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Review article

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FOOD-BASED STRATEGIES TO MODULATE THE COMPOSITION OF THE INTESTINAL MICROBIOTA AND THEIR ASSOCIATED HEALTH EFFECTS

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The most well known food-based strategies to modulate the composition of the intestinal microbiota are the dietary use of prebiotics, probiotics and their combination, synbiotics. Currently established prebiotic compounds are mainly targeting the bifidobacteria population of the colon microbiota. A good illustration of the importance of high colonic bifidobacteria levels is the observation that breast milk creates an environment in the colon (because of its high amount in galacto-oligosaccharides with prebiotic activity) favouring the development of a simple flora, dominated by bifidobacteria to which various health benefits have been ascribed. Currently, high colonic bifidobacteria levels has been considered favourably at all ages and strategies to augment their presence have been demonstrated in placebo-controlled intervention studies; *e.g.* in toddlers to reduce sickness events, in adults to reduce the risk for developing gastrointestinal diseases and in the elderly to re-enhance their declining immune activity. The intestinal microbiota can be considered as a metabolically adaptable and rapidly renewable organ of the body. However, unbalances in its microbial community and activities are found to be implicated in disease initiation and progression, such as chronic inflammatory bowel diseases and colonic cancers. Restoration of this balance by increasing bifidobacteria levels has demonstrated to reduce disease severity of patients and to improve well-being in healtly volunteers. New emerging evidence on the difference in the composition of the colonic microbiota between obese and lean volunteers has opened new areas for pre-, pro- and synbiotic research. Additionally, as knowledge will increase about the microbial bio-conversion of polyphenolic compounds into bioactive metabolites in the colon and whether food-based strategies can augment such bioconversion into more potent compounds with anti-oxidant and/or anti-inflammatory activity new areas of research will be discovered. This paper provides an up-to-date review of the health benefits associated to the induction of high bifidobacteria levels in the colon by the use of prebiotics (inulin and oligofructose). New areas of emerging science will be discussed as well.

Key words: *inulin-type fructans, prebiotics, intestinal microbiota, obesity, phytonutrient metabolisation*

INTRODUCTION

The term 'prebiotic' was first defined in 1995 by Gibson and Roberfroid as 'a non-digestible food ingredient that selectively stimulates growth and/or activity of one or a limited number of bacteria in the colon, thereby improving host health'. As research progressed, three criteria were accepted which a food ingredient should fulfil before it can be classified as prebiotic: firstly, it should be non-digestible and resistant to gastric acidity, hydrolysis by intestinal (brush border/pancreatic) digestive enzymes, and gastrointestinal absorption; secondly, it should be fermentable and; thirdly, it should in a selective way stimulate growth and/or metabolic activity of intestinal bacteria that are associated with health and wellbeing (1). Well established prebiotic compounds nowadays are inulin and oligofructose (or fructo-oligosaccharides), galacto-oligosacchardies and lactulose, however extensive research is ongoing to strengthen the scientific basis of promising new candidates.

Inulin-type fructans are naturally occurring oligosaccharides that represent the carbohydrate reserve in plants. Plants containing inulin-type fructans primarily belong to the Liliales, *e.g.* leek, onion, garlic and asparagus; or the Compositae, such as Jerusalem artichoke (*Helianthus tuberosus*), dahlia and chicory (*Cichorium intybus*). Inulin is a polydisperse carbohydrate material consisting of β (2 ->1) fructosyl - fructose links (*Fig. 1*). A starting glucose moiety can be present. Inulin-type fructans can be represented as both GF_n and F_m. In chicory inulin, the number of fructose units linked to a terminal glucose can vary from 2 to 70 units. By means of an endo-inulinase inulin is hydrolysed into a DP between 2 and 8 (average DP=4) called oligofructose.

Other interesting classes of dietary substances that arrive to a great extent in the colon and are metabolised by the microbiota in the colon are the polyphenols. Most polyphenols are in the form of esters, glycosides or polymers (proanthocyanidins) and have to be hydrolysed by intestinal enzymes or by the colonic microflora before absorption can occur (2-6). This complex group of plant derived-polyphenolic compounds has been the focus of much research given their interesting anti-oxidant properties which have been related to the protecting effect of diets rich in fruits and vegetables against several chronic diseases such as cardiovascular diseases and certain cancers (2, 7). Polyphenols can be classified in different groups including

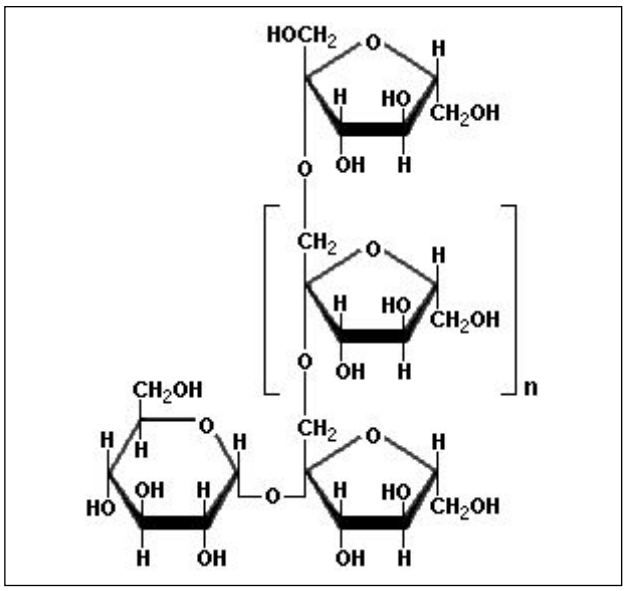

Fig. 1. Chemical structure of inulin compounds.

phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids and the less common stilbenes and lignans. The flavonoids can be further divided in flavones, isoflavones, anthocyanidins, flavanones, flavanols and their polymers the proanthocyanidins (*Fig. 2*). The main dietary sources are fruits (*e.g.* citrus fruit, apples, grapes and berries), wine, tea, soy and cacao. Polyphenols are also found in vegetables (*e.g.* onions, artichokes) but are less abundant. Foods mostly contain complex mixtures of polyphenols (2, 3, 8, 9). To understand their impact on human health, their nature, origin, amount in the diet, bioavailability and microbial metabolisation in the colon need to be investigated. In this respect, gaining understanding of the metabolisation pathways of polyphenols by the microbiota and the kind of bioactive metabolites that are formed during this process is of paramount importance. Also in turn, the effects of such metabolites on the composition of the microbiota might be subject of investigation. As such, in the future, strategies that enhance bioactive formation by colonic microbiota manipulation could be an important tool to enhance anti-oxidant or anti-inflammatory properties of polyphenols.

INTESTINAL FUNCTION, METABOLISM AND MICROBIOTA

Studies in ileostomised volunteers have demonstrated that orally ingested inulin enters the colon almost quantitatively (>90%) where it is subsequently completely metabolized by the endogenous colonic microbiota (10). In the colon, inulin-type fructans are completely converted by the microbiota into bacterial biomass, organic acids, like lactic acid and short-chain fatty acids (SCFA: acetic, propionic and butyric acid) and gasses (CO_2, H_2, CH_4). SCFA and lactate contribute to the host's energy metabolism.

Inulin-type fructans, through their presence and subsequent fermentation in the large bowel, influence the colonic metabolism in its lumen and the integrity and functioning of the epithelial cell lining. Apart from their stool bulking effect which has been demonstrated in randomised, double-blind and placebo-controlled human studies in subjects with low stool frequency patterns or constipated patients (11-13), more recently also a significant decrease in the intensity of digestive disorders in patients with minor functional disorders was found in a randomised and double-blind controlled, multicentre study set-up (14). An increase in stool frequency with the administration of a synbiotic supplement (*Bifidobacterium animalis* and an oligofructose-enriched inulin) has been demonstrated in elderly subjects also to be associated with an improved well-being and the quality of life (15 - CROWNALIFE project, 'Crown of Life' Project on Functional Foods, Gut Microflora and healthy Ageing, QLK1-2000-00067).

The intestinal microbiota can be considered as a metabolically adaptable and rapidly renewable organ of the body. Administration of oligofructose to post-weaning infants has been shown to increase the numbers of bifidobacteria (up to 9.5 log of colony-forming units per gram of faeces) (16). Also in adults and elderly subjects, administration of inulin and oligofructose alone or as synbiotic has been demonstrated to selectively increase numbers of bifidobacteria in the luminal as

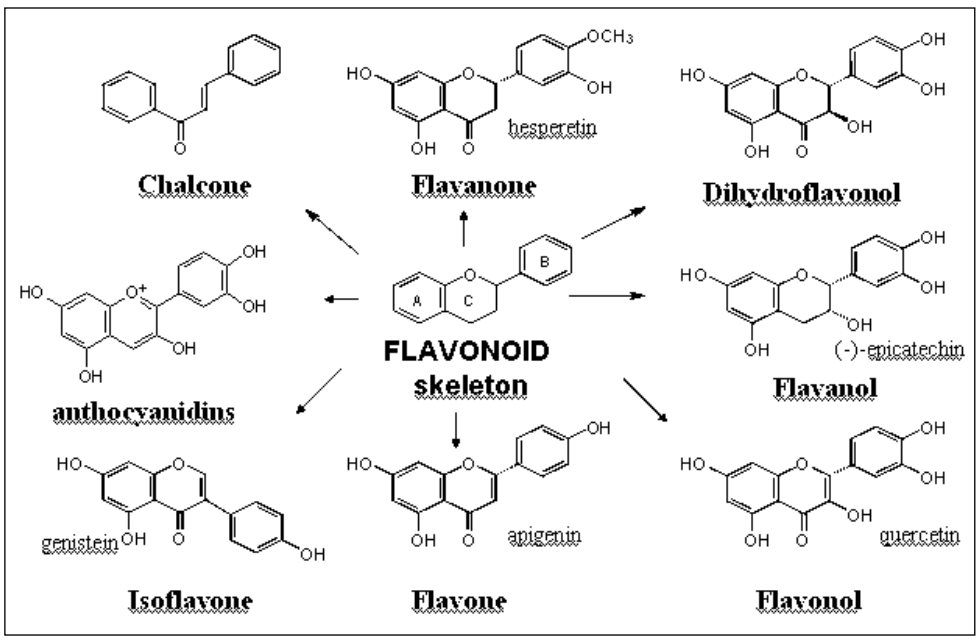

Fig. 2. Subclasses of flavonoids.

well as the mucosa-associated microbiota (11, 12, 15, 17-20), typically representing a prebiotic effect.

In certain conditions such as old age, the use of antibiotics or in case of (critical) illnesses (acute or chronic such as inflammatory bowel diseases and cancer) the intestinal barrier is functioning less and gastro-intestinal dysfunction can occur. As a result, increased bacterial translocation may happen and leading to systemic illness. The interdigestive intestinal motility (*e.g.* migrating myoelectric/motor complex) is one physiological mechanism that prevents bacterial overgrowth and translocation in the gut and a relationship appears between the intestinal motility and the composition of the intestinal microflora. Administration of *Lactobacillus rhamnosus* GG, *Bifidobacterium lactis* Bb12 and oligofructose-enriched inulin to elderly rats regularised the occurrence of intestinal contractions of high amplitude which are more effective in propelling the residual food, debris, secretions and bacterial cells (21). Other animal experiments to test the potential of modulating the microbiota to efficiently discriminate and eliminate pathogenic organisms showed decreased translocation of bacteria (total aerobic, anaerobic and the *Enterobacteriaceae*) to the mesenteric lymph nodes and liver, after oral administration of probiotics (*Bifidobacterium infantis*) and/or prebiotics (oligofructose-enriched inulin) in DSS-colitis induced rats (22). These data are indicative of an improved epithelial barrier function and in agreement with earlier studies. In mice infectioned (intra-peritoneally) with virulent strains of systemic pathogens (*Listeria monocytogenes* and *Salmonella typhimurium*) mortality rates were much lower upon inulin feeding (23). Other studies in rats, also infected with *Salmonella*, showed lower pathogen colonization in the intestines with oligofructose, however, the authors observed an increase in translocation rate to the spleen and liver. These observations can most likely be ascribed to the low calcium diet used in this model which by itself damaged barrier function as the authors demonstrated that increasing the calcium level of the diets was accompanied by a decrease in the rate of translocation (24).

In humans, no effect on barrier function of (high dose) oligofructose was found in healthy volunteers. Barrier function was measured by the levels of intestinal epitheliolysis and the excretion of O-linked oligosaccharides in stools. The latter refers to the production of glycoproteins which build up the mucus gel layer that is covering the intestinal epithelium. The authors, however, did observed a lower level of cytotoxicity of the faecal water with oligofructose (25). In the SYNCAN Project, Synbiotics and Cancer Prevention (QLK-1999-00346), the effect of oligofructose-enriched inulin (as synbiotic) on the epithelial barrier function was studied. The trans-epithelial resistance (*ex vivo*) of cell lines subjected to the faecal water from polypectomized volunteers supplemented with oligofructose-enriched was measured as an indicator of barrier functioning. The faecal water is the faecal fraction in most intimate contact with the colonic epithelium and mediates its functioning. A common observed effect of tumour promoters is the reduction in barrier function of the epithelium inducing lower protection of the mucosa to carcinogenic substances. Interestingly, the synbiotic intervention increased the barrier function of the epithelium which was showed by the significantly increased percentage in trans-epithelial resistance of the Caco-2 cell monolayer when subjected to the faecal water of polyp patients receiving the synbiotic (26).

PREBIOTICS AND BUTYROGENIC EFFECT

Studies (both *in vitro* and *in vivo*) have demonstrated that the colonic fermentation of inulin-type fructans increases the production of butyrate, which is the so-called 'butyrogenic effect'. However, as bifidobacteria are primarily lactate and acetate producers, this effects remained until recently unclear. Fermentation studies (*in vitro*) using simple and complex bacterial cultures or faecal slurries offer a valuable tool to study individual bacterial metabolism and interspecies interactions. Kinetic analyses of co-cultures with *Bifidobacteria sp*. and butyrate-producing colonic bacteria in the presence of oligofructose revealed distinct types of cross-feeding reactions which were strain-dependent. In such studies, butyrate-producing bacteria (*e.g. A. caccae* and *R. intestinalis*) were unable to degrade oligofructose, whereas in the presence of bifidobacteria and/or fermentation metabolites (acetate) or breakdown products, degradation did occur with corresponding butyrate production (27). Studies with stable isotopes, enabling to follow carbon flows, showed that indeed *Bifidobacterium sp.* in the presence of oligofructose produce lactate (and/or breakdown products) which in turn are converted into butyrate in the presence of butyrate-producing bacteria (*e.g. R. intestinalis* and *E. halli*) (28). Faecal batch cultures found that the addition of oligofructose significantly increased butyrate production. About 80% of the newly synthesised butyrate derived from oligofructose fermentation originated from the inter-conversion of extracellular acetate and lactate. Also, Duncan *et al.* found that the contribution of external acetate to butyrate formation from oligofructose fermentation ranged from 82% (faecal slurry batch culture) to 87% (continuous cultures). The increased flux of extracellular acetate to butyrate upon oligofructose fermentation in mixed (faecal) slurries is in agreement with butyryl CoA: acetyl CoA transferase being the dominant butyrate-producing pathway. It appears that this pathway is selectively activated upon oligofructose fermentation, with concomitant butyrate production. These cross-feeding mechanisms could play an important role in the colonic ecosystem and contribute to the combined bifidogenic and butyrogenic effect observed after addition of inulin-type fructans to the diet (29).

INTESTINAL MICROBIOTA, INFECTION, INFLAMMATION AND IMMUNITY

Exploratory *in vitro* work with fecal slurries, starting in the early nineties, indicated that inulin and oligofructose are completely fermented by the colonic microbiota and selectively stimulate bifidobacteria and lactobacilli growth and activity at the expense of pathogenic bacteria (*e.g.* clostridia). Protective effects of bifidobacteria have been demonstrated in (gnotobiotic) quails against the development of necrotizing enterocolitis (NEC)-like lesions when inoculated with a pathogenic flora (containing *Clostridium butyricum* and *Clostridium perfingens*) from premature newborns. Lesions occurred rapidly after establishment of the NEC-flora (*e.g.* thickening of the caecal wall with gas cysts, hemorrhagic ulcerations, necrotic areas), whereas did less in the presence of *Bifidobacterium infantis* and *Bifidobacterium longum* (30). Supplementing the quails' diet with oligofructose induced an increase in the level of bifidobacteria which prevented overgrowth of bacteria implicated in NEC (*e.g. Escherichia coli*, *Clostridium perfringens*, *Clostridium difficile*, and *Clostridium ramosum*) and reduced NEC-like lesions caused by polymicrobial infection (31). Other experiments in mice showed that supplementation with inulin-type fructans reduced intestinal yeast densities after oral challenge of mice with *Candida albicans*, resulting in an enhanced survival rate (23). A combination of oligofructose and *Lactobacillus paracasei* also showed to suppress pathogens (*Clostridium*, enterococci and enterobacteria) in weaning pigs (32). Furthermore, in pigs with cholera toxin-induced secretory

diarrhea, oligofructose suppressed the presence of pathogens and increased lactobacilli numbers (33).

Clinical studies in humans have also shown that inulin-type fructans can protect against pathogen colonization and infection. Critically ill patients have a gut microbial ecology that is in dysbalance and is characterized by high numbers of potential pathogens. Such patients, at risk for developing sepsis (at intensive care unit), when receiving oligofructose (as a synbiotic), had lower numbers of pathogens in their nasogastric aspirates. Treatment with antibiotics, on the other hand, also changes the gut microflora and disrupts normal ecological balance, which often leads to antibiotic-associated diarrhea. In the study of Orrhage *et al*. antibiotic treatment of patients induced a marked decrease in the anaerobic microflora, mainly with a loss of bifidobacteria and an overgrowth in enterococci. Oligofructose administration (as synbiotic) in those patients restored their numbers of lactobacilli and bifidobacteria (34). Also, in patients with *Clostridium difficile*-associated diarrhea, which frequently occurs after antibiotic-therapy, oligofructose suppressed colonization with *C. difficile* and increased bifidobacteria levels. These changes were accompanied with a lower relapse of diarrhea and reduced length of hospital stay (35).

Chronic inflammatory bowel diseases such as ulcerative colitis, Crohn's disease and pouchitis are though to have their etiology to some extent linked to the composition of the colonic microbial community and its activities. Although members of the gut microbiota normally do not induce disease, in genetically susceptible hosts, an altered immune response towards normal commensal organisms is estimated to drive the inflammatory process towards a state of chronic inflammation (36). The effect of inulin-type fructans in modulating the disease process has been repeatedly demonstrated in experimental models in which inflammation was induced by chemical agents such as DSS (37) or TNBS (38). In each of these, administration of inulin-type fructans (alone or as symbiotic) to the diets of animals reduced the inflammatory process (*e.g.* MPO, IF-γ, PGE_2), improved clinical and histological markers with a reduction in corresponding lesions. The HLA-B27 transgenic (TG) rat is a well-characterised model of chronic intestinal inflammation. The model spontaneously develops colitis. Oral administration of oligofructose-enriched inulin to HLA-B27 TG rats decreased gross cecal and inflammatory histological scores in the caecum and colon and altered mucosal cytokine profiles (decreased IL-1β and increased TGF-β levels). Cytokine responses of mesenteric lymph node (MLN) cells were also studied *in vitro* by their response to cecal bacterial lysates (CBL). Stimulation of MLN cells by CBL from oligofructose-enriched inulin-treated TG rats induced a lower interferon-γ response (39).

In humans suffering from ulcerative colitis, it has been described that bifidobacteria populations are about 30-fold lower compared to that in healthy individuals. This let to the hypothesis that restoring bifidobacteria populations in these patients by the use of pre- or synbiotics may influence the disease process. Supplementation of the diet of patients with ulcerative colitis with oligofructose-enriched inulin together with a probiotic (*Bifidobacterium longum*) for 1 month resulted in a 42-fold increase in bifidobacteria numbers in mucosal biopsies. Clinical intervention study in ulcerative patients supplemented with the same synbiotic as indicated above; showed improvement of the clinical appearance of chronic inflammation, evidenced by a reduction in sigmoidoscopy scores, reduction in acute inflammatory activity (TNF-α and IL1-α) and regeneration of the epithelial tissue (40). In another placebo-controlled clinical trial in patients with ulcerative colitis, oligofructose-enriched inulin lowered the levels of calprotectin in the faeces thereby improving the patients' response to therapy by mitigating intestinal inflammation (41). A reduction of the inflammation and associated factors was observed also in patients with an ileal pouch-anal anastomosis after therapy with inulin-type fructans (42). Moreover, in patients with active ileo-colonic Crohn's disease, dietary intervention with a combination of inulin and oligofructose has been shown to lead towards an improvement of the disease activity (reduction in Harvey Bradshaw Index) and enhanced lamina propria denritic cell IL-10 production and TLR2 and TLR4 expression. Strikingly different changes in mucosa microbiota following inulin supplementation were observed between patients who entered remission and those that did not. Patients who entered remission had an increase in mucosal levels of bifidobacteria (43).

MICROBIOTA AND COLONIC CANCER

Diet has a strong influence on the etiology of colorectal cancers and intestinal bacterial metabolism can generate substances derived from food with genotoxic, carcinogenic, and tumour-promoting potential. Administration of weanling rats with different types of inulin-type fructans induced a reduction in the number of aberrant crypt foci (ACF) in the proximal, distal and total colon. ACF are pre-neoplastic lesions found in the etiology of most colon cancers. Such reductions in the distal parts of the colon (and the whole colon) were most pronounced when rats were fed oligofructose-enriched inulin and resulted in the lowest numbers of colonic ACF (44). Long-term studies with probiotics, prebiotics and synbiotics in rats with AOM-induced colon cancer showed a reduction in the number of colon carcinomas when supplemented with oligofructose-enriched inulin either alone or given as a synbiotic (with *Lactobacillus rhamnosus* GG and *bifidobacterium lactis* Bb12) (45). Treatment with the carcinogen AOM suppressed the rats' natural killer (NK-) cytotoxicity in the Peyer's patches (PP). NK cells are involved in both the recognition and subsequent elimination of tumour cells. Suppression of this NK-cell activity may subsequently contribute to tumour growth. Interestingly, the changes in tumour formation upon the intervention coincided with a stimulation of immune functions within the gut-associated lymphoid tissue (GALT) and PP which are the primary lymphoid tissues responsive upon oral intake of prebiotics or synbiotics. The supplementation with oligofructose-enriched inulin (alone or as a synbiotic) prevented such carcinogen-induced NK-cell suppression in PP. After 33 weeks of treatment, immunological investigation of the rat's PP revealed significant higher NK cell-like activity after intake of the pre- or synbiotic. Other immunological markers in PP cells that differed upon both interventions were the stimulation in IL-10 production. This increase in IL-10 cytokine production in PP was also found in a previous study of the same authors after short-term exposure of AOM-rats to prebiotics, probiotics and synbiotics (45).

A phase-II anticancer study, randomised, double-blind and placebo-controlled in 80 patients with a history of colon cancer or polyps, and supplemented with a synbiotic (oligofructose-enriched inulin and *Bifidobacterium lactis* Bb12 and *Lactobacillus rhamnosus* GG) for 12 weeks, showed increased levels of bifidobacteria and lactobacilli. This was accompanied by a decrease in the numbers of pathogens (coliforms and *Clostridium perfringens*). The altered composition of the colonic bacterial ecosystem beneficially affected the metabolic activity in this organ. This was obvious from the decreased DNA damage in the colonic mucosa (measured by the comet assay) and the tendency to lower the level of colorectal proliferation (surrogate biomarker for colon cancer risk) in polyp patients (no measures were taken in cancer patients). Other effects were the decreased cytotoxicity of the faecal water. The fecal water of synbiotic-fed polyp patients also showed a lower level of cell necrosis as demonstrated by the lower cytotoxic potential in (HCT116 cell types). This indicates that the synbiotic effectively prevented cell

death of the colonic epithelium (26 - SYNCAN project, Synbiotics and Cancer Prevention started, QLK-1999-00346).

INTESTINAL MICROBIOTA, ADIPOSE TISSUE AND INFLAMMATION

Obesity and metabolic disorders (insulin resistance, hyperlipaemia) are tightly linked to a chronic low-grade state of inflammation (elevated levels of circulating inflammatory markers such as IL-6, and C-reactive protein). It is hypothesized that an altered gut microbiota in the obese state could contribute towards low grade inflammation resulting in the development of metabolic diseases associated with the condition (*e.g.* diabetes, cardiovascular disease, *etc*.) (46). However, the factors triggering such metabolic alterations remain to be determined.

In the obese lower levels of Bacteroidetes and higher levels of the phylum Firmicutes in the colonic microbiota as compared to lean counterparts are found (47). These observations have been associated with increased gut fermentation and calorific bioavailability to the host. Moreover, feeding high fat diets have been demonstrated to alter dramatically the microbiota composition in mice with reducing the quantities of dominant Gram-positive groups, *e.g. Bifidobacterium spp*. and *E. rectale - C. coccoides* groups, and the murine Gram-negative group, *Bacteroides* MB (48). Recent studies in animal models have shown that such changes within the microbial ecology or functional activities of the gut microbiota can induce a metabolic shift towards a pro-inflammatory phenotype, whole-body, liver and adipose tissue weight gain and impaired glucose metabolism. Factors of microbial origins (*e.g.* bacterial lipopolysaccharides) are hypothesized to lie at the basis of such effects. In mice, high-fat feeding let to (low level of) metabolic endotoxemia, low inflammatory tone, increasing macrophage infiltration in adipose tissue and dysregulating lipid and glucose metabolism. Multiple correlation analyses showed that the level of endotoxaemia was negatively correlated with *Bifidobacterium spp.*, but no relationship was seen between any other bacterial groups. On the other hand, restoration of the levels of bifidobacteria in the intestine of mice upon oligofructose supplementation lowered endotoxaemia and the level of microbial toxins and improved mucosal barrier function. Interestingly, the lower body weight and visceral adipose tissue mass in the oligofructose group (compared with the not supplemented high-fat fed mice) showed a positive correlation with the endotoxin plasma levels and negatively with the levels of bifidobacteria. Moreover, levels of mRNA of IL-1, TNF-α, and plasminogen activator inhibitor type-1 (Pai-1, or Serpine-1) in adipose tissue were increased in high-fat fed mice, whereas the levels were blunted with oligofructose feeding. In addition, a normalisation of IL-1α and IL-6 cytokines was observed upon oligofructose feeding. These data indicate that a lower fat mass and body weight 'only' are not a prerequisite for a lower inflammatory tone and that this effect is accompanied by prebiotic changes in the microbiota. Plasma cytokines were positively correlated with plasma endotoxin levels and negatively with bifidobacteria levels (48). In diabetic mice, feeding oligofructose reduced hepatic levels of phosphorylate IKK-β and NFKB, suggestive of a reduction in the hepatic inflammatory status which might relate to an improvement of the insulin sensitivity (49).

MICROBIOTA AND INTESTINAL METABOLISATION OF PHYTONUTRIENTS

Polyphenol aglycones and a few glucosides (*e.g.* quercetin 3-glucoside) can be absorbed in the intestine, but the efficiency of polyphenol absorption is generally low and differs widely depending on the type and structure of the polyphenol. An extensive review comparing bioavailability and bioefficacy of polyphenolic compounds showed that polyphenols which have high absorption (after intake of 50 mg dose) are gallic acid (C_{max}= 4 μM), followed by isoflavones glycosides (daidzin, genistin) (C_{max}= 2 μM), flavanones and quercetin glucosides. Proanthocyanidins and anthocyanidins are poorly absorbed (C_{max}= 0.02 μM) (8). Oral administrations of chlorogenic and caffeic acid supplements, found that these phenolic acids are absorbed for about 33 and 95 %, respectively. However, cholorogenic acid accounts for 0.3% in urine and caffeic acid was found for 11% in urine. Thus after absorption chlororgenic and caffeic acid are metabolised extensively in other compounds (50).

Non absorbed polyphenols reach the colon. In the colon, the microbiota (*e.g. Escherichia coli*, *Bifidobacterium sp.*, *Lactobacillus sp.*, *Bacteroides sp.*, *Eubacterium sp.*) hydrolyses the glycosides to aglycones, which can further be metabolised to aromatic acids like phenylacetic, phenylpropionic, phenylvaleric and benzoic acid. Those phenolic acids are well absorbed through the colonic epithelium (2-6). With respect to the bioavailability of dietary polyphenols and their colonic metabolites, more research is currently needed in order to clarify the contribution of these different metabolites to *in vivo* anti-oxidant efficacy.

The importance of the colonic metabolisation has already been demonstrated for some polyphenolic compounds in different studies. First, for the hydroxycinnamic acids, which are naturally esterified in plant products, metabolisation is carried out by the gut microflora (2, 3, 51). Bacterial species like *Escherichia coli*, *Bifidobacterium lactis* and *Lactobacillus gasseri* express cinnamoyl esterase activity and are responsible for the cleavage of the ester bond between caffeic and quinic acid in chlorogenic acid (51). Secondly, regarding the flavonoid group, the microbiota enzymes from *Bacteroides distasonis*, *B. uniformis* and *B. ovatus* are important (*e.g.* α-rhamnosidases hydrolyse rutinoside to quercetin). *Enterococcus casseliflavus* and *Eubacterium ramulus* metabolise quercetin-3-O-glucoside to form formate, acetate, lactate, the aglycone quercetin, butyrate, ethanol and 3,4-dihydroxyphenylacetic acid. Strains belonging to the *Clostridium*, *Bacteroides* and *Eubacteria* genera are also mentioned to cleave the C-ring of quercetin resulting in 3,4-dihydroxyphenylacetic acid and protocatechuic acid (2). *Eubacterium ramulus* has also an impact on naringenin, apigenin and the isoflavone genistin (7, 9). In another study, the role of gut microflora in the absorption and metabolism of isoflavones and lignans was investigated using germ-free rats and rats associated with human faecal bacteria. Soy and soy products contain the isoflavones genistein and daidzein usually in the form of glycosides (genistin and daidzin). Germ-free rats fed soy-isoflavone only excrete the aglycones daidzein and genistein. Hydrolysation of the isoflavone glycosides occurs in the proximal intestinal tract. In contrast, the metabolites equol, O-desmethylangolensin and the lignan enterolactone were only detectable in the urine of human flora associated (HFA) rats. This demonstrates the importance of the gut microbiota in the metabolisation of isoflavones and lignans. The colonization of germ-free rats with faecal flora from human subjects, capable to convert daidzein to equol, results in the excretion of the metabolites. In the urine of HFA rats associated with a faecal flora from a low-equol producing subject no detectable equol quantities were found. This indicates that some subjects are unable to produce equol due to the lack of specific components of gut microbiota (52).

Apart from inter-individual variation in daily intake of polyphenols, inter-individual differences in the composition of the human microbiota may lead to differences in bioavailability and bioefficacy of polyphenols and their metabolites. Research is needed to understand the role of the colonic microflora in the

metabolisation of polyphenols and to evaluate the biological effects, including the anti-oxidative effects of these microbial metabolites.

In this respect, dietary strategies that modulate the composition of the microbiota enhancing metabolisation of polyphenols are hypothesized to improve bioavailability of polyphenols and could potentate their activity. In ovariectomized rats, feeding simultaneously soy isoflavones and fructo-oligosaccharides increased plasma levels of genistein, daidzein, and equol compared to isoflavone feeding alone. This effect also maximised the protective effects of isoflavones agains gonadal induced osteopenia (53). Inulin-type fructans have also been shown to increase plasma and urinary concentrations of soy-derived genistein and daidzein and their aglycone forms in humans. In post-menopausal women who were asked to consume a conjugated form of soybean isoflavones together with inulin it was found that 24 hr plasma levels (measured as the area under the curve) were resp. 38% for daidzein and 91% for genistein higher when compared to the isoflavone intake alone (54).

OUTLOOK AND PERSPECTIVES

The number of publications about food-based strategies to modulate the composition of the microbiota and their associated health effects has increased steadily over the last decade. This is expecting to continue since the importance of a well balanced colonic microbiota and its activities, as being a key factor in the modulation of human immunity, anti-oxidant defence, metabolism and endocrine activities, is more and more recognized. As new insights are being elucidated about the composition of the microbiota and its species diversity, the metabolic pathways of substrate degradation and the role in health and disease, interest will continue to rise. Together with this, it is of paramount importance to develop strategies to modulate this microbiota in a way to reduce the risk of developing disease through dietary means and the use of functional foods offers great value in this regard.

Conflict of interests: None declared.

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