of Cholesterol and Bile Acids by the Gut Microbiota. *Pathogens*. 2014 Mar;3(1):14–24.

126. Sadeghpour A, Rappolt M, Misra S, Kulkarni CV. Bile Salts Caught in the Act: From Emulsification to Nanostructural Reorganization of Lipid Self-Assemblies. *Langmuir*. 2018 Nov 13;34(45):13626–37.
127. Srinivasan V. Probiotics in Pediatric Severe Sepsis: The Time Has Come to Trust Our ‘Gut’! *Crit Care Med*. 2018 Oct;46(10):1707–8.
128. Wassef L, Wirawan R, Chikindas M, Breslin PAS, Hoffman DJ, Quadro L.  $\beta$ -Carotene-Producing Bacteria Residing in the Intestine Provide Vitamin A to Mouse Tissues In Vivo. *J Nutr*. 2014 May 1;144(5):608–13.
129. Culligan EP, Sleator RD, Marchesi JR, Hill C. Metagenomic Identification of a Novel Salt Tolerance Gene from the Human Gut Microbiome Which Encodes a Membrane Protein with Homology to a brp/blh-Family  $\beta$ -Carotene 15,15'-Monooxygenase. *PLOS ONE*. 2014 Jul 24;9(7):e103318.
130. Tydeman EA, Parker ML, Wickham MSJ, Rich GT, Faulks RM, Gidley MJ, et al. Effect of Carrot (*Daucus carota*) Microstructure on Carotene Bioaccessibility in the Upper Gastrointestinal Tract. 1. In Vitro Simulations of Carrot Digestion. *J Agric Food Chem*. 2010 Sep 8;58(17):9847–54.
131. Verrijssen T, Balduyck L, Christiaens S, Van Loey A, Van Buggenhout S, Hendrickx M. The effect of pectin concentration and degree of methyl-esterification on the in vitro bioaccessibility of carotenoid-enriched emulsions. *Food Research International*. 2014;57:71–8.
132. Fukunaga T, Sasaki M, Araki Y, Okamoto T, Yasuoka T, Tsujikawa T, et al. Effects of the soluble fibre pectin on intestinal cell proliferation, fecal short chain fatty acid production and microbial population. *Digestion*. 2003;67(1–2):42–9.

133. Bishop RE, Cambillau C, Privé GG, Hsi D, Tillo D, Tillier ERM. Bacterial Lipocalins: Origin, Structure, and Function [Internet]. Madame Curie Bioscience Database [Internet]. Landes Bioscience; 2013 [cited 2021 Mar 24]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK6135/>
134. Åkerstrom B, Flower DR, Salier JP. Lipocalins: unity in diversity. *Biochimica et Biophysica Acta, Protein Structure and Molecular Enzymology*. 2000;1482(1/2):1–8.
135. Grizotte-Lake M, Zhong G, Duncan K, Kirkwood J, Iyer N, Smolenski I, et al. Commensals Suppress Intestinal Epithelial Cell Retinoic Acid Synthesis to Regulate Interleukin-22 Activity and Prevent Microbial Dysbiosis. *Immunity*. 2018 Dec 18;49(6):1103-1115.e6.
136. Cha H-R, Chang S-Y, Chang J-H, Kim J-O, Yang J-Y, Kim C-H, et al. Downregulation of Th17 Cells in the Small Intestine by Disruption of Gut Flora in the Absence of Retinoic Acid. *The Journal of Immunology*. 2010 Jun 15;184(12):6799–806.
137. Liu J, Liu X, Xiong X-Q, Yang T, Cui T, Hou N-L, et al. Effect of vitamin A supplementation on gut microbiota in children with autism spectrum disorders - a pilot study. *BMC Microbiol* [Internet]. 2017 Sep 22 [cited 2019 Jul 15];17. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5610466/>
138. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. *Cell*. 2009 Oct 30;139(3):485–98.