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Human gut microbiota: does diet matter?

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The human oro-gastrointestinal (GI) tract is a complex system, consisting of oral cavity, pharynx, oesophagus, stomach, small intestine, large intestine, rectum and anus, which all together with the accessory digestive organs constitute the digestive system. The function of the digestive system is to break down dietary constituents into small molecules and then absorb these for subsequent distribution throughout the body. Besides digestion and carbohydrate metabolism, the indigenous microbiota has an important influence on host physiological, nutritional and immunological processes, and commensal bacteria are able to modulate the expression of host genes that regulate diverse and fundamental physiological functions. The main external factors that can affect the composition of the microbial community in generally healthy adults include major dietary changes and antibiotic therapy. Changes in some selected bacterial groups have been observed due to controlled changes to the normal diet e.g. high-protein diet, high-fat diet, prebiotics, probiotics and polyphenols. More specifically, changes in the type and quantity of non-digestible carbohydrates in the human diet influence both the metabolic products formed in the lower regions of the GI tract and the bacterial populations detected in faeces. The interactions between dietary factors, gut microbiota and host metabolism are increasingly demonstrated to be important for maintaining homeostasis and health. Therefore the aim of this review is to summarise the effect of diet, and especially dietary interventions, on the human gut microbiota. Furthermore, the most important confounding factors (methodologies used and intrinsic human factors) in relation to gut microbiota analyses are elucidated.

Gut microbiota: Human gut: Diet

In the past 10 years, there has been a wealth of studies in which the relationship between the human gut microbiota and human health has been investigated. Moreover, recently there have been several human health-related microbiota studies with partly contradictory results regarding e.g. obesity-related microbiota and abundance of bifidobacteria in the faecal microbiota of babies. As it is likely that at least some of the differences may be explained by the methodology applied, it is of utmost importance that when reading articles related to human gut microbiota studies the most important confounding factors are known.

Human gut microbiota

In an adult human individual, resident bacteria outnumber human cells by a factor of ten; each adult harbours on average 10^{13} mammalian cells and 10^{14} microbial cells[1]. Most of the microbes, typically 10^{11}–10^{12} microbes/g, can be found in faeces and from the large intestine[2–4], which is considered to be a complex fermentor with a metabolic potential to rival that of the liver[5]. The environmental determinants, namely temperature, pH, redox potential, atmospheric composition, water activity, salinity and light, within each region of the human oro-gastrointestinal (GI) tract are very

Abbreviations: GI, gastrointestinal; DF, dietary fibre; FISH, fluorescent *in situ* hybridisation; FOS, fructo-oligosaccharide; MZ, monozygotic.
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different, and therefore each region has its own distinctive microbiota[6]. Since digestive enzymes are not secreted by the mucosa of the large intestine, further breakdown of dietary constituents is carried out by the resident microbiota[6]. Carbohydrates are mainly fermented in the proximal colon, whereas the fermentation of proteins takes place mainly in the distal colon. Transit time of digesta through the colon strongly influences the activities of the gut microbiota. The mean transit time of the oro-GI tract has been reported to be approximately 70 h in UK people consuming a normal daily diet[7,8]. The primary activity of the caecum and colon microbiota is the breakdown of carbohydrates not digested in the ileum to SCFA, which are then rapidly absorbed. The principal products of carbohydrate fermentation are SCFA (acetate, propionate and butyrate), hydrogen and CO_2 gasses and bacterial cell mass (biomass)[9]. The amount of energy derived from SCFA accounts for up to 10 % of the total energy requirement of human subjects[10]. From a nutritional point of view, the SCFA are important since they not only provide the body with energy but are also metabolised in different tissues[9].

The microbiota in the colon and faeces is extremely diverse and on the basis of estimations from culture-based and molecular studies more than 1200 prevalent bacterial species altogether reside there. Each individual harbours at least 160 such species[11,12]. Under normal circumstances, predominant intestinal microbiota of an adult individual is fairly stable. However, in studies where the long-term temporal stability of the predominant microbiota has been assessed from healthy subjects, the number of subjects has been limited[13–16]. The human GI-tract, although harbouring a vast number of microbes, has only a limited diversity at the phylum level. Microbes from seven bacterial phyla (Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, Verromicrobia and Cyanobacteria-like) and one archael phylum (Euryarchaeota) have been detected in the human intestine[17–21]. However, the majority of the GI-tract population are representatives of three phyla: the Firmicutes (Families Lachnospiraceae and Ruminococcaceae), the Bacteroidetes (Bacteroidaceae, Prevotellaceae and Rikenellaceae) and the Actinobacteria (Bifidobacteriaceae and Coriobacteriaceae).

Faecal microbiota is dominated by bacteria from Families Lachnospiraceae (*Eubacterium rectale* group) and Ruminococcaceae (*Clostridium leptum* group) and Bacteroidaceae/Prevotellaceae[3,4,17,18,22], which together account for 60–80 % of the faecal bacteria of healthy adults[22–24]. The bacteria belonging to the Family Lachnospiraceae comprise on average 10–45 % of the total faecal bacteria as detected with quantitative hybridisation-based methods[3,4,22–30] and the bacteria within Ruminococcaceae 16–27 %, thus co-dominating with the bacteria within Lachnospiraceae[22–25,30–32]. Many members of the Lachnospiraceae and Ruminococcaceae are polysaccharolytic and produce butyrate. In addition, they are obligately anaerobic. Members of the Bacteroidetes phylum comprise 12–60 % of the total faecal bacteria depending on which method has been used for the quantification of this group[22–24,33,34]. *Bacteroides* spp. are saccharolytic bacteria that contain a wealth of polysaccharide-degrading enzymes. They have an excellent ability to ferment simple and complex sugars and polysaccharides, producing acetate and succinate as major metabolic end products. In addition, most species are weakly proteolytic[35]. Most of the bacteria within Prevotellaceae and Porphyromonadaceae have been isolated from the oral cavity and are commonly found in oral sites in culture-based studies, whereas with DNA-based molecular studies members of Prevotellaceae and Porphyromonadaceae have also been found in faeces.

Bifidobacterium spp., *Lactobacillus*-group and bacteria within the Family Coriobacteriaceae although found in most human subjects, comprise smaller populations among faecal bacteria in adults (bifidobacteria 0·5–6 %, lactobacilli <1–3 % and Coriobacteriaceae 1–5 % of the total faecal bacteria)[3–4,22–24,30]. Moreover, there are several bacteria/bacterial groups, which usually comprise <1–2 % of the total faecal microbiota of healthy adults, such as *Akkermancia muciniphila*, 'true clostridia' (Family Clostridiaceae), enterobacteria, streptococci, bacteria within the Families Peptostreptococcaceae, Erysipelotrichaceae, Veillonellaceae, Eubacteriaceae, in addition to sulphate-reducing bacteria and/or methanogens[3,22–23,27,36–39]. Besides bacteria, low levels of viruses, archaea (<1 %) and eukaryotes (≤2 %) are also found from the large intestine[39–41].

The effect of sampling and used analysis methods on the results obtained from gastrointestinal-tract samples

Conditions during sample transportation have a major impact on sample quality[42–44] and therefore the time between sampling and further processing and storing should be limited to the minimum. It should be kept in mind that regardless of how sophisticated the applied method for characterisation of the microbial community is, the result can be only as good as the sample that arrives in the laboratory. In addition, when molecular techniques are applied to study microbial communities of the GI tract, oral bacteria may be detected with molecular techniques from faeces as well, since the human oro-GI tract is an open system. In studies with snapshot samples, it is difficult to confer that the detected bacteria are resident and not just transient. Therefore to be able to establish resident microbiome, follow-up samples would be needed.

Most of the steps in the processing of faecal samples may affect the microbiological results obtained. The sampling and storage conditions affect the results[42–44], although not as extensively as the following steps, especially the DNA extraction[45–48]. One week storage at −20 °C decreases the detected numbers of Gram-negative *Bacteroides* spp. by over one logarithmic unit as compared with the fresh samples, whereas the storage temperature does not have such a significant effect on the Gram-positive bacteria (e.g. Firmicutes and Actinobacteria)[47]. Since the analysis of fresh faecal

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samples, especially in the case of large cohorts, is impractical and mostly also impossible, the effect of the storage conditions on the results obtained should be taken into account when the results are discussed.

When DNA extraction is performed using solely chemical treatments combined with heat treatments, the results for some bacterial groups may be as much as four logarithmic units lower as compared with a DNA extraction method in which rigorous mechanical disruption is applied[47]. Actinobacteria, in particular, are underestimated with many currently used techniques, most probably due to their high guanine–cytosine content[49]. In a study of Nakamura *et al.*[50] it was shown that when the same samples were examined with quantitative PCR (enzymatic DNA extraction) and fluorescent *in situ* hybridisation (FISH), the difference in results was enormous. The proportions of bifidobacteria with quantitative PCR was 0·1–1·7 %, whereas the proportion with FISH was 28–84 %[50]. Moreover, the number of bacteria within Family Coriobacteriaceae has been shown to be 5–6 logarithmic units higher in a sample that was initially stored at +4 °C for 2 d and thereafter at −70 °C and when DNA extraction was performed mechanically than on the same sample that was stored at −70 °C and the DNA extraction was performed enzymatically[47]. This may also explain why in some studies members of Coriobacteriaceae are not considered to be the part of the normal dominant microbiota[17], whereas in others, especially those conducted with FISH, bacteria within Coriobacteriaceae constitute 1–8 % of the total population of the human gut microbiota[23,24,38,51].

Another methodological aspect that may cause confusion is that different probes/primers target different bacterial populations, such as in the detection of Bacteroidetes and Firmicutes. In metagenomic/16S rRNA-based next-generation sequencing studies the authors usually refer to Bacteroidetes, whereas in studies conducted with FISH/PCR, the studied bacterial population is usually narrower (e.g. *Bacteroides* spp. or *Bacteroides-Prevotella* group)[52]. The same controversy applies to Firmicutes. In many next-generation sequencing studies, Firmicutes are discussed as a single group, whereas in fact it contains several different bacterial classes e.g. Bacilli, Clostridia, Erysipelotrichia, which all have different metabolic properties. Furthermore, when PCR/FISH is used, each of these groups is usually studied separately. Moreover, since the phylum Bacteroidetes is more thoroughly characterised than the phylum Firmicutes, it is possible that the representation of Bacteroidetes in metagenomic studies is overestimated.

When microbial communities are examined, especially using several different techniques and/or next-generation sequencing, the quantity of data generated is enormous. Therefore the use of suitable algorithms e.g. measurement of microbial diversity and correct interpretation of the results are of utmost importance[53–61]. It should also be borne in mind that even if statistical significance is achieved, the observed changes may not necessarily be of biological significance.

Factors that affect the gut microbiota

There are several factors that affect the composition of the human GI microbiota, such as genetics, sex, ethnicity, age, medication, diseases/disorders and last but not least the diet.

Genetics of the host and geography

Twins, especially monozygotic (MZ) twins, have been reported to have more similar interindividual faecal microbiota than unrelated people. In addition, twins and their mothers have a more similar microbiota than unrelated individuals. These findings have led to the conclusion that the host genotype affects the development of the gut microbiota and gut bacterial composition[14,21,62,63]. However, the aforementioned studies have mainly been conducted with MZ pairs concordant for leanness or obesity. Simoes *et al.*[64] studied MZ twins concordant and discordant for obesity, and found that the concordant normal-weight MZ twins had more similar bacterial populations than the MZ twins discordant for obesity. These findings also address the importance of the diet in addition to the genetic drivers[64]. Although the genetics or the shared environmental factors during upbringing result in more similar bacterial populations, viromes have been shown to be unique to individuals regardless of their degree of genetic relatedness[40].

Besides genetics, the effect of the geographic origin (which may also include genetic differences and environmental sources of variation) has also been studied in relation to the gut microbiota composition. Even in Europe some differences may be found between different countries, e.g. proportions of bifidobacteria have been found to be 2- to 3-fold higher in an Italian adult study population than in other European study populations[23,24]. In addition, when 6-week-old infants across Europe were studied, geography was a more prominent factor than delivery mode, breast-feeding and antibiotics. In infants, children from Northern European countries had more bifidobacteria, whereas the Southern European infants were associated with more diverse microbiota and higher numbers of *Bacteroides* than the Northern European children[65]. In the studies involving people from different continents, the bacterial population differences have been more distinct. In a few recent studies, there have been consistent results that the European and North-American faecal microbiota differ significantly from the Southern-American, African and Chinese faecal microbiota[66–68]. However, in all of these studies it is not possible to exclude the impact of other possible confounding factors, and therefore dietary habits and genetics may also contribute to the differences.

Age

Bacterial colonisation of the infant GI tract is influenced by e.g. mode of delivery, prematurity, type of feeding (breast feeding *v.* formula feeding), antibiotic treatment of the child or the mother, lifestyle and geographics[65,69–74]. The earliest colonisers are usually facultative anaerobic bacteria such as Enterobacteriaceae,

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streptococci and staphylococci, whereas later colonisers tend to be strict anaerobes e.g. bifidobacteria, clostridia and *Bacteroides* spp. regardless of the infant's geographical origin and methods used for the detection[65,75–80]. Immediately after birth, the rectal microbiota of vaginally delivered babies resembles their own mother's vaginal microbiota, whereas the rectal microbiota of babies delivered by Caesarean section resembles that of the skin[81]. The gut microbiota of preterm infants is less diverse than those of full-term babies[70,73,82–84]. There are numerous studies in which the predominance of bifidobacteria in exclusively breast-fed infants has been found[65,66,74,85–88]. At age 3–6 weeks, exclusively breast-fed infants harbour higher numbers of bifidobacteria, whereas formula-fed babies have more diverse microbiota, lower numbers of bifidobacteria and higher numbers of *Bacteroides*, Lachnospiraceae, *Lactobacillus* group, *Clostridium difficile* and Coriobacteriaceae[65,71,74,87,89,90]. By the end of the first year of life, when the child has already started to eat the same foods as the adults, the gut microbiota starts to converge towards a profile characteristic of the adult microbiota[76,90]. However, the faecal bacterial diversity is still lower. By the end of the second to third year, the phylogenetic composition evolves towards the adult-like composition[66,91].

The GI microbiota evolves with age[92]. Dental deterioration, salivary function, digestion, slower intestinal transit time and changes in diet and physical activity may affect the GI microbiota of ageing people. Interest, as well as the number of studies, in the GI microbiota of elderly people has grown as life expectancy in the Western world has rapidly increased. The elderly have been reported to have relatively stable microbiota[93–95]. However, the microbiota of the elderly has been reported to be more diverse and to contain partly different core microbiota as compared with younger adults[93,94,96,97]. Moreover, inter-individual variation is greater in elderly people as compared with younger adults[2,94,98].

Medication

In addition to dietary components, the other important external factor affecting the microbiota is antibiotic use. However, different types of antibiotics have different types of action mechanisms and thus different effects on the human microbiota[99,100]. In addition, individual human responses may be different. Bifidobacteria are typically susceptible to the majority of clinically relevant antibiotics such as penicillins (β-lactam antibiotics), cephalosporins and macrolides[101–106], and most of the commensal gut bacteria (e.g. bifidobacteria, *Bacteroides* spp.) to amoxicillin (β-lactam antibiotic) and clavulanate[102].

The effect of diet on human gut microbiota

The importance of the metabolic activities of the gut microbiota from the host's perspective

From the host's perspective, there are numerous activities of the commensal gut microbiota that are of great importance to health. Carbohydrates are mainly fermented in the proximal colon, whereas the fermentation of proteins takes place mainly in the distal colon. However, the metabolic output of the microbial community depends not only on available substrates, but also on the gut environment, with the pH playing a major role. For example, at pH 6·7 *Bacteroides* spp. predominate, whereas at pH 5·5 bacteria related to *E. rectale* predominate[107–109]. The main saccharolytic genera in the human GI-tract are *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Eubacterium*, *Lactobacillus* and *Ruminococcus*. The saccharolytic genera are able to produce SCFA, which have both local and systematic beneficial biological effects. A wide range of bacteria have proteolytic activities, such as clostridia, and species within genera *Propionibacterium*, *Prevotella*, *Bifidobacterium* and *Bacteroides*. Protein metabolism, however, is not as favourable to the host as carbohydrate metabolism; some of the end-products of amino acid metabolism may be deleterious to the host, e.g. ammonia, amines and phenol compounds. Some species of the genera *Bacteroides*, ruminococci and *Akkermansia* are able to break down mucin. Moreover, several *Eubacterium* spp. and *Clostridium* spp. are able to dehydroxylate bile acids, some *Clostridium* spp. transform conjugated bilirubin, *Eubacterium coprostanoligenes* is able to convert cholesterol to coprostanol and *Bacteroides* spp. inactivate tryptic activity, have dipeptidase activity and play a key role in the enterohepatic circulation of bile acids[7,35,110–114].

In addition to the individual activities, cross-feeding between the gut microbes and the metabolic networks thus created are also of great importance. For example, it has been shown *in vitro* that lactate produced by *Bifidobacterium adolescentis* as a fermentation product from fructo-oligosaccharide (FOS) and starch was further utilised by butyrate producers, which were not able to grow solely on FOS and starch[115]. In addition, *Roseburia intestinalis* and *Anaerostipes caccae* were able to grow with *Bifidobacterium longum* using FOS; *R. intestinalis* was able to grow on the FOS-supplemented medium when acetate, a major fermentation product of *B. longum*, was added to the medium, whereas *A. caccae* was able to utilise fructose that was released during the bifidobacterial fermentation of FOS[116]. Besides other survival mechanisms, gene transfer within and from outside the gut microbiota has also been shown to occur. In Japan, where consumption of marine algae is high, a Japanese gut bacterium (*Bacteroides plebeius*) has acquired genes coding for porphyromonases, agarases and associated proteins and thus the ability to utilise marine algae. These algae are not readily fermentable by Western gut microbiota[117].

Dietary interventions v. *habitual diet*

The main external factors that can affect the composition of the microbial community in generally healthy adults include major dietary changes and antibiotic therapy. Changes in some selected bacterial groups have been observed due to controlled changes to the normal diet e.g. high-protein diet[118,119], prebiotics[97,120–122], probiotics[123–125], weight-loss diet[20,126,127] and berries[128]. More specifically, changes in the type and quantity of non-digestible carbohydrates in the human diet influence both the metabolic products formed in the lower regions of the GI tract and levels of bacterial populations detected in faeces[129]. The interactions between dietary factors, gut microbiota and host metabolism are important for maintaining homeostasis and health[130].

The impact of habitual diet on faecal microbiota has been studied for decades. In older culture-based studies, it was found that numbers of bacteroides were lower and numbers of enterococci and *Escherichia coli* higher in Ugandan, Indian and Japanese people on a high-carbohydrate diet as compared with people on a Western diet[131,132]. However, when English people on a strictly vegetarian diet were studied, their microbiota resembled those of people on a Western diet more than the microbiota of other vegetarian people from different continents[131]. Similarly, numbers of bacteroides and clostridia were lower in the Nigerian Maguzawa tribal people (predominantly cereal diet) than in the other dietary groups[133]. In more recent molecular studies, in which gut microbiota from different parts of the world has been compared, a significant correlation between habitual diet and faecal microbiota has also been found. African children consuming a diet low in fat and animal protein and rich in starch, fibre and plant polysaccharides (predominantly vegetarian) had significantly more Bacteroidetes and Actinobacteria and less Firmicutes and Proteobacteria than European children, who had a diet high in animal protein, fat, sugar and starch, but low in fibre. Moreover, members of the genera *Prevotella* and *Xylanibacter* were found exclusively from the African children[68]. Partly similarly to these findings, *Prevotella* enterotype was previously found to be associated with high consumption of carbohydrates and with both vegetarian and vegan habitual diets, whereas *Bacteroides* enterotype was associated with high consumption of animal protein, amino acids and saturated fats[134]. Strict vegetarians have higher percentages and/or numbers of Lachnospiraceae and *Clostridium ramosum* group (Clostridial cluster XVIII) bacteria than omnivorous people[135,136], whereas vegans have lower faecal numbers of *Bacteroides* spp., bifidobacteria and Enterobacteriaceae[137]. In older culture-based studies, no fusobacteria were found in faecal samples of vegetarian people. In addition, the total count of anaerobic streptococci, *Peptostreptococcus* spp., *Actinomyces* spp. and *Lactobacillus* spp. were higher in strict vegetarians as compared with people on a traditional Western diet[2,132,138,139].

Hospitalisation of Scottish elderly people has resulted in a decrease of faecal *Bacteroides–Prevotella* group and *Ruminococcus albus* prevalence, while increasing the *Enterococcus faecalis* prevalence, as compared with healthy elderly people. Moreover, when the hospitalisation was combined with antibiotic treatment, reductions in other bacterial groups were also observed[140]. Long-term residential care of Irish elderly people has been associated with a higher proportion of bacteria belonging to the phylum Bacteroidetes and several genera from other phyla, whereas community-dwelling elderly people had higher proportions of bacteria belonging to the phylum Firmicutes. In addition, the microbiota of elderly people in long-term care was significantly less diverse. However, the results also correlated with the diet; if the elderly people were grouped according to their diet, the clustering was similar to that based on the residence location. After 1 month residential care, the diet was converged to 'long-term diet', but it took 1 year for the microbiota to clearly cluster within the long-term residential type. Collectively this indicates that the composition of the faecal microbiota is determined rather by the composition and diversity of the diet than by the location of residence[98].

Since it has not always been clear which part of the habitual diet induces the changes observed in the GI-tract microbiota, numerous dietary interventions have been conducted to elucidate the changes induced by specific nutritional substances, e.g. different types of fibres, proteins, fats and polyphenols. In addition, the effects of probotics and prebiotics on the GI-tract microbiota have been widely studied. Therefore, the effects of the aforementioned nutritional substances on GI-tract microbiota are addressed in more detail.

Fermentable dietary carbohydrates

Dietary components that escape digestion by endogenous enzymes in the upper GI tract become available substrates in the large intestine[7]. Dietary fibre (DF) is a normal constituent of most foods derived from plants[141]. These 'non-digestible' dietary carbohydrate substrates include resistant starch, plant cell-wall material (non-starch polysaccharides) and oligosaccharides[7]. In the human colon, DF is metabolised by the microbiota to SCFA, comprising mainly acetic, propionic and butyric acids. SCFA have been implicated to have both local and systemic beneficial biological effects in the human body; acetate is readily absorbed and transported to the liver; propionate is a substrate for hepatic gluconeogenesis; butyrate is the preferred fuel of the colonocytes and also plays a major role in the regulation of cell proliferation and differentiation[7]. Several excellent review-papers already exist[130,142–147], and therefore we will not go into detail with the dietary interventions with fibre.

In vitro and *in vivo* evidence indicate that a bacterial group related to *Faecalibacterium prausnitzii*, *Roseburia* and *E. rectale* plays a major role in mediating the butyrogenic effect of fermentable dietary carbohydrates[107,148–150]. In addition, it has been shown in numerous studies that as dietary carbohydrate content is reduced in the diet the count of *F. prausnitzii* declines, respectively[129,151]. However, some lactate-utilising bacteria within Lachnospiraceae produce less butyrate in the presence of lactate-utilising sulphate-reducing bacteria. Moreover, in the presence of higher abundance of lactate, the formation of butyrate was reduced even more and the formation of hydrogen sulphide was promoted[152].

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Cereal grains are a good source of DF. The main DF components of cereal grains are arabinoxylan, cellulose, β-glucan, fructan, resistant starch and lignin[153]. The gut microbiota stimulating activities of arabinoxylan (stimulates *Bacteroides* spp. and *Roseburia* spp.), resistant starch (stimulates bifidobacteria, *Bacteroides* spp., *Ruminococcus bromii, E. rectale* and *Roseburi*a spp.), β-glucan (stimulates bifidobacteria) and fructan (stimulates bifidobacteria, *Bacteroides* spp., lactobacilli and butyrate-producers) are well recognised[144,154–161]. In addition, arabinoxylan-oligosaccharides, which are enzymatic hydrolysis products of arabinoxylan, have been shown to stimulate the growth of bifidobacteria in some studies[156]. The effects of cellulose and lignin on gut microbiota are less well known[162,163]. However, it should be noted that e.g. the size of the grain flakes may lead to different bacterial responses: i.e. smaller-sized whole-oat grain flakes (0·53–0·63 mm) resulted in a significant increase in the numbers of *Bacteroides–Prevotella* group bacteria, whereas in a fermentation with larger oat flakes (0·85–1·00 mm) bifidobacterial numbers increased[164]. It has also been shown in *in vitro* model studies using different substrates that the majority of the bacteria attached to wheat bran belonged to Lachnospiraceae and some bacteria were *Bacteroides* spp., whereas *R. bromii*, *B. adolescentis*, *Bifidobacterium breve* and *E. rectale* were found attached to starch. When mucin was used as a substrate, the most commonly found bacteria were *Bifidobacterium bifidum* and an uncultured relative of *Ruminococcus lactaris*[165].

Prebiotics

Prebiotics are non-digestible (by the host) food ingredients that have a beneficial effect through their selective metabolism in the intestinal tract. The prebiotics that currently fulfil the prebiotic criteria are inulin, FOS, galacto-oligosaccharides and lactulose[166]. The best sources of naturally occurring prebiotics may be found in vegetables such as artichokes, onions, chicory, garlic and leeks[167]. There are numerous studies in which the bifidogenic properties of prebiotics are shown[121,122,166,168–171]. In addition, increase in abundance of lactobacilli[121,122] and *F. prausnitzii*[170,172] has been shown. Moreover, in infant formulas, galacto-oligosaccharides + FOS supplementation of cow's milk-based formula has led to a bifidobacterial population which resembled more that of breast-fed infants than purely formula-fed infants[173]. FOS have also positive effects on the intestinal barrier function[174].

Protein

Endogenous protein sources make up approximately one-third of the exogenous dietary protein pool [175,176]. Bacterial amino acid catabolism in the human gut occurs via a number of mechanisms involving either deamination or decarboxylation reactions. The types of SCFA produced from amino acids are dependent on the chemical compositions of the substrates. In addition to SCFA, branched chain-fatty acids and aromatic compounds, namely phenol, indole and a range of phenolic and indolic substituted fatty acids derived from phenylalanine, tyrosine and tryptophan may be formed. Moreover, branched-chain amino acids are slowly fermented by colonic bacteria, with the main acidic products being branched chain-fatty acids one carbon atom shorter than the parent amino acid[177]. Many metabolites produced by amino acid fermentation are harmful to the host. Phenolic and indolic compounds are also thought to act as co-carcinogens, while amines serve as precursors of nitrosamine production[177].

Culture-based studies have shown[118] that the counts of *Bacteroides* spp. and clostridia increased significantly, whereas counts of *B. adolescentis* decreased significantly during a high-beef diet as compared with a meatless diet (fat and fibre contents were essentially the same in both diets). In addition, sulphide concentrations were high on a high-beef diet[178]. Since hydrogen sulphide is toxic to the colonic epithelium and sulphide inhibits butyrate oxidation, dietary sulphide may selectively stimulate the growth of a single group of bacteria, namely sulphate-reducing bacteria, with potentially harmful effects on the epithelium[9,179]. In addition, an intervention diet with a high protein and low carbohydrate content reduced the numbers of *Roseburia*/*E. rectale* group, while increasing proportions of branched-chain fatty acids and concentrations of phenylacetic acid and *N*-nitroso compounds[119]. Moreover, it should be noted that the World Cancer Research Fund released in May 2011 a report based on 1012 clinical trials, in which red and processed meat were convincingly associated with increased risk, whereas foods containing DF, in particular cereal fibre and whole grains, were associated with decreased risk of colorectal cancer[180].

Fat

Fats are composed of fatty acids that are divided into SFA and unsaturated fatty acids. Dietary SFA are mainly obtained from animal products, such as meats and dairy foods, but may also be obtained from some plant sources, such as coconut, cottonseed and palm kernel oils. The major dietary MUFA is oleic acid. Oleic acid is the primary component of olive oil, but may also be found in hazelnut, rapeseed and peanut oils. A carbon chain that contains two or more *cis* double bonds characterises the families of *n*-3 or *n*-6 PUFA. These families cannot be synthesised by the human body[181]. Linoleic (*n*-6 PUFA) and α-linolenic (*n*-3 PUFA) acids form the majority of PUFA in most Western diets. The long-chain *n*-3 PUFA EPA and DHA are found in seafood, especially oily fish[182].

High intake of dietary fat may increase the quantities of bile acids and fat that reach the colon. It has been suggested that the gut microbiota may metabolise dietary fats (producing diacylglycerols from polyunsaturated fats), convert primary bile acids into secondary bile acids and impact on the enterohepatic circulation of bile acids and fat absorption from the small intestine[183]. However, there are only a few human studies in which the effect of high-fat diet on the human intestinal microbiota has been investigated, and especially those in which the correlations between the different types of dietary fat and intestinal microbiota have been investigated. In a study of Brinkworth *et al.*[184], it was shown that a very low-carbohydrate, high-fat diet resulted in a significant reductions in bifidobacterial numbers, concentrations of butyrate and total SCFA, defecation frequency and faecal excretion as compared with isoenergetic high-carbohydrate, high-fibre and low-fat diet.

High MUFA-containing dietary intervention that lasted 4 weeks reduced the total bacterial numbers but did not affect the specific bacterial groups[183]. Conversely, high habitual intake of MUFA has been associated with lower numbers of bifidobacteria and slightly higher numbers of *Bacteroides* spp.[64]. In a recent metagenomic study in healthy volunteers, the *Bacteroides* enterotype was found to be highly associated with the consumption of MUFA and SFA[134]. These observations suggest that the consumption of fat and animal-derived products, typically present in the Western diet, are associated with increased *Bacteroides* spp. prevalence in the human gut microbiota.

Habitual *n*-3 PUFA intake has been shown to have a significant positive association with *Lactobacillus* group abundance[64]. The increase in *Lactobacillus* group bacterial numbers in stool after *n*-3 PUFA intake has also been reported in a mouse study[185]. In addition, in a human study by Santacruz *et al.*[186] the numbers of lactobacilli remained at the same level, even though the ingested amount of total PUFA was greatly reduced. The increase in *n*-3 PUFA is effective in supporting epithelial barrier integrity by improving transepithelial resistance and by reducing IL-4-mediated permeability[187], and several lactobacilli enhance the function of the intestinal barrier[188,189]. Maternal salmon (marine *n*-3 PUFA) consumption before delivery has also lowered the number of Coriobacteriaceae in bottle-fed infants[190].

Higher habitual *n*-6 PUFA intake has been associated with decreased numbers of bifidobacteria[64]. It has also been reported that high *n*-6 PUFA intakes decrease certain immune functions, such as antigen presentation, adhesion molecule expression, proinflammatory cytokines and T-helper 1 and T-helper 2 responses[191]. Furthermore, genomic DNA of some bifidobacterial strains is able to stimulate the production of T-helper 1 and proinflammatory cytokines, interferon-γ and TNF-α[192]. Overall, these results indicate an association between dietary fat types and their distinct effect on the faecal microbiota. As a consequence, it seems that balanced diet with regard to fat consumption is critical not only for the host's health, but also for the gut microbiota.

Polyphenols

Plant foods contain significant amounts of phenolic compounds[193]. Plant polyphenols are a class of chemically diverse secondary metabolites that possess many different biological activities both within the plant and in the human subjects eating these plants. Plant polyphenols have the potential to affect certain risk factors of CVD, as well as being antioxidants, have antimicrobial properties and possessing inherent free radical scavenging abilities[194]. The main dietary sources of polyphenols are berries, fruits, beverages (e.g. coffee, tea and wine), chocolate, whole-grain cereals, vegetables and legume seeds[193].

The human gut microbiota has extensive hydrolytic activities and breaks down many complex polyphenols into smaller phenolic acids, which can be absorbed across the intestinal mucosa[194]. Daily consumption of red wine polyphenols for 4 weeks significantly increased numbers of bacteria within genera *Enterococcus*, *Prevotella*, *Bacteroides*, *Bifidobacterium*, *Eggerthella* and Family Lachnospiraceae[195], whereas consumption of high cocoa flavanol drink for 4 weeks significantly increased the bifidobacterial and lactobacilli numbers but significantly decreased clostridial counts[196]. Human dietary intervention with ellagitannins, which are polyphenols abundant in strawberries, raspberries and cloudberries, induced changes in the composition of Lachnospiraceae and Ruminococcaceae[128]. Tea phenolics (e.g. epicatechin, catechin, gallic acid and caffeic acid) significantly repressed certain bacteria such as *Clostridium perfringens* and *C. difficile* and members of the *Bacteroide*s spp., whereas bifidobacteria, *Lactobacillus* spp. and nonpathogenic *Clostridium* spp. were less severely affected[197]. Moreover, many phenolic compounds have *in vitro* antimicrobial activities towards pathogenic bacteria, such as *Salmonella* spp., *C. perfringens*, *C. difficile*, *E. coli* and *Staphylococcus aureus*[197–199].

Probiotics

Probiotics are live micro-organisms which when administered in adequate amounts confer a health benefit on the host, according to the widely accepted definition by Food and Agriculture Organisation WHO[200]. Most of the currently used probiotics belong to the genera *Bifidobacterium* and *Lactobacillus*. However, probiotic preparations containing species of the genera *Enterococcus*, *Pediococcus*, *Streptococcus*, *Lactococcus*, *Propionibacterium*, *Bacillus* and *Saccharomyces* are also used[201]. The past two decades have seen a marked increase in the inclusion of probiotic bacteria in various types of food products, especially in fermented milks[202]. During recent years probiotics have also been increasingly incorporated into non-dairy foods such as fruit and berry juices and e.g. cereals[201]. In good quality products, the daily dose should be approximately 10^9 colony-forming units/d[203]. Probiotics do not usually colonise the GI tract, and therefore the products should be consumed daily for the health benefits[167]. In most of the studies, probiotics have not caused any significant changes in the predominant faecal microbiota of healthy adults. However, there are very few studies in which e.g. *Lactobacillus rhamnosus* GG has modulated the faecal microbiota and increased overall bacterial diversity in infants[123,204]. In addition, *Bifidobacterium animalis* subsp. *lactis* Bb12 has reduced the numbers of Enterobacteriaceae and *Clostridium* spp. in preterm infants[124].

Conclusions

To answer the question posed in the title: does diet matter in regard to human microbiota? Yes, it does. From the host's perspective, there are numerous activities of the commensal gut microbiota that are of great importance to health. Moreover, by choosing what we eat, we can decide which bacteria we feed. However, even though diet matters, the results from dietary interventions are not always straight forward. It should be remembered that the detected effect is dependent on the study subjects, study protocols, used DNA-extraction techniques, used methodologies, and in case of next-generation sequencing also the algorithms used for cleaning and analysing the data. In addition, individual variation in human intestinal microbiota is so wide that the subtle changes may not be detected if the study cohort is not large enough. Therefore, studies in which different habitual diets have been compared with each other, usually get clearer correlations with nutrients *v.* bacteria than those observed in dietary interventions.

In order to get as much information from future dietary interventions there would be need for big enough group sizes, long enough trials, long enough wash-out periods in cross-over designs, sufficient background information (i.e. baseline 7-d food records and clinical parameters) and last but not least more interdisciplinary research across microbiology, nutrition, immunology, genetics, epigenetics, proteomics, transcriptomics, metabolomics and human physiology.

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Conflicts of Interest

None.

Authorship

J. M. drafted the manuscript and undertook the literature searches. M. S. reviewed the literature on polyphenols, proteins and fats. J. M. and M. S. reviewed and revised the manuscript.

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