Non-Digestible Food Ingredients, Colonic Microbiota and the Impact on Gut Health and Immunity: A Role for Metabolomics

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Abstract: Increasing health issues related to immune and gut function such as inflammatory disorders, resistance to infections and metabolic syndrome demand modern analytical approaches to accelerate nutritional research aimed at health promotion and disease prevention. Gut microbial-human mutualism endows the host ‘superorganism’ with a fitness advantage including nutritional, immune and intestinal health aspects. The gut microbiome enlarges our genome and enhances our metabolic potential. Dietary modulation can significantly alter the microbiota community and metabolic activity, and consequently impacts on nutrient bioavailability and host metabolism. Although in an early stage, microbial metabolites generated during colonic fermentation of food stuffs may have beneficial or deleterious effects on intestinal health and immunity, as summarized in this review. However, current evidence is largely based on in vitro and animal studies while substantiation in humans is lacking. The challenge to establish coherent links between the bioconversion of non-digestible food ingredients, their bioavailability and their downstream effects on the host metabolism may be achieved by metabolomics. In this review, metabolomics studies focusing on microbe-host mutualism have demonstrated that metabolomics is capable of detecting and tracking diverse microbial metabolites from different non-digestible food ingredients, of discriminating between phenotypes with different inherent microbiota and of potentially diagnosing infection and gastrointestinal diseases. Integrative approaches such as the combined analysis of the metabolome in different biofluids together with other ‘omics technologies will cover exogenous and endogenous effects and hence show promise to generate novel hypotheses for innovative functional foods impacting gut health and immunity.

Keywords: Metabolomics, colonic microbiota, immunity, intestine, non-digestible food ingredients, microbial metabolites, short chain fatty acids, polyphenols, phenolic acids.

INTRODUCTION

Within the last few years advances in microbial ecology, immunology and metabolomics have shown that the contribution of the intestinal microbiota to the overall health status of the host has been underestimated. The profound impact on our health should not surprise us considering we have co-evolved together with our intestinal microbes over millions of years, and these have been programmed to manipulate networks of genes [1,2]. Our digestive tract offers a nutrient rich and relatively non-hostile environment to trillions of gut microbes. Humans benefit from this mutualistic relationship whereby the microbiota contribute to host health by performing roles in host nutrition, immune modulation, pathogen colonization resistance, intestinal epithelial development and activity, and energy metabolism [3,4].

The presence of the gut microbiota is generally highly valuable for the development and maintenance of gut and immune health which is especially apparent in studies with germ-free animals, whereby the mucosal associated lymphoid tissue remains underdeveloped, cell mediated immunity is defective, and mucosal cell turnover and cytokine production is reduced amongst other effects [5-7]. Moreover, the recognition of the commensal microbiota by Toll-like receptor (TLRs), a family of host pattern recognition receptors that recognize pathogens, plays a crucial role in the maintenance of intestinal homeostasis and protection from injury [8]. At the interface between the gut microbes with the luminal contents and the host tissues, the intestinal epithelium must integrate pro- and anti-inflammatory signals to regulate innate and adaptive immune responses [9,10]. The healthy intestinal microbiota and luminal contents essentially prime the mucosal immune response and keep it in a state of “controlled physiological inflammation” [10].

Increasingly novel insights reveal cell signaling between the microbiota, the epithelium cells and the mucosal immune system as well as how the disturbance of this dialogue may contribute to chronic and physiological inflammation [10-12]. Nevertheless, there are still numerous gaps in our understanding of the mechanisms underlying this interaction, as well as the systemic effects of gut microbial activity on the immune system. Diet is known to be an important factor in modulating the immunocompetence of an individual [13,14]. Consequently, understanding the role of the human gut microbiota in relation to diet and the immune system is essential to support the design of functional foods or novel nutritional strategies that will maintain and promote health.

In this review, the current evidence on the impact of microbial metabolites from non-digestible food ingredients on gut and immune function is summarized. From this follows that the development of nutritionally enhanced or functional foods requires more understanding of the mechanisms of action, the identification of biological actives, and demonstration of efficacy of these actives. In this respect, metabolomics provides a systems approach to understanding metabolic regulation of the host with its commensal microbiota [15,16]. Here, we review metabonomic studies focusing on the microbe-host mutualism and describe how the metabolomics approach may be applied to advance our understanding of the interactions between the microbiota and the host in particular with respect to gut and immune health.

THE GUT MICROBIOTA

An estimated $10^{13}$ intestinal microorganisms, collectively termed the gut microbiota, are distributed along the human intestinal tract and reach a remarkable density in the colon. The sheer complexity of the gut ecosystem has required the development of modern molecular biology techniques as most of the microbes present have not yet been cultured and are only known as a result of their detection via 16S ribosomal RNA (rRNA) or DNA (rDNA). The number of different species comprising the human gut microbiota is controversial with many authors referring to previous estimates of 400-500 species based on culture studies, while more recent estimates are reaching into the thousands [1,17]. A combination of sequence analysis of 16S rRNA gene- and metagenomic-libraries, and fluorescent in situ hybridization approaches targeting...
the 16S RNA has shown that the most abundant bacterial groups in the human intestine belong to, in order of numerical importance, the phyla of the **Firmicutes** (including the large class of **Clostridia** and the lactic acid bacteria), and **Bacteroidetes** which dominate the ecosystem, followed by, **Actinobacteria** (including **Collinsella** and **Bifidobacterium** spp.), **Proteobacteria**, and **Archea**, and finally bacteriophages that outnumber the microbes by 10 to 1 (Table 1) [18-21].

Currently, members of the genera bifidobacteria and lactobacilli are the main members of the gut microbiota which are recognised as being beneficial for health and this is addressed in previous reviews [22,23]. There is considerable circumstantial evidence that colonic bifidobacteria impact positively on the host via various mechanisms and consequently bifidobacteria are the target of probiotic functional foods and supplements. A prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating growth and/or modifying the metabolic activity of one or a limited number of bacterial species in the colon that have the potential to improve host health [24]. Furthermore, specific probiotic strains, often of the **Bifidobacterium** and **Lactobacillus** genera, may be ingested in food or in supplements, and there are some convincing interventions which indicate positive health effects on the host for those specific strains [25]. Probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” [26].

In accordance with a common microbial diversity at the division level, a core human microbiome, i.e. collection of gut microbiota genomes, with a high functional uniformity is present in the vast majority of individuals once they are weaned [27,28]. Nevertheless, the gut microbiota of each individual is unique and is influenced by a combination of factors, including the legacy acquired during birth, genotype of the individual, host physiological status, lifestyle and diet [21,29,30]. The assembly of the gut microbiota commences at birth and is initially simple and unstable. Post weaning a highly complex microbiota arises which is relatively stable during a considerable part of human lifespan.

Although in its infancy, several recent metagenomic studies show imbalances in the diversity of the microbiota in disorders which have an immune or inflammatory aspect (reviewed by [31]). Metagenomics is the study of microbial communities through sequence-based, compositional and/or functional analyses of all the combined microbial genomes contained within an environmental sample. Diverse conditions such as antibiotic-associated diarrhoea, Crohn’s disease, ulcerative colitis, obesity, and poutchitis have been correlated with large-scale imbalances in gastrointestinal microbiota, or ‘dysbiosis’. Compositional metagenomic studies involving healthy individuals as well as with inflammatory bowel disease (IBD), including ulcerative colitis and Crohn’s disease, revealed statistically different gut microbiota, with reduced complexity of the bacterial phyla **Firmicutes**, **Bacteroidetes** and **Lachnospiraceae** as well as over-representation of species such as uncultured Porphyromonadaceae as a signature of the faecal microbiota in these patients [32,33]. Studies with obese subjects (note obesity is currently viewed as an inflammatory disease [34]) demonstrated a lower abundance of **Firmicutes** and a higher level of Bacteroides [33,35]. The fecal microbial communities in patients with recurrent antibiotic-associated diarrhoea due to **Clostridium difficile** were highly variable in bacterial composition and were also characterized by markedly decreased diversity [36]. Addressing these large-scale imbalances in gut microbial ecology may play a role in ameliorating these conditions.

The gut microbiome extends our own genome, and greatly enhances our metabolic potential such that we may be described as a human-microbial superorganism [37,38]. Superorganism metabo-

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<th>Major Phyla</th>
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<td><strong>Bacteroidetes</strong></td>
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*The fermentation end-products are only indicative of some cultured representatives of a family as the vast majority has not been isolated and researched; N.R. not reported
lism involves integration of metabolic processes encoded by the human host with those of the microbiome which results in transgenonomic co-metabolism of dietary compounds. Dietary modulation of the gut microbiota can dramatically alter the microbiota community and its activity, and consequently, nutrient bioavailability and metabolism.

**COLONIC MICROBIOTA METABOLITES FROM FOOD INGREDIENTS**

Food passes relatively quickly through the stomach and the small intestine. Transit of undigested and indigestible food components through the colon is much slower allowing for the development of diverse microbiota in the large intestine. Microbial metabolism in the colon is influenced by the amount and type of dietary compounds that survive small intestinal digestion. In general, microbial metabolites including digested dietary compounds absorbed by the gut as well as non-nutrient compounds produced by the microbiota are co-metabolized by host enzymes in the liver. These modified metabolites are returned to the colon by the bile and possibly by other secretory fluids for further metabolism and/or excretion. The commensal-derived metabolites can have various effects on the host immune system [39]. Fig. (1) and Fig. (2a) describe schematically the metabolic route of non-digestible food ingredients in humans and the potential impact of microbial metabolites on gut and immune function. Disturbances in the homeostasis between bacteria- and host-derived signals at the epithelial cell level can lead to breaching of the intestinal barrier function and to the development of mucosal immune disorders in genetically susceptible hosts [10].

![Fig. (1). Scheme describing the potential impact of non-digestible food ingredients on the gut and immune function through the production of microbial metabolites.](image)

The actual number of metabolites produced by diverse gut microorganisms is not known due to the incomplete annotation of the microbial genome sequences and their functions. Likewise, among the hundreds of already identified metabolites, the number of metabolites that have the ability to exert an effect on the host is also unknown but is assumed to be limited considering that many metabolites are present only transiently and in very low concentra-

**Microbial Metabolites from Non-Digestible Food Ingredients**

**Fig. (2).** (a) Generalized scheme of metabolic route of non-digestible food ingredients in humans. Microbial metabolites originating from diverse non-digestible food ingredients are excreted in faeces and/or further co-metabolized by the host to commensal metabolites which can be excreted in urine. The bioconversion of these ingredients can be traced by integrating the information present in human faecal water extract and urine, as well as in supernatants from *in vitro* faecal batch fermentation. Furthermore, the combination of different profiling techniques allows for monitoring a wide-range of different compounds in parallel for zooming into specific compound classes. This approach is in particular useful for following the bioconversion of natural mixtures or a composition of different functional food ingredients. Typical NMR-based metabolite profiles (b) and GC-MS-based profiles of phenolic acids (c) from urine, faecal water extract and supernatants from *in vitro* batch fermentation are displayed exemplarily for the bioconversion of polyphenols. (PPA: 3-phenylpropionic acid; 3HPPA: 3-(3-hydroxyphenyl)propionic acid; 4HPPA: 3-(4-hydroxyphenyl)propionic acid; 3HPAA: 3-hydroxyphenylacetic acid; 4HPAA: 4-hydroxyphenylacetic acid; VA: vanillic acid; HA: hippuric acid; SA: syringic acid; 3HHA: 3-hydroxyhippuric acid; 4HHA: 4-hydroxyhippuric acid; AC: acetic acid; PA: propionic acid; BA: butyric acid; ALA: alanine).
substrates to specific metabolic patterns [42]. Nevertheless, there is substantial inter-individual variation between the gut microbiota of different individuals and these global, regional and personal differences are likely to lead to significant differences in response to dietary actives that may impact on health [21,29]. For example, the inability of some subjects to produce equol, a fermentation product of soy-isoflavones, has been shown to be a consequence of the lack of specific components of the gut microbiota [43-46]. Similarly, the absence of colonization of the gut microbe *Oxalobacter formigenes*, which transforms oxalate to formate, is a risk factor for the formation of calcium-oxalate stones in individuals lacking this microorganism [47].

In particular ‘non-digestible’ dietary carbohydrates including polysaccharides, oligosaccharides, lignin and associated plant material, are the preferred energy source for colonic microbiota. They are metabolized to short chain fatty acids (SCFAs), primarily to acetate, propionate, and butyrate which are considered to be health promoting (Table 1) [48,49]. Other SCFAs and organic acids such as valerate, caproate, succinate, malonate, fumarate, formate and lactate are produced at lower concentrations. Notably, considerable evidence has been accrued suggesting that the consumption of bifidogenic prebiotics inulin, fructo-oligosaccharides and galacto-oligosaccharides have significant health effects. In particular, positive effects on mineral absorption, and to a lesser extent on lipid metabolism, anti-inflammatory and other immune effects such as prevention of atopic disease, and putative anti-cancer properties have been documented [23,50]. Much of the non-digestible dietary carbohydrates are fermented in the proximal colon. The presence of fermentable carbohydrates influences proteolytic fermentation in the colon. Dietary protein can also reach the colon, and in addition to endogenous secretions such as mucin, provide other substrates for fermentation and deamination of amino-acids, that can result in a range of metabolites including the branched-chain fatty acids (BCFAs) isobutyrate, isovalerate and isocaproate, and also indoles, sulphides, ammonium, phenols, histamine, oxalacetate amongst others. These putrefactive components are generally considered to be toxic and to cause adverse effects on the colonic epithelium. A strong causal relationship between proteolytic fermentation in the (distal) colon and the occurrence of colon cancer [51] and inflammatory bowel disease (IBD) [52] is hypothesized.

Besides the complex polysaccharides, many secondary plant metabolites or phytochemicals including polyphenols persist to the colon where they undergo extensive bioconversion and metabolism by the gut microbiota. Polyphenols are highly abundant in fruits and vegetables [53]. They are classified into several groups including flavonoids, stilbenes, lignans and phenolic acids. According to their chemical structures, the flavonoids themselves are divided into flavonols, flavones, isoflavones, flavanones, anthocyanidins, anthocyanins, and flavanols including catechins and proanthocyanidins [54]. The uptake of polyphenols by the gastrointestinal tract is incomplete and their circulating levels in plasma low. Polyphenols that were initially absorbed in the ileum can subsequently be excreted as conjugates in bile and pass through the small intestine before they reach the colon. Alternatively, non-absorbed polyphenols reach the colon by directly passing through the small intestine [55-57]. In the colon, polyphenols are deconjugated by bacterial glycosidases, glucuronidases, and sulfatases and further fermented to a wide range of low-molecular-weight phenolic acids [58]. Thus, the gut microbiota plays a key role in the bioavailability of polyphenols and has been shown to modulate the health promoting activity through transformation to more active derivatives [58-60]. For example, depending on the inter-individual differences in the intestinal transformation potential, intestinal conversion of isoxanthohumol upon moderate beer consumption can lead to increased exposure of 8-pregnynaringenin, one of the most potent phytoestrogens [61].

Further to food ingredients which influence gut homeostasis via mediating metabolic or biochemical factors such as intra-colonic pH, production of bile acid metabolites, and fermentative production of metabolites, disturbances of the gut ecology can be caused by a variety of factors including antibiotics, environmental toxins, infections, stress, and genetic predisposition. In particular, the use of antibiotics is speculated to be linked to several disorders such as obesity, insulin resistance, diabetes, irritable bowel syndrome and diarrhoea [15,62,63]. Modern microbiota-targeted therapies envisage combinations of antibiotics, probiotics, prebiotics and perhaps laxatives to restore the homeostasis of gut ecology in the host [39].

**EFFECTS OF FOOD MICROBIAL METABOLITES ON GUT AND IMMUNE FUNCTION**

There is growing evidence that several diseases related to the intestine and/or the immune system are associated with variations in the composition [1,3,4,33] and consequently activity of the gut microbiota [64]. Recent evidence also indicates that the bacterial conversion of foods leading to the formation of a large number of compounds may have beneficial effects (e.g. inulin [23,65]) or deleterious effects on the host health (such as a protein-rich diet, [51,52]) [66]. Understanding the health effects of specific food microbial metabolites is key to optimise the diet in order to aid restoration of a healthy homeostasis in patients, to increase the robustness of healthy homeostatic control and to help healthy subjects stay healthy and thus to properly target interventions aiming at modulating the microbiota. However, distinguishing the direct impact of the food component on host health from indirect effects through the microbial metabolites or the microbiota itself remains a major challenge. In the following section, effects of food microbial metabolites on the gut and immune function of the host are re-reviewed with a focus on those studies which tested specifically single metabolites (or mixes) and measured their effects on specific aspects of intestinal health and immunity.

**EVIDENCE FROM Fecal WATER**

The first evidence on a causal relationship between microbial metabolites and specific aspects of intestinal health has been obtained by testing *in vitro* the effect of faecal water. For example, faecal water from patients with colorectal adenomas had a higher capacity to induce proliferation of colonic cancer cells compared to faecal water from control subjects [67]. Similarly, treatment of intestinal epithelial CaCo-2 cells *in vitro* with faecal water from several subjects could inhibit the adhesion of pathogenic intestinal bacteria to the cells, and this inhibition was positively correlated to the genotoxic potential of the various faecal water samples tested [68]. More recently, by measuring the transepithelial resistance (TER), it was shown that faecal water from healthy adults (40+/−9.7 yr) improved the barrier function of intestinal epithelial cells CaCo-2 *in vitro* [69]. In contrast, faecal water from healthy elderly subjects (76 +/− 7.5 yr), which contained lower levels of SCFAs, slightly impaired this barrier function. Together, these examples indicate that faecal metabolites can directly affect various aspects of intestinal function.

**EFFECT OF SCFAs**

The microbial metabolites that have been mostly studied for their effects on intestinal health and immunity are undoubtedly the SCFAs acetate, propionate and butyrate, which primarily originate from the fermentation of carbohydrates [48,70] but to a lesser extent also from proteins [71,72].

Butyrate is the major energy source for the colonocytes, but besides this more generic role, it has been extensively investigated for its potential protective effects against colon carcinogenesis, against intestinal inflammation, against oxidative stress and for its contribution in improving the intestinal barrier (e.g. reducing the intestinal permeability or modulating the mucus barrier) [48,70,73].
Although the *in vitro* and animal studies strongly suggest a protective role of butyrate against colon carcinogenesis, the direct evidence in humans is still lacking. Anti-inflammatory effects of butyrate enemas in patients with UC are more convincing, even though there is some inconsistency between studies, which may be partly explained by variations in dose, volume or duration of treatment [70]. Furthermore, *in vitro* or animal studies have clearly found beneficial effects of butyrate against oxidative stress and on various components of the intestinal barrier (including mucus, epithelial permeability and migration), trefoil peptides (anti-microbial peptides) and heat shock proteins [48,70], which contribute to improving the colonic barrier defence. However, these effects still have to be confirmed in humans. At the molecular level, it is known that butyrate is a histone deacetylase inhibitor (HDAC), leading to changes in chromatin conformation and gene expression [74]. This mechanism is assumed to be crucial for the ability of butyrate to modulate the expression of numerous genes involved in colonic health [75-78]. Apart from acting on epithelial cells, butyrate is also capable of interacting with immune cells: a transmembrane receptor for SCFAs called GPR43 has been identified on immune cells [79,80], and butyrate has anti-inflammatory properties in IFN-γ-stimulated macrophages *in vitro* [81], suggesting a likely role for butyrate in modulating host immunity.

Besides butyrate, all organic acids produced in the colon also contribute to the acidification of the colonic luminal environment, which is considered beneficial for the host, because pathogens have a reduced survival at lower pH [82,83]. Beyond butyrate there is less evidence for the impact of other SCFAs on gut and immune function of the host. For example, many *in vitro* studies have found that acetate, propionate, valerate and/or caproate were either not effective at all or at least not as potent as butyrate in modulating cell proliferation and in inhibiting HDAC (e.g. [84-86]), or in modulating colonic mucin gene expression [77]. Recently, faecal water obtained from fermentation of apple pectin and apple juice, has been found to have HDAC inhibitory properties in colonic cells *in vitro* [87]. In this study, the HDAC activity of the faecal water was strongly correlated to its butyrate levels and not to the levels of acetate and propionate. However, in another study propionate showed a similar anti-inflammatory effect as butyrate in colon organ cultures [88].

In summary, SCFAs produced mostly from carbohydrate fermentation have been extensively studied for their potential health effects on the colon and to a lesser extent on the immune system. Butyrate had consistent beneficial effects *in vitro* and in animal studies, but evidence in human intervention studies is less consistent. Other SCFAs have been studied to a much lesser extent and, as far as can be concluded at this stage, seem to have less potent effects. It is important to note that while butyrate is mostly consumed by the colonic epithelium, acetate and propionate can reach the systemic circulation.

**MICROBIAL METABOLITES FROM PROTEINS**

Protein and amino acids are fermented by the microbiota to a variety of other important metabolites including mostly branched chain fatty acids (BCFAs), ammonia, hydrogen sulfides, polyamines, indolic and phenolic compounds, as well as nitric oxide, nitrite and N-nitroso compounds (for a recent review of effects on the colonic epithelium see [71]).

Unlike the SCFAs, BCFAs, such as isobutyrate or isovalerate, are produced exclusively from protein metabolism in the colon. Although there are few studies, there is evidence that they may interfere with ion movements through the colonic epithelium and thus may be involved in diarrhoea regulation [71]. In contrast to butyrate, they clearly do not have measurable stimulatory effects on the proliferation of colonocytes *in vitro* [85,89]. The potential effects of branched-chain fatty acids on intestinal or immune health have so far been insufficiently investigated.

The colonic microbiota also produces N-nitroso compounds which can be involved in ion exchanges across the mucosa and more importantly in exacerbation of colonic inflammation [71]. The large intestine harbours the highest concentration of ammonia in the body, and levels produced by the microbiota seem to be modulated by fibres [90,91]. Ammonia has been shown to affect the colonic histology in some studies, but results are rather inconsistent: ammonia caused histological damage when infused in rat colon [92], yet had no effect on pig isolated crypts although it was applied at higher concentrations [93]. Similarly, the effects of ammonia on colonic proliferation remain controversial, for example, inhibiting proliferation in a colonic cancer cell line [94] but stimulating it in isolated colon tissue [95].

There is substantial evidence that hydrogen sulfide can have deleterious effects on the colon. This includes its likely role in the pathology of intestinal inflammatory diseases such as UC, as well as its ability to interfere with butyrate β-oxidation by colonic cells [96,97]. However, the deleterious effects of hydrogen sulfide on the colon appear to depend on many factors such as the luminal concentrations of unbound sulphide, on the enzymatic detoxification potential of the mucosa as well as the adaptive capacities of the epithelium towards this metabolite [71].

Polyamines (such as putrescine, agmatine, cadaverine, tyramine, histamine, spermidine) are produced by the microbial fermentation of proteins, but are also present in the diet as such. This makes it difficult to specifically evaluate the health effect of the polyamines produced by the microbiota. Depletion of the microbiota with antibiotics suggested that bacterially-derived polyamines are less important than those derived from the diet [98]. Overall, polyamines are considered to be important for gut health and are known to be involved in gut maturation and fluid secretion by colonic crypts. Moreover, polyamines have also shown to be involved in the differentiation of immune cells for an effective response and in the regulation of the inflammatory response [99]. However, polyamines have also been implicated to have deleterious effects on postprandial motility [99] and, moreover, they are likely to be involved in the formation of pre-neoplastic colonic lesions [71].

Protein and amino acid fermentation by the microbiota is also known to result in production of phenolic and indolic acids. Among the indolic acids, p-cresol has been mostly studied, and is considered to have negative effects on the host. High levels of urinary p-cresol have been associated with various disease conditions such as acute infections [100], IBD [52] and colon cancer [101]. *In vitro*, p-cresol has a negative impact on granulocyte functional capacity [100], and p-cresylsulphate, the main metabolite of p-cresol (formed in the colonic mucosa), has been shown to activate leucocyte free radical production [102].

Overall, protein metabolites produced by the microbiota are mostly known for having deleterious effects on the colonic physiology. However, like for SCFAs, the direct proof of such effects in humans is lacking. Furthermore, the effects are presumably rather complex and depend on various factors such as the luminal concentrations or absorption, the enzymatic detoxification potential of the mucosa as well as the adaptive capacities of the epithelium towards the metabolites. For example, on the one hand it has been established that polyamines can be deleterious but they are also necessary for the intestinal maturation.

**MICROBIAL METABOLITES FROM POLYPHENOLS**

In comparison to microbial metabolites generated from carbohydrates and proteins, the impact of metabolites from polyphenol metabolism or bioconversion on gut and immune function has been far less studied until now. However, the potential number of gut microbial metabolites generated by the activity of the gut microbiota may be far greater due to the substantial diversity of polyphenols.
Most research has focused on the phytoestrogen equol, a major microbial metabolite of the soy isoflavones daidzein and genistein. Several recent reviews have summarized the evidence on the effects of equol on various aspects such as cardiovascular disease, hot flashes or bone health in post-menopausal women as well as various types of oestrogen-related cancer [103-107]. However, within the scope of this review, there is only limited evidence for the impact of equol on gut or immune health. Briefly, several in vitro studies [108-111], indicated that equol consistently showed a stronger anti-oxidant capacity than its precursors genistein and daidzein [107]. So far, only a few human intervention studies have assessed whether the capacity to produce equol is associated with the effects on markers and inflammation [112,113]. In these studies, the anti-inflammatory effect of isoflavones was mostly limited to a moderate reduction in C-reactive protein levels, and was not linked to the capacity of the microbiota to produce equol.

Aspart from isoflavones and their metabolite equol, only few studies have recently started to compare the effects of other polyphenols to their microbial metabolites for potential effects on intestinal or immune cells.

Antioxidant Capacity

Several studies have compared the in vitro antioxidant activity of several microbial metabolites with their precursors. Compared to the high antioxidant activity of major ellagitannin of pomegranate called punicagin, its three microbial metabolites [namely, 3,8-dihydroxy-6H-dibenzo[f,c,d]pyran-6-one gluconuride, an unidentified aglycone (tentatively, trihydroxy-6H-dibenzo[b,d]pyran-6-one) and hydroxy-6H-dibenzo[f,c,d]-pyran-6-one gluconuride] showed no antioxidant activity (either isolated or mixed in urine or plasma matrix) [114]. In contrast, ellagitannin germi and one out of four of its microbial metabolites, namely 3,8,9-trihydroxy-6H-dibenzo[b,d]pyran-6-one, had comparable antioxidant capacity [115]. With respect to flavonoids, 5-(3`,4`,5`-trihydroxyphenyl)-valerolactone, a microbial metabolite of epicatechin present in tea, had lower antioxidant capacity than its precursor, but higher capacity than L-ascorbic acid [116]. In comparison, 3,4-dihydroxyphenylactetic acid (3,4DHPPA) showed a stronger antioxidant capacity than its precursor quercetin (both in immune polymorphonuclear cells and in cell-free assays). However, much lower antioxidant activity was observed for similar phenolic acids such as 3-hydroxyphenylacetic acid (3HPAA) and 3-(4-hydroxyphenyl)-propionic acid (4HPPA) [117]. Together, these results support the hypothesis that not only the food polyphenols but also their microbial metabolites must be taken into account when assessing the impact of polyphenols on the host.

Inflammatory Mechanisms

The potential effects of microbial metabolites from polyphenols on inflammatory mechanisms have recently been assessed. Human faecal water from vegetarian volunteers was shown to inhibit COX-2 protein level and enzymatic activity, as well as prostaglandin E2 (PGE2) production in colon cancer cell line HT-29 stimulated with TNFα [118]. Three out of the five main phenolic compounds found in these faecal water samples [i.e. 3-phenylpropionic acid (PAA), 3HPAA and 4HPPA, but not 4-hydroxy-3-methoxycinnamnic acid (4H3MCA) and 3,4 DHPPAA] decreased the protein levels or the activity of COX-2 in HT29 cells [118]. Thus, some phenolic acids (but not all) seem to be able to regulate COX-2 levels and activity in colonic cells, which could be interpreted as a benefit against intestinal inflammation and possibly cancer. In another study, quercetin but none of its metabolites including taxifolin, alpinitin and 3,4DHPPA induced anti-inflammatory effects in a mouse intestinal epithelial cell line as shown by the inhibition of TNFα-induced interferon-γ-inducible protein 10 (IP-10) and macrophage inflammatory protein 2 (MIP-2) expression [119]. One of the microbial metabolites of epigallocatechin gallate (EGCG), namely 5-(3`,4`,5`-trihydroxyphenyl)-γ-valerolactone, was shown to inhibit NO production but had no effect on arachidonic acid release by RAW264.7 murine macrophages [120]. Furthermore, when comparing blueberry polyphenols to their microbial metabolites (obtained in vitro with faecal flora from two different volunteers), blueberry polyphenols stimulated in vitro prostaglandin production by fibroblasts, and their microbial metabolites showed either an enhanced prostaglandin production or a decreased production, depending on the volunteer from which the faecal microbiota was used [121].

Anti-Proliferation Activity

The anti-proliferation activity of microbial metabolites from polyphenols has also been assessed in a few recent studies. Among a variety of different microbial metabolites identified from the in vitro microbial conversion of various supplements including green tea, black tea, citrus fruits with rutin, or soy flavonoids, only 3,4DHPPA exhibited anti-proliferative activity in prostate and colon cancer cell lines [122]. Furthermore, this metabolite also showed potent cytotoxicity against tumour cell line than 4-hydroxyphenyl acetic acid (4HPAA) and the precursor quercetin [123]. In another study, 5-(3`,4`,5`,5`-trihydroxyphenyl)-γ-valerolactone, one of the microbial metabolites of EGCG, was demonstrated to be less effective than EGCG in inhibiting the growth of human cell lines, and that its effect depended on the cell line being assessed [124-126].

Histone Deacetylase (HDAC) Inhibition

One of the mechanisms via which polyphenols metabolites may modulate cell proliferation is the inhibition of HDAC. Phenylacetic and phenylbutyrate have been described as potential HDAC inhibitors [127,128]. The HDAC inhibition capacity of microbial metabolites from polyphenols [including phenylacetic acid (PAA), 3,4DHPPA, 3HPAA, PPA, 4HPPA, caffeic acid, trans-cinnamic acid and p-coumaric acid] was recently compared to the HDAC inhibition potential of SCFAs (namely, acetate, propionate, butyrate, valerate, iso-butyrate and is-valerate) [86]. As expected, butyrate (1-2mM) was the most potent HDAC inhibitor among the SCFAs. Amongst the metabolites from polyphenols, only 3-(3-hydroxyphenyl) propionic acid (3HPPA) and 4HPPA showed a relevant HDAC inhibitory activity in the whole cell tests (in which the intracellular availability of these metabolites is taken into account, as opposed to tests with nuclear extracts only). However, 3HPPA and 4HPPA inhibited HDAC at a relatively high concentration range (10-20mM), which is much higher than the concentration range found in faecal water (~1-450 μM) [129].

In summary, the research exploring the effects of microbial metabolites from polyphenols on the health of the mucosal epithelia cells, intestine and immune health is still in its infancy. The well-known equol originating from soy isoflavones shows anti-oxidant capacity, but its potential role in gut or immune health is not clear. So far, other polyphenol metabolites have been investigated even less. As may have been expected, the first in vitro results indicate that their potential impact on gut and immune function depends not only on the metabolites produced, but also on the function being investigated; for example, the flavonoid metabolite 3,4DHPPA showed anti-oxidant [117] and anti-proliferative effects [122] but no anti-inflammatory effects [118,119].

Overall, non-digestible food ingredients can have an indirect impact on the host through their microbial metabolites acting in an either beneficial or deleterious manner depending on a variety of factors including the luminal concentrations of food components, gut microbial diversity and host response. The current evidence is limited and basically restricted to in vitro and animal studies. Evidence in humans and an overview on the complex response induced by microbial metabolites are lacking. More insight into the interaction between microbial and host metabolism is a further step towards a clearer understanding of the role of the gut microbiota on
gut and immune health. Therefore, metabolomic studies focusing on microbe-host mutualism are reviewed in the following section.

METABOLICOMICS AND GUT MICROBIAL METABOLITES

Besides the structural components of their cells, the gut microbiota is assumed to communicate with the host via a characteristic secretion pattern and thus participate in the host metabolic network. This secretome or small-molecule metabolome is accessible in faeces and, after passage through and modification by the host, in urine [39] (Fig. 2a). The advances of profiling techniques such as 1H-nuclear magnetic resonance (NMR) spectroscopy, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) allow the simultaneous monitoring of changes in metabolites with diverse chemical properties and at a wide range of concentrations [130,131]. Fig. (2) demonstrates how NMR and GC-MS profiling can be applied to retrieve different information on the secretome. Therefore, metabolite profiling in combination with multivariate pattern recognition techniques is a novel approach to examine the microbial and mammalian metabolic cooperation with respect to phenotype, disease and diet [15].

METABOLITE PROFILING OF FAECES

SCFAs and/or organic acids are the prevailing microbial metabolites in faeces. Their production in relation to diet has been typically measured in faeces from human subjects [73,132-136] or after in vitro faecal fermentation [137,138] using targeted GC analysis. In comparison, NMR-based metabolite profiling is a rapid, non-targeted and comprehensive technique to detect a variety of different fermentation products in faeces [139] including SCFAs (e.g. acetate, propionate, butyrate, isobutyrate, isovalerate, malate), organic acids (e.g. succinate, pyruvate, fumarate, lactate), amino acids, uracil, trimethylamine (TMA), ethanol, glycerol, glucose, phenolic acids, cholate and lipid components.

Metabolite profiling of faeces can be effectively applied to detect species-dependent differences and to differentiate diseased from healthy states. Comparing the faecal metabolite composition in humans, mice and rats showed that SCFAs and BCFA were common to the three species, whereas other metabolites like β-alanine and malonate were exclusively present in rat and humans, respectively [140]. This species-dependent microbiota activity may hamper the transfer of the results from the transplantation of human gut microbiota into surrogate rats or mice. However, the application of human microbiota-associated gnotobiotic mice or rats may be used to overcome this [30]. In addition, the transplantation of human gut microbiota into germ-free piglets produced a donor-like microbial community with minimal individual variation and ageing patterns similar to those observed in humans. This represents an improved human-microbiota mimicking model for research on gut ecology in human metabolism, nutrition and drug discovery [141].

The potential of NMR-based profiling of faeces as a non-invasive diagnostic tool for inflammatory bowel disease has been demonstrated on patients with Crohn’s disease (CD) and ulcerative colitis (UC) [142]. The faecal extracts of both CD and UC patients were characterized by reduced levels of butyrate, acetate, methyamine, and TMA in comparison with a control population, suggesting changes in the gut microbial community. Furthermore, the elevated levels of amino acids found in the faeces of both disease groups have been ascribed to malabsorption caused by the inflammatory disease or to excessive loss of proteins into the gastrointestinal tract. In another study investigating the gut ecosystem in subjects with colorectal cancer and with adenomatous polyposis (known to be at high risk of developing colorectal cancer), significant alterations including increased diversity of the Clostridium leptum and Clostridium cocoides subgroups and elevated levels of amino acids were observed in both patient groups when compared to the control group [143]. The authors have speculated that the higher clostridial species may be involved in the dietary protein degradation potentially leading to the production of potential carcinogens such as phenols, ammonia and indoles.

Faecal metabolite profiling may also be useful to explore the fate of diverse food components or compositions, considering that many metabolites present in faeces derive from non-digestible food ingredients and that this method is capable of covering a broad range of different compound classes. Recently, we have optimized NMR-based metabolite profiling of human faeces using samples from a placebo-controlled cross-over intervention trial, in which healthy human volunteers consumed a mixture of wine and grape extracts over a period of four weeks [139]. The polyphenols induced a few consistent metabolite changes suggesting that polyphenols are able to modulate the microbial ecology in healthy humans. The effect, however, was subtle and apparently covered by large intra- and inter-individual metabolite variation possibly arising from the uncontrolled diet. Consequently, controlling the diet in the intervention trial may be useful to reduce the variation and thus to observe subtle nutritional effects. Moreover, considering that the kinetics of the fermentation process varies considerably between subjects, multiple sampling is recommended in order to establish an unambiguous link between diet and metabolic response. In this respect, it is noteworthy that the metabolite concentration present in faeces reflects the fermentation processes in the distal colon rather than in the proximal part of the colon where fermentation of most non-digestible complex polysaccharides takes place. Microbial processes occurring in the proximal colon may be investigated in the caecum of animals [144,145] and in vitro gut fermentation models that mimic the colon [52,146,147]. For example, in vitro culture models of the human colonic microbiota have been used to investigate the extensive metabolism of natural polyphenols to detect potential biomarkers of colonic metabolism [148] and to associate changes of bacterial species with the formation of phenolic acids [149].

To summarize, NMR-based metabolite profiling in faeces is an efficient technique to characterize the microbial activity in vivo. Metabolite profiling on supernatants from sophisticated in vitro culture models, like the Simulator of the Human Intestinal Microbial Ecosystem SHIME [150], the University of Reading 3-stage model [151], and the TIM-2 in vitro model of the human intestine [152], will assist analysis on the metabolic processes in the gut. Furthermore, the combination of microbial diversity as well as metabolome analysis of other biological fluids will help to bridge the gap between microbiota composition and host metabolism.

METABOLITE PROFILING OF URINE

In contrast to the relatively recent studies on metabolomics in faeces, metabolite profiling in urine is well established. Moreover, a range of metabolites related to microbial activity have already been identified in urine using the metabolomics approach. Several studies have shown that the composition of the microbial metabolites in urine depend on the composition of the microbial population. In these studies, the differences in the intestinal microbiota were mainly manifested in the excretion of phenolic acids. For example, the different excretion levels of hippuric acid (HA) and 3HPPA discriminated conventional rats after antibiotic administration [153]. Genetically homogeneous rats maintained under identical conditions were distinguishable by different excretion levels of HA and two metabolites from chlorogenic acids, namely 3HPPA and 3-hydroxyxynamic acid (3-HCA) [154]. In another study investigating the acclimatization of germ-free rats to standard conditions, HA, PA and 3HPPA were used to monitor the period until a stable gut microbiota was established [155]. Furthermore, the development of an obesity-prone phenotype in rats was linked to several different differential mammalian-microbial metabolites [HA, PPA, 4HPPA, N-phenylacetylglucose (PAG), p-cresol, 4-ethyl-phenol] [156]. The authors have concluded that the inter-subject (phenotypic) variations associated with different compositions of the gut microbiota
predispose the host to different patho-physiological outcomes upon dietary alteration or chemical stimulus [156]. In another study, the correlation of the gut microbiota fingerprints with subsequent identification of the microbial phytootypes in combination with urinary NMR-based metabolite profiles from Chinese individuals allowed characterization of the key functional members of the microbiome that have the most influence on the host metabolism and possibly health [29]. Notably, the population variation of *Faecalibacterium prausnitzii* was associated with the modulation of eight urinary metabolites of diverse structure, indicating that this species is a highly functionally active member of the microbiota, influencing numerous host pathways.

Metabolite profiling of urine in combination with pattern recognition strategies has also proven to be useful to uncover diagnostic markers of infection and to elucidate the complex host-parasite cross-talk. A range of microbial metabolites, such as HA, p-cresol glucuronide, PAG and TMA were associated with a *Schistosoma japonicum* infection in Syrian hamsters [157] and with *Schistosoma mansoni* infection in mice [158]. However, the production or utilization of 4HPPA was inhibited in *S. japonicum* infected hamsters, when compared to an *S. mansoni* infection in a mouse. In another study, *Helicobacter pylori* infection in gerbils perturbed the carbohydrate, energy and amino acid metabolisms and modified the gut microbiota as highlighted by the changes of microbial metabolites such as indoxyl sulphate and HA present in urine [159]. Furthermore, NMR-based metabolomics has been applied to cerebrospinal fluids and has revealed that metabolites of microbial and host origin were responsible for the separation of bacterial or fungal meningitis patients from patients with viral meningitis and control subjects [160]. Diagnosis of subtle effects of infections may suggest greater insights in complex conditions of unknown etiology believed to have a microbial factor such as intestinal enteropathy/tropical sprue [161].

Several metabolomic studies have shown a notable effect of diets on the urinary excretion of microbial metabolites. Changes in human urinary profiles upon dietary intervention showed that the excretion of creatinine, creatine, trimethylamine-N-oxide (TMAO), taurine and 1- and 3-methylhistidine was associated with a high meat diet and the excretion of 4HPAA with a vegetarian diet [162]. Whereas 4HPPA may originate from polyphenols present in fruit and vegetables, the presence of TMAO may be ascribed to dietary choline which is converted to TMA by gut microbiota and subsequently detoxified to TMAO in the liver via the flavine monooxygenase system [163]. The conversion of choline into methylamines by microbiota in mice on a high-fat diet has been shown to reduce the bioavailability of choline and to mimick the effect of choline-deficient diets, causing non-alcoholic fatty liver disease [164]. The microbiota-related reduced choline bioavailability has been suggested to alter the lipid metabolism of the host. The endogenous host response to probiotic intervention was investigated in a germ-free mouse model colonized with human infant microbiota by performing statistical analysis of diverse compartmental metabolic fluctuations in various biological fluids, tissue and cecum. The probiotic exposure modified the microbiome, affected the levels of amino acids, methylamines and SCFAs, and altered hepatic lipid metabolism coupled with lowered plasma lipoprotein levels and apparent stimulated glycolysis [163]. In a crossover metabolomic study in pigs, a whole-grain diet induced higher urinary excretion of betaine and HA, whereas the higher creatinine excretion was associated with the non-whole-grain diet [165].

Acute changes in urinary metabolite profiles have been shown to occur after the consumption of dietary phytochemicals [166]. The urinary excretion of creatinine and 3-methylhistidine was associated with a low-phytochemical diet, whereas the excretion of HA, the glycine conjugate of benzoic acid, was associated with phytochemical intake. It has been suggested that the absence and presence of 3HPPA and HA, respectively, in rat urine is due to a combination of differences in dietary precursors of substrates for glycine conjugation and a dietary dependent modulation of the gut microbiota [167]. In another dietary intervention study demonstrating persistent dietary effects, perturbations in microbial metabolites in urine of healthy volunteers were found after the daily consumption of chamomile tea over a period of two weeks [168]. After a further two weeks of abstaining from chamomile tea, the metabolic signature was still different from the baseline condition, indicating that the effect of chamomile tea was not rapidly reversible. In humans, HA and 1,3-dihydroxyphenyl-2-O-sulphate have been identified as indicative metabolites of tea flavonoids with significant inter-individual variation on their excretion levels [169,170]. Interestingly, green and black tea intake had a different impact on the endogenous metabolism. In comparison to black tea, green tea caused a stronger increase in urinary levels of several citric acid cycle intermediates, suggesting that green tea flavanols affect the human oxidative energy metabolism and/or biosynthetic pathways [170]. In another human study, a dietary intervention with soy isoflavones has resulted in subtle changes in urinary metabolic profiles that were associated with osmolyte fluctuation and energy metabolism [171]. These biochemical changes were more significant following ingestion of unconjugated soy isoflavones suggesting that the chemical composition of the isoflavones present in soy-based foods affects the biological efficacy in vivo.

The gut microbiota has also been shown to be involved in the response to cold stress in rats [172]. Among several other metabolic pathways including catecholamines, glucocorticoids, the triarboxylic acid (TCA) cycle and tryptophan, the urinary excretion levels of several microbial metabolites (4-methyl-phenol, 4HPAA, and HA) were altered upon exposure to cold stress. The authors have indicated that changes in endogenous metabolism may be a result of gastrointestinal tract motility and secretions in response to cold stress. Interestingly, when orally administered with ginsenosides known for their anti-inflammatory activity, green tea intake had no effect on the urinary excretion of microbial metabolites, whereas the green tea containing chamomile tea did alter the microbial metabolites, suggesting that the ginsenosides have a protective effect in restoring homeostasis of the metabolic network in cold-stressed rats [172].

In summary, a variety of microbial metabolites present in the urine can be detected by NMR spectroscopy and linked to different phenotypes, diseased or infected states and dietary intake. Moreover, the simultaneous detection of microbial and host metabolites is advantageous to link the exogenous and endogenous metabolism, in particular when combined with the metabolome in faeces and plasma. However, the NMR profiles of urine contain many unidentified signals possibly originating from commensal microbial metabolites and the identification of these metabolites is still the major bottleneck in the metabolomic workflow. Whereas the metabolomic studies have generally addressed the microbial-host metabolism, metabolomic studies have not been specifically designed yet to assess the impact of non-digestible food ingredients on gut health and immunity. In the following, we describe strategies on how metabolomics can be further developed and applied to establish the causal link between the non-digestible food ingredient, the microbial activity, the host metabolism and the immune system.

**PERSPECTIVES**

Non-digestible food ingredients can induce various complex responses on the host through their microbial metabolites and evidence is increasing for the impact of these metabolites in intestinal health and immunity. SCFAs, the main metabolites of carbohydrates fermentation by the colonic microbiota, have potential beneficial effects on colonic health, whereas microbial metabolites from proteins, such as indoles and hydrogen sulfide appear to have deleterious effects. Moreover, emerging evidence has shown that some microbial metabolites of polyphenols have the same *in vitro* antioxidant, anti-inflammatory and anti-proliferation activity as their precursor suggesting that these polyphenols may exert benefi-
cional effects on the host via their microbiobioconverted derivatives. Nevertheless, current findings are predominantly substantiated by *in vitro* and some animal studies. Evidence in humans is scarce, and certainly the challenges to access and study microbial metabolites in humans have created a major limitation here. Moreover, the studies were designed to test specific hypothesis leading to scattered results.

Integrative –omics approaches overlap various processes and thus provide holistic views useful to generate well-founded, novel hypotheses. In this review, various metabolomic studies focusing on microbe-host mutualism have clearly demonstrated that NMR-based metabolomics is capable of detecting and tracking diverse microbial metabolites from different non-digestible food ingredients, of discriminating between phenotypes with different inherent microbiota and of potentially diagnosing infection and gastrointestinal diseases. In particular, the combined analysis of the metabolome in different biological fluids (Fig. (2)) including faecal water extract, plasma and urine is a feasible strategy to establish coherent links between the bioconversion of non-digestible food ingredients, their bioavailability and their downstream effects on the host metabolism. In view of personalized nutrition, the inter-individual variation and kinetic aspect are important to be taken into account when following this approach.

Complementary to NMR-based metabolite profiling, LC-MS and GC-MS-based metabolite profiling can detect metabolites in lower levels. In particular, targeted profiling of specific compound classes is an alternative approach to access microbial metabolites. For example, GC-MS-based profiling of phenolic acids in faecal water has been demonstrated to be useful to investigate the bioconversion of dietary polyphenols [129]. This method has been extended to diverse biological fluids including urine, plasma, faecal extract and supernatants from *in vitro* faecal fermentation models [173]. In comparison to GC-MS, LC-MS-based methods are also capable of detecting a variety of different phenolic acids and, beyond it, larger phenolic acids and polyphenols [174,175]. Comparing the levels of a variety of different phenolic acids in various biological fluids of the same individual allows for tracing the microbiota-mediated bioavailability of polyphenols in humans.

Besides the exogenous metabolism, targeted metabolite profiling focusing on the lipidome or on sub-metabolomes directed to specific biological processes such as energy metabolism, oxidative stress and inflammation is a promising approach to observe endogenous effects on the host metabolism using mass spectrometry. With respect to gut and immune function, a metabolomic study on an IBD mouse model, has revealed a shift in the serum lipid profile as response to dextran sulphate sodium (DSS) treatment used to induce acute colitis. Interestingly, the changes on lysophosphatidylcholines (LPCs) occurred prior to the symptoms of acute colitis and correlated well with elevated expression of proinflammatory cytokines [176]. Furthermore, the results from this study suggested functional differences among the LPC's regarding the activation of the immune system. In addition to LPC's, oxidized phospholipids appear to have a fundamental role in innate immunity [177]. Prostaglandins are also known to play an important role in the regulation of inflammatory responses, are involved in the regulation of motility and epithelia barrier function [178] and hence may be interesting for targeted profiling. Thus, the combination of various targeted profiles will be useful to link the exogenous and endogenous metabolism or to zoom in on specific endogenous processes.

Complementary to metabolomics, stable isotope-aided metabolic flux analysis (MFA), including the calculation and analysis of the flux distribution of the entire biochemical reaction network in the system using stable isotopes, provides more detailed information on the dynamics of metabolic processes and the biosynthetic pathway of the metabolite [40]. Regarding the interaction of human and gut microbial metabolism, MFA has been predominantly used to characterize the fate of microbial metabolites like SCFAs [179-181] and amino acids [182]. However, the applicability of MFA to phytochemicals like polyphenols may be limited due to their lower abundance and the laborious isotope-labelling. Nevertheless, MFA combined metabolomics may be applied to shed light on the dynamics between microbial activity and host metabolism. Another approach that may be used to couple microbial diversity to metabolic function is *in situ* stable isotope probing (SIP), a technique that relies on the incorporation of a substrate enriched in a stable isotope, such as 13C, to identify microorganisms that can metabolize this substrate [183]. For example, RNA-SIP in combination with NMR-based metabolite profiling has allowed the identification of both the metabolites and bacteria involved in the fermentation of glucose under conditions simulating the human intestine [184].

The integration of metabolomics with other –omics techniques is a further step towards a more coherent understanding of the complex microbe-host mutualism. With respect to gut and immune function, metabolomics combined with analysis of the gut microbiota composition is essential to correlate the microbial species to their activity. Further downstream, the link between metabolomics and transcriptional data would strengthen the scientific evidence on the metabolic and immune regulation of the host. Of course, the novel hypothesis generated by the –omics approach need to be validated furthermore using targeted hypothesis testing approaches involving the measurement of relevant immune function markers.

In conclusion, holistic –omics approaches are indispensable to cover the complex interactions between the gut microbial ecosystem and the host. In particular, metabolomics, albeit in an early stage with respect to the microbe-host mutualism, holds great potential to better understand the fate of non-digestible food ingredients on gut health and immunity. Considering the increasing intestinal diseases including the enteropathy in the developing countries and that in the Western world obesity is associated with immune imbalance [14], further knowledge on population-specific metabolic regulation will help to develop novel food concepts aiming at improved intestinal well-being and immune strength.

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**ABBREVIATIONS**

SCFA = Short chain fatty acid  
GC = Gas chromatography  
LC = Liquid chromatography  
MS = Mass spectrometry  
MFA = Metabolic flux analysis  
NMR = Nuclear magnetic resonance  
IBD = Inflammatory bowel disease  
HDAC = Histone desacytelase  
CD = Crohn’s disease  
UC = Ulcerative colitis  
TMA = Trimethylamine  
TMAO = Trimethylamine-oxide  
COX-2 = Cyclooxygenase-2  
TNFα = Tumour necrosis factor α  
3HPAA = 3-hydroxyphenylactic acid  
4HPAA = 4-hydroxyphenylactic acid  
3,4DHPAA = 3,4-dihydroxyphenylactic acid  
PPA = 3-phenylpropionic acid
3HPPA = 3-(3-hydroxyphenyl)propionic acid
4HPPA = 3-(4-hydroxyphenyl)propionic acid
PAG = N-phenylacetylglycine
HA = Hippuric acid
EGCG = Epigallocatechin gallate

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