

Gut microbiota, diet and obesity-related disorders – the good, the bad and the future challenges

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List of abbreviations: inflammatory bowel disease (IBD), gastrointestinal (GI), fluorescence *in situ* hybridization (FISH), quantitative PCR (qPCR), denaturing gradient gel electrophoresis (DGGE), high-fat diet (HFD), whole grain (WG), fructooligosaccharides (FOS), galactooligosaccharides (GOS), resistant Starch (RS), high-protein diet (HPD), toll-like receptors (TLRs), glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), free fatty acid receptor (FFAR), histone deacetylase (HDAC), γ -aminobutyric acid (GABA), colorectal cancer (CRC)

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Abstract:

Diet has been shown to be a major factor in modulating the structure of the mammalian gut microbiota by providing specific nutrient sources and inducing environmental changes (pH, bile acids) in the gut ecosystem. Long-term dietary patterns and short-term interventions have been shown to induce changes in gut microbiota structure and function, with several studies revealing metabolic changes likely resulting from the host-microbiota cross-talk, which ultimately could influence host physiology. However, a more precise identification of the specific dietary patterns and food constituents that effectively modulates the gut microbiota and brings a predictable benefit to the host metabolic phenotype is needed to establish microbiome-based dietary recommendations. Here we briefly review the existing data regarding gut microbiota changes induced by different macronutrients and the resulting metabolites produced via their respective fermentation, including their potential effects on obesity and associated metabolic disorders. We also discuss major limitations of current dietary intervention studies as well as future needs of applying cutting-edge ‘omic’ techniques and of progressing in functional microbiota gene discovery to establish robust causal relationships between the dietary-microbiota-induced changes and metabolic health or disease.

Introduction:

Recent evidence suggesting a role of intestinal dysbiosis in promoting or aggravating different diseases such as obesity, type 2 diabetes and inflammatory bowel disease (IBD) [1] has sparked a revolutionary shift in regarding the gut microbiota as a significant player in human health rather than just being a commensal hitchhiker. Components of the gut microbiota are now considered to play significant roles in areas as diverse as the regulation of intestinal function, metabolism, behavior, blood vessel formation, and immune function [2]. Many interactions of the commensal gut microbiota with host physiology have been defined as beneficial, such as providing vitamins and essential nutrients, improving the digestibility of nutrients (e.g. complex polysaccharides), maintaining normal gut motility and immune function and releasing chemicals potentially involved in cancer prevention [3-5]. In contrast, emerging evidence has suggested a contributory role of intestinal dysbiosis and specific microbial metabolic products in the development of diseases such as metabolic syndrome, cardiovascular diseases, IBD and some cancers. Intestinal health and its impact beyond the gut is now viewed as at least partially dependent on the composition and function of the gut microbiota and its respective metabolic products that can interact with and influence host physiology [3]. This concept has reinforced the necessity to identify the environmental factors that can modify the gut microbiota and understand how these changes affect the microbiota metabolic output and ultimately host physiology and health.

Diet has emerged as an instrumental factor in defining and shaping the mammalian gut microbiota [6, 7]. Although the gut microbiota is relatively stable in healthy adult human populations [8], short-term alterations in diet have been demonstrated to rapidly change microbial composition, which can occur within 24

hours of diet intervention [9, 10], although more profound changes could require longer dietary modifications [8]. Long-term dietary patterns appear to have a substantial effect on shaping the human gut microbiota [10], as common microbial features are observed in humans from geographically distinct countries with similar diets higher in plant-derived polysaccharides (South America, Malawi, Africa/Burkina Faso) compared to humans from countries with typical Western diets rich in fat and protein (U.S. and Europe) [11, 12]. Dietary effects on human gut microbiota have also been directly demonstrated recently in dietary intervention studies of different durations (reviewed in [13]). The gut microbiota has also been demonstrated to some extent to be resilient, whereby microbial compositional changes in mice have been shown to revert back to the original structures after short dietary disturbances are removed and the animals are returned to the original diet [14]. Recently, however, the long-term impact on mice fed low-fiber diets over successive generations was shown to cause a progressive loss of certain fiber-fermenting bacteria that could not be restored by fiber-rich diets [15], thus demonstrating the potentially serious effects that sustained diets can have on modulating the microbiota diversity.

The mammalian gut environment is profoundly different in distinct compartments traveling along the length of the gastrointestinal (GI) tract, creating specific environments for different species or functional bacterial assemblages. For instance, the small intestine has faster transit times, higher bile acid concentrations and greater oxygen availability than the large intestine [16], thus allowing more bile-resistant facultative anaerobes to thrive in these areas of the gut compared to the colon. These differences are pronounced when comparing the proximal and distal regions of both the small and large intestines and also locally between the intestinal lumen and the mucosal surfaces [3]. Intestinal pH, which varies significantly along different regions of

the GI tract [17], is another critical factor in shaping bacterial species compositions and metabolic output [18]. Thus, environmentally diverse subcompartments within the human GI tract likely facilitate heterogeneous bacterial assemblages, and hence accurate representation of the gut microbiota is largely dependent on very specific regions of the gut that are sampled. Since human fecal samples are largely the choice for analyzing the microbiota because intestinal biopsy samples are difficult or impossible to obtain, much of the present work characterizing the human gut microbiota is biased towards the community present in the lumen of the distal large intestine. Furthermore, the number of studies that correlated gut microbiota dietary-induced changes with their potential physiological and clinical consequences is very limited, thus precluding the understanding of their significance to human health [19].

In this review, we analyze the existing data regarding how dietary interventions with different types of macronutrients (fats, carbohydrates/fibers, and proteins) affect the mammalian gut microbiota composition. We then discuss the metabolic intermediate and end products of bacterial metabolism associated with each of the three aforementioned macronutrients and their possible role or influence in the development of obesity and related metabolic disorders. Finally we discuss the future challenges existing in this research area and suggest potential ways to overcome these limitations.

1. Dietary fat and high-fat diets (HFDs)

Ingestion of dietary fats leads to a release of digestive enzymes including lipase that aids in breaking down complex triacylglycerol molecules to free fatty acids and monoglycerides. Bile acids are released into the duodenum and associate with free fatty acids and monoglycerides to form micelles, which facilitate transport to the enterocyte

plasma membrane and eventual absorption of the freely dissolved fatty acid. Dietary fatty acids are mostly absorbed and utilized in the small intestine, although a small percentage is able to reach the colon and can be excreted in feces [13].

1.1. Lipid-degrading bacteria

Although a large focus of study has been conducted on the relationship of the gut microbiota and diet-related diseases such as obesity, surprisingly little is known regarding dietary fat degradation *in vivo* within the mammalian gut, as well as the dominant active lipid-degrading bacterial species present in the gut. It is known that microbes from ruminants are able to biohydrogenate certain PUFAs such as linoleic acid into the saturated fatty acid stearic acid [20], and similar biotransformation of linoleic acid has been observed in numerous strains of human gut bacteria *in vitro* [21]. In this latter study, substantial linoleate isomerase activity was detected in the bacterial groups *Roseburia* spp., *Butyrivibrio fibrisolvens* and *Propionibacterium freudenreichii* subsp. *shermani*, and a range of metabolic products such as conjugated linoleic acids, vaccenic acid and hydroxy-18:1 fatty acid were detected [21]. Evidence for the ability of gut bacteria to metabolize dietary PUFAs *in vivo* was also recently demonstrated in mice [22, 23].

A great deal of work on known lipid-degrading bacteria has also been conducted in the biotechnology sector, with a focus on bacterial lipases for commercial enzymatic use. From this work, bacterial lipases have been identified in numerous bacteria, including some common gut microbial genera or species: *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Bacillus*, *Pseudomonas*, *Enterococcus*, *Lactobacillus*, *Propionibacterium*, *Proteus vulgaris*, *Staphylococcus* and *Serratia marcescens* [24, 25]. However, most of these genera are not dominant members of the mammalian gut

microbiota, and little is known regarding degradation of lipids by the more dominant bacterial members. Although not directly isolated from the gut, Čipinytė et al. [26] found that *Enterobacter aerogenes*, a common human gut bacterium, has very high lipase activity *in vitro* and is capable of degrading different types of fatty acids, ranging from saturated (palmitic and stearic) and unsaturated (oleic and linoleic) fatty acids to triglycerides. Theoretically, the fraction of the dietary fat that reaches the colon could be partially metabolized by gut bacteria although direct evidence is lacking, due to the fact that the main energy sources are known to be primarily carbohydrates and then protein products. Although it is well-known that cholesterol is degraded by gut microbiota to the metabolic end product coprostanol increasing its excretion in feces [27], the ultimate consequences on human health are poorly understood. Further work on identifying lipid-degrading bacterial strains is needed in order to improve the existing knowledge of the microbiota's primary role in fat metabolism.

1.2. Effects of HFD on microbiota composition

Recent observations in animal studies have found that HFDs stimulate substantial changes in certain taxonomic groups from the gut microbiota compared to control diets (Table 1). In contrast, very few controlled human interventional studies examining the effects of HFDs on gut microbiota composition have been carried out to date (Table 1). Among these human studies, Wu et al. [10] examined the changes in the gut microbiota of ten individuals given either a high fiber/low fat diet (LFD) or a low fiber/HFD and found that interindividual variation in microbial composition masked possible variation from short-term dietary changes. During this 10-day study, the enterotype identities (characterized by increased abundance of specific genera, namely *Bacteroides* and *Prevotella*) that were assigned to each individual remained stable

despite rapid changes in microbiota composition within a single day of dietary intervention [10]. Duncan et al. [28] also carried out a controlled human study that examined changes in the gut microbiota after a shift from a weight maintenance diet (30% of total calories from fat) to a high fat/protein, low carbohydrate diet (protein:carbohydrate:fat = 30%:4%:66% of total calories) labeled a HPLC diet. However, the primary goal of this study was to examine the effect of changing carbohydrates and protein content instead of fat. In fact, this study reported reductions in common fiber-fermenting bacteria such as *Bifidobacterium*, *Roseburia* spp. and *Eubacterium rectale*. This trend in reduced fiber-fermenting bacteria has also been observed in mice subjected to HFD studies that reduced the carbohydrate (and possibly fiber) percentages at the expense of increasing the fat percentages in the HFDs [29].

Other dietary factors, such as the type of fat (saturated vs. mono-unsaturated or PUFA), also appear to affect microbiota composition, although only a few studies have compared the effects of different dietary fat types. In mice, microbial diversity was decreased in diets high in saturated fatty acids, but not affected by diets high in PUFAs [30]. Saturated fat, but not PUFAs, has also been demonstrated in mice to indirectly lead to a 'bloom' of the undesired sulfite-reducing bacterium *Bilophila wadsworthia* via stimulation of taurine conjugation of hepatic bile acids, which ultimately increase organic sulfur availability and modulate microbiota composition [31].

Habitual fat consumption may also affect microbiota composition, although few observational studies theoretically reflecting long-term dietary effects have been conducted. Examination of the effects of habitual diets show that the long-term intake of animal fat-rich diets are associated with increases in *Bacteroides* [10]. Short-term consumption of solely animal-based diets also yielded an increase in the abundance of *Bacteroides*, as well as *Alistipes* and *Bilophila*, with a concomitant decrease in certain

Firmicutes groups (*Roseburia*, *Eubacterium rectale*, *Ruminococcus bromii*) known for their role in fiber fermentation [9].

1.3. Dietary fat and related effects mediated by bacterial metabolites

High dietary fat intake is associated with increased adiposity, chronic low-grade inflammation, insulin-resistance and increased bile acid production [32-35], which can consequentially lead to diseases such as obesity, metabolic syndrome, type 2 diabetes and colon cancer. However, little is known regarding potentially bioactive metabolites produced as a consequence of the direct or indirect effects of dietary fat on gut bacteria and its relationship with obesity and related metabolic alterations or symptoms.

Haghikia et al. [36] recently discovered that long chain fatty acids (LCFAs) commonly found in Western diets are involved in enhancing differentiation and proliferation of T helper 1 (Th1) and/or Th17 cells, as well as reducing SCFAs in the gut, thus favoring a pro-inflammatory environment that could be adverse in obesity and its associated comorbidities. Furthermore, gut microbiota were found to be crucial for this observed effect on Th cells [36], suggesting that the primary effect on these immune cells possibly stems from a bacterial-related compound. Gut microbiota have also been implicated in stimulating fatty acid absorption and enhancing lipid droplet formation in the intestinal epithelium and liver of zebrafish [37], providing a mechanistic role of gut microbiota in host adiposity in animal models. Furthermore, bacteria from the phylum Firmicutes and their associated metabolic products were demonstrated to increase the number of lipid droplets while lipid droplet size was associated with other bacterial types [37], supporting evidence for distinct mechanisms of microbial species in regulating fatty acid absorption in the host. Interventions in HFD-induced obesity in mice with the supplementation of the bacterium *Bacteroides uniformis* CECT 7771 also

showed a reduction in dietary fat absorption in enterocytes, supporting the notion that specific components of the human microbiota could interfere with dietary lipid absorption, although the mechanism or bacterial components mediating this effect were not investigated [38].

1.4. Role of dietary fat and microbiota in inflammation

Bacterial-induced inflammation in the host associated with obesity and type 2 diabetes are currently thought to stem from two factors related to HF diets: increased lipopolysaccharide (LPS) concentration and increased permeability of the intestinal barrier. LPS, which is a microbial compound found in the outer membranes of Gram-negative bacteria that can induce inflammatory responses, is largely thought to be responsible for the observed increase in inflammation associated with obesity and type 2 diabetes. LPS levels are particularly associated with increased consumption of HF diets in mice [39] and in humans [40]. HF diets further have been linked to reduced intestinal barrier via direct activation of an inflammatory response by saturated fat [41]. Increased intestinal permeability induced by HF diets may result from decreased integrity of gut epithelial tight-junction proteins. In mice, reduced expression of several genes encoding tight (zona occludens-1 and occludin) were reduced in groups undergoing HFD feeding [32]. Furthermore, gut bacteria may have a role in affecting intestinal permeability, as antibiotic administration reduced gut permeability, systemic inflammation and metabolic endotoxemia [32]. Everard et al. [42] revealed that the abundance of the common mucus-degrading bacterium *A. muciniphila* actually displays a direct relationship with the thickness of the mucus layer, and administration of this bacterium to obese mice reversed HF diet-induced metabolic disorders. Activation of the endocannabinoid system by the gut microbiota may also contribute to increased

permeability of the intestinal epithelium, increased plasma LPS and inflammation, as well as the regulation of adipogenesis [43]. Although some correlations between specific gut bacterial groups and changes in intestinal barrier function have been made, the definitive evidence and the mechanisms associated with these changes remain to be clarified [44].

Microbial interactions with dietary lipids may also play an important role in inflammation in adipose tissue as well as a role in type 2 diabetes. Mice fed saturated fats had increased activation of Toll-like receptors (TLRs), which was at least partially mediated by the microbiota, leading to white adipose tissue inflammation and reduced insulin sensitivity [45]. This effect was not observed in mice fed unsaturated fish oil, suggesting that dietary fat content is instrumental in driving these microbiota-associated changes in inflammation.

1.5. Dietary fat, microbiota and bile acids

High dietary fat consumption is known to stimulate primary bile acid release in the small intestine [46]. Primary bile acids are synthesized in the liver and are conjugated to glycine or taurine, facilitating their uptake in the distal ileum and transport back to the liver. However, bacteria can deconjugate and dehydroxylate these primary acids in the distal ileum to prevent uptake, allowing their entry into the colon where they are further metabolized to secondary bile acids via the gut microbiota [1]. These bacterial activities may modify solubilization and absorption of dietary lipids throughout the intestine and increase bile fecal excretion. This, in turn, increases neosynthesis of additional bile acids from cholesterol in the liver, thereby regulating cholesterol levels via interactions with enterohepatic farnesoid X receptors [47]. Bacterial activities on bile acids could also affect the bile acid-induced activation of the

G-protein coupled membrane receptor TGR5 on enteroendocrine L cells, which leads to glucagon-like peptide-1 (GLP-1) secretion, an enteroendocrine peptide that regulates appetite, insulin sensitivity and glucose metabolism [48]. Recent work has also shown that bacteria from the phylum Firmicutes are primarily involved in bile acid metabolism in humans [49]. Secondary bile acids have been shown to alter the cecal microbiota composition in rats, inducing similar changes in microbiota composition to those induced by high-fat diets [50]. Along with fatty acids, secondary bile acids are also implicated in gastrointestinal cancers due to genotoxic and cytotoxic damage of cells [51]. Finally, bile acids have been demonstrated to select for certain groups of bacteria, including the sulfite-reducing bacterium *B. wadsworthia* described earlier. This scenario can lead to increased production of sulfide and ultimately increases in inflammation in genetically susceptible hosts [31].

2. Dietary fiber

Digestion of carbohydrates is a complicated process using numerous enzymes and is highly dependent on the specific type of carbohydrate ingested. The majority of digestible dietary carbohydrates are utilized and absorbed in the small intestine, whereas non-digestible carbohydrates reach the colon and are fermented by anaerobic gut bacteria to produce a range of metabolic end products. To date, most of the studies investigating the role of specific nutrients on gut microbiota have been focused on the effects of fibers since this is the preferred carbon source for the microbiota in the large intestine. Nevertheless, many of the initial studies assessed the effects of fiber only in a limited number of bacterial groups generally considered to confer benefits for humans, such as *Bifidobacterium* or *Lactobacillus* species. More recent studies have now

revealed that specific groups of Firmicutes (mainly butyrate-producers) and occasionally bacterial groups from Bacteroidetes and Actinobacteria are also increased in response to greater fiber consumption, but specific effects largely vary depending on the type of fiber evaluated (Table 2) as well as on the individual's microbiota composition. The following sections discuss impacts of common types of non-digestible carbohydrates on microbiota structure.

2.1 Effects of dietary fiber on microbiota

2.1.1. Whole grain (WG)-rich food

WG cereals undergo minimal processing after harvesting in order to preserve the natural proportions of the endosperm, germ, and bran, the latter with the highest fiber content of all grain elements. Controlled dietary interventions on humans with WG cereals have reported a bifidogenic effect as well as an increase in several groups of Firmicutes (*Blautia*, *Lactobacillus*, butyrate-producers) and several Actinobacteria groups (*Collinsella*, *Atopobium* spp.) (Table 2). Interestingly, in contrast to WG wheat diets, *Bifidobacterium* was not observed to increase when subjected to wheat bran diets [52, 53], suggesting a high degree of specialization accordingly to the particular carbon sources present in the diet. The above is further exemplified with recent studies outlining the increase of *Blautia* and *Roseburia* species as a consequence of a dietary intervention with WG barley, and the increased abundance of butyrate-producers from *Clostridium* cluster IV during a dietary interventional study using WG rye [54, 55].

2.1.2. Inulin, fructooligosaccharides (FOS), and galactooligosaccharides (GOS)

Inulin is made of a heterogenous mixture of up to 60 fructose monomers with a terminal glucosyl moiety that can be broken down to produce shorter chain FOS (degree

of polymerization between two to nine). GOS, on the other hand, are composed of chains of galactose units with variable chain length and types of linkages between monomers. These structural differences largely affect the outcome in influencing changes in microbiota structure [13]. Inulin, FOS and GOS all have been demonstrated to increase the number of *Faecalibacterium prausnitzii* (Table 2) detected in human feces [56-59]. Other well-described effects of inulin and FOS consist of elevating the abundance of *Bifidobacterium* and *Lactobacillus* (Table 2) with the concomitant improvement of certain metabolic parameters [60, 61]. Formula milk supplemented with both FOS and GOS produces a notable bifidogenic effect, causing a shift in the gut microbiota of formula-fed infants towards that observed for breast-fed children [62-64]. However, despite the recognized bifidogenic effect of fructans and galactans, this outcome is not always observed, and a reduction of *Bacteroides*, *Prevotella* and *F. prausnitzii* species has also been associated with FOS/inulin administration [65].

One of the many claimed positive effects of dietary fructans and galactans is the ability to reduce potentially ‘undesirable’ bacterial groups that may cause detrimental effects to host health. For example, FOS administration has been shown to lower both *Escherichia coli* and *Salmonella* sp. numbers [66] while GOS reduced *Clostridium histolyticum* and *Desulfovibrio* abundances [67, 68]. The latter is of particular interest since *Desulfovibrio* has been linked to negative effects on human health, such as inducing LPS endotoxemia [69]. However, non-digestible oligosaccharides such as inulin have also produced opposite results in 9-12 week old pigs, as pathobionts such as *Klebsiella* spp. has been observed to use these carbon sources to proliferate [70]. Clearly, further studies are required to assess the potential ability of these oligosaccharides to shift the composition of the gut microbiota and particularly their health consequences.

2.1.3. Resistant Starch (RS)

Non-digestible starch, known as resistant starch (RS), is assumed to have an extensive impact on the gut microbiota given its ability to resist degradation in earlier sections of the GI tract to reach distal portions of the colon. In particular, *R. bromii* seems to be a keystone species for the degradation of type RS3 in the human colon, and its abundance and that of closely-related species can reach proportions of 17% of the fecal microbiota of humans following a RS-supplemented diet [7, 71]. In addition to ruminococcal bacteria, other gut microbial species such as *Bifidobacterium adolescentis*, *Eubacterium rectale*, *Prevotella* spp., *Parabacteroides distasonis* and *Oscillibacter* relatives can be positively influenced by the intake of different types of RS (Table 2) [7, 72]. Similar to studies with WG supplements, variation in gut microbiota patterns seems to be influenced by the subtypes of the RS used [72, 73].

2.2. Dietary fiber effects on gut microbiota metabolites and host physiology

High-fiber diets have beneficial effects on host health, including a direct impact on glucose and lipid metabolism by regulating nutrient absorption [74] and indirectly via production of SCFAs by gut microbiota fermentation. Most microbial production of SCFAs from carbohydrate degradation results from multiple alternative pathways (reviewed in [75]), which use the common glycolytic intermediate phosphoenolpyruvate as a substrate. *Bifidobacterium* spp., on the other hand, use a unique central fermentative pathway called the fructose-6-phosphate shunt, or ‘bifid’ shunt, in order to ferment sugars to SCFAs and other organic compounds [76].

Predominant SCFAs produced by gut bacteria in response to fiber uptake are acetate, propionate, and butyrate. These SCFAs are formed primarily in the colon, in

which 95% are subsequently absorbed by colonocytes [77] and used as energy sources, being preferably used in the order butyrate > propionate > acetate [78]. Although butyrate is largely expended as energy by colonocytes, propionate and acetate travel to the liver via the portal vein, whereas acetate can reach the peripheral tissues after entering systemic circulation. Propionate and acetate can be used as substrates for gluconeogenesis and lipogenesis, whereas acetate is also a substrate for cholesterol biosynthesis [1, 79]. Propionate conversely inhibits cholesterol synthesis, as well as decreases the level of hepatic triglycerides and reduces food intake by triggering intracellular signaling to release anorexigenic peptides [80-82].

Numerous studies have attempted to correlate several different gut immune disorders with the reduction of SCFA availability (primarily butyrate) to colonocytes. Indirect evidence has shown a depletion of microbial butyrate-producers in the feces of patients with inflammatory bowel diseases [83-85]. Butyrate has been demonstrated to increase endogenous production of the glucagon-like peptide-2 (GLP-2), whose production may improve mucosal barrier function by increasing the rate of crypt cell proliferation and villus elongation, and reduce apoptosis [86-88]. Butyrate has been demonstrated to play a pivotal anti-inflammatory role via numerous varied mechanisms: by suppressing inflammatory responses at the colon by inducing Treg (regulatory T) cells [89], modulating the function of intestinal macrophages to down-regulate the production of pro-inflammatory cytokines and the TLR4 receptor [90, 91], interfering with differentiation and maturation of monocyte-derived dendritic cells [92], and activating the production of the anti-inflammatory cytokine IL-10 via binding to GPR109A receptors on intestinal macrophages and dendritic cells [93].

Butyrate and propionate have recently been demonstrated to have a protective role against diet-induced obesity and insulin resistance in mice [81]. The free fatty acid

receptors FFAR3 (GPR41) and FFAR2 (GPR43) are well known to mediate the cellular response to SCFAs, while the stimulation of GLP-1 by SCFAs seems to be FFAR3 independent in mice [81]. SCFA-dependent signaling via these receptors affects different functions, depending on the cellular type [1]. This is in agreement with the differential pattern of expression described for genes encoding such receptors [94]. Therefore, a global model for cellular responses to SCFAs may not be possible and the potential pleiotropic effects must be carefully considered when proposing SCFA-based anti-inflammatory or anti-obesity therapies.

Beyond characterization of cell surface receptors by which SCFAs exert their anti-inflammatory activity and protection against diet-related diseases, it is worth mentioning that different studies have been recently carried out in order to shed light on the intracellular molecular mechanisms driving the response to SCFAs. Particularly, SCFAs have been described as inhibitors of histone deacetylases (HDACs), thus participating in activation/repression of gene expression [95]. One example of this role is the SCFA-mediated differentiation of T cells into Th17, Th1, and IL-10⁺ Treg cells promoted by an attenuated HDAC activity leading to the expression of p70S6 kinase and activating the mTOR pathway [96]. Similarly, butyrate has been shown to have a protective role against type 1 diabetes in rats by altering the acetylation pattern of H3 and H4 histones and inducing beta-cell proliferation by inhibiting the p38/ERK apoptotic pathway [97]. Additionally, SCFAs seem to modulate the activity of the peroxisome proliferator-activated receptor γ -dependent (PPAR γ), a key player to control adipogenesis and the fat oxidative metabolism in mitochondria. SCFAs induce lower expression of PPAR γ thus promoting activity of the uncoupling protein 2 (UCP2), thereby stimulating oxidative metabolism in liver and adipose tissue, insulin sensitivity, and weight loss [98, 99]. Although there is no direct evidence regarding the molecular

mechanism by which SCFAs induce down-regulation of the PPAR γ nuclear receptor, one plausible explanation can be the inhibitory role of SCFAs on HDACs given that these enzymes have been shown to control expression of PPAR γ during adipogenesis [100]. However, whether or not SCFAs, in particular butyrate, produced in the gut can reach peripheral tissues to induce such effects remains to be elucidated.

3. Dietary protein and high-protein diets (HPDs)

3.1. Fate of dietary protein and proteolytic bacteria

Ingested dietary and endogenous protein are first digested in the small intestine by pancreatic enzymes and peptidases from enterocytes. A significant amount of oligopeptides and amino acids are then transported to the portal bloodstream via enterocyte transporters where they are used as amino acid precursors for protein synthesis or metabolized for fuels or precursors necessary for intestinal mucosal metabolites [101]. Typically ~10% of the ingested protein reaches the large intestine [13], and then undergoes further proteolysis by the colonic microbiota, yielding levels of some amino acids as high as millimolar concentrations as detected from human intestinal contents extracted from the small and large intestines [102]. Unlike enterocytes, colonocytes do not absorb amino acids to any significant extent and so the remaining amino acids in the colon are fermented by resident bacteria to a wide variety of metabolic products in which some metabolites can be utilized by colonocytes while others are excreted as waste products in feces [101].

Bacterial proteolytic activity in the colon has mainly been attributed to the genera *Bacteroides*, *Clostridium*, *Propionibacterium*, *Fusobacterium*, *Streptococcus* and *Lactobacillus* [103]. Other common proteolytic bacterial genera found in the human

gut include *Peptostreptococcus*, *Actinomyces*, *Peptococcus*, *Ruminococcus*, *Bacillus*, *Staphylococcus*, *Megasphaera*, *Acidaminococcus* as well as some Enterobacteria [101, 104, 105]. As most of the work on amino acid-degrading bacterial strains has been carried out in the 1980's / early 1990's, there is a need to further examine the amino acid/protein-degrading capabilities of the numerous dominant anaerobic bacterial taxa that may not have been previously detected or cultured/isolated in these studies.

Numerous factors affect the availability of protein in the human colon, such as the amount and type of protein consumed as well as the amount of undigested carbohydrates that reach the large intestine. As protein ingestion increases, the amount of residual protein entering the colon subsequently increases [106]. Since fermentable carbohydrates are preferentially utilized over proteins by most bacteria in the small intestine and proximal colon, most fermentation of amino acids as an energy source occurs in the distal colon, where carbohydrates are depleted [107]. However, several bacterial groups (*Peptococcus*, *Acidaminococcus*, and *Veillonella* and several strains of Clostridia, *Fusobacterium* and *Eubacterium*) display weak or no fermentation capacity of carbohydrates [108], and thus may be relatively unaffected by carbohydrate availability.

3.2. Effects of high-protein diets (HPDs) on microbiota

Very few studies have examined the effects of HPDs on changes in gut microbial composition in mammals. Among these studies, reductions in *Clostridium coccoides*, *C. leptum* and *Faecalibacterium prausnitzii* have been observed in rats [109], while reductions in *Roseburia* spp., *Eubacterium rectale*, *Collinsella aerofaciens*, *Bacteroides* spp., and *Oscillibacter* relatives (Table 3) have been observed in humans [7, 28, 110]. Unfortunately, HP diets that attempt to maintain similar caloric levels

between diets have similar inherent design problems as HF diets, in that the relative concentrations of carbohydrates and/or fats need to be reduced in order to maintain similar energy levels. For example, in Liu et al. [109], digestible carbohydrates were reduced (54% reduced sucrose and corn starch) in order to maintain an isocaloric diet compared to the normoproteic diet. Therefore, in the current experimental designs for these studies, it is difficult to identify the critical dietary factor (i.e. change in protein or carbohydrate or fat content) that is predominantly controlling the observed compositional shift in specific bacterial groups. In any case, decreased relative percentages of carbohydrates found in the HP diets likely contributed to reductions of many bacterial groups, particularly known fiber-fermenting bacteria such as *Roseburia* and *Eubacterium*, in several studies [28, 110].

It is important to note that most of the current studies examining changes in the microbiota via a HPD have only examined selected bacterial groups using techniques such as FISH and qPCR with group-specific (i.e. class, genera or species) probes [28, 109, 110], leaving much of the microbial diversity unexplored in these studies. Liu et al., (2014) further used DGGE to partially examine changes in the dominant microbial groups under HPDs, while Walker et al. [7] is the only known study to investigate the microbial community in a controlled HP diet intervention using 16S rRNA gene clone libraries. Therefore, there is a current need for more studies employing next-generation sequencing techniques (16S rRNA gene amplicon and metagenomic studies) to analyze the entire microbial composition changes under the effects of increased dietary protein.

3.3. Dietary protein effect on gut microbiota metabolites and host physiology

3.3.1. Bacterial metabolites from protein fermentation

Bacterial metabolites produced from protein fermentation after elevated consumption of protein are abundant and diverse, including hydrogen sulfide (H₂S), ammonia, aromatic compounds (phenol, *p*-cresol, indole), polyamines (agmatine, putrescine, spermidine, spermine, cadaverine), SCFAs, branched-chain fatty acids (isobutyrate, 2-methylbutyrate), organic acids (formate, lactate, succinate), ethanol, gases (H₂, CO₂, CH₄), and compounds with potential neuroactive activity (GABA, serotonin, histamine, L-DOPA, tryamine, nitric oxide, tryptamine, phenethylamine) among others [101]. Although much work has been conducted analyzing the physiological impacts that these compounds elicit on the host during high protein fermentation (reviewed in [111]), many studies have not included an examination of the gut microbiota nor have they provided a direct link of microbiota-derived metabolites from protein fermentation to the observed physiological effects in the host. Metabolomic studies are attempting to establish associations between controlled dietary regimens and microbially-produced metabolites in an effort to investigate the role of dietary interventions on the microbial metabolome and its subsequent effect on host health [112]. However, difficulties in attributing bacterially-derived versus host-derived metabolites confound many of these associations, as numerous genetic pathways for amino acid metabolism are conserved across bacteria and mammals [113]. On the other hand, some gut microbe-specific metabolic products, such as phenolic and indolic compounds (phenol, phenylacetate, phenylpropionate, indole, idoleacetate, indolepropionate, *p*-cresol) [114], can be easily traced in host systems. The following sections briefly discuss the current knowledge of positive and negative impacts of HP diets on host physiology and the possible role that bacterial metabolites generated from increased protein fermentation can play in these associated changes.

3.3.2. Beneficial effects of dietary protein on host health

HP diets are well-known diets used for body weight reduction and are considered to be possible strategies for preventing or mitigating weight gain and obesity. Many studies analyzing the physiological impact of these diets in humans and rodents have found that HP diets reduce body weight, blood pressure, triglyceride levels and fat mass, as well as improve cardiometabolic risk factors (reviewed in [115-117]). These beneficial effects on host health have been partially attributed to alterations in energy metabolism and decreased appetite, which ultimately lead to a reduction in energy assimilation and therefore weight loss [118]. Increased energy expenditure has been observed after consumption of acute HP diets, presumably due to increases in postprandial thermogenesis and resting metabolism [115].

So far, a direct link between bacterial metabolites and beneficial changes in the host physiology is lacking, although current evidence strongly supports possible associations. For instance, several amino acid-derived compounds that can be produced only by gut bacteria (indole) or by gut bacteria and mammalian host (tyramine, tryptamine and SCFAs) have direct or indirect impacts on satiety and gut motility in mammals via effects on the incretin GLP-1 and serotonin secretion from enteroendocrine cells [119-121]. Furthermore, recent work on the impact of gut microbiota on the host central nervous system suggests that several microbial amino acid-fermented metabolites (i.e. GABA, serotonin, histamine, L-DOPA, tryamine, nitric oxide, tryptamine) may be neuroactive compounds that can produce substantial effects in the host, such as regulation of anxiety, mood, cognition, satiety, and immunity (reviewed in [121-123]). However, the physiological effect (if any) on the host intestinal and periphery tissues of the majority of these compounds is still not well

understood. In addition, the distinction between human- and bacterial-origins of many of these compounds has not been well established yet, and further work with *in vivo* studies is necessary to validate such effects.

3.3.3. Adverse effects of dietary protein on host health

High protein consumption and increased protein fermentation have been linked to several important bowel diseases such as colorectal cancer (CRC) and ulcerative colitis (UC), as well as impaired renal and mucosal function (reviewed in [6]). Evidence for the link between protein fermentation and CRC and UC stems from production of potentially carcinogenic and genotoxic metabolites (sulfides, phenols, polyamines and ammonia) during protein fermentation, as well as higher protein fermentation in local areas where these bowel diseases frequently occur. Epidemiological studies have further suggested a link of prolonged consumption of red and processed meats to increased risks of colorectal cancer (reviewed in [111]). However most of the support for the toxic effects of many of these metabolites is based on *in vitro* studies, and the evidence for a direct role of protein fermentation in the etiology of bowel diseases may still not be clear [111]. Recent discovery shows that gut microbial metabolism of the amino acid derivative L-carnitine commonly found in red meat yields trimethylamine, which is further metabolized to trimethylamine-N-oxide in the liver, which can increase atherosclerosis in mice [124].

Several metabolites produced by the bacterial fermentation of aromatic amino acids have been associated with negatively affecting the intestinal mucosal barrier of the host as well as causing DNA damage to gut epithelial cells. Phenol has been demonstrated *in vitro* to increase paracellular permeability and decrease the epithelial barrier function of Caco-2 monolayers [125]. HP diets have been shown to increase the

bacterial-derived genotoxic metabolite *p*-cresol in feces, along with concomitant increases in DNA damage to rat colonocytes and decreases in the integrity of the colonic mucosal barrier [126]. Furthermore the type of protein consumed has been demonstrated to affect the level of rat colonocyte DNA damage observed, as casein caused higher levels of damage compared to cooked lean red beef [127] and red beef was higher than chicken [128]. In contrast, a human intervention study with HP diets revealed that fecal water genotoxicity did not correlate with protein fermentation [129]. Thus, a clear role of *p*-cresol produced during increased protein ingestion in humans has yet to be established in *in vivo* studies.

Increased ingestion of dietary protein can also lead to increases in metabolites such as *p*-cresyl sulfate and indoxyl sulfate that may cause a potentially detrimental effect on host kidney function [131]. Sulfide, another metabolite produced by increased protein fermentation by sulfate reducing bacteria, has been demonstrated to inhibit colonocyte mitochondrial respiration and is genotoxic to colonocytes (reviewed in [101]). Although the effects of many bacterial metabolites from protein fermentation have been investigated *in vitro*, further studies, particularly *in vivo* studies in healthy human populations, are necessary to determine the true potential toxicity of many of these metabolites and their relationship to increased protein ingestion.

4. Future Challenges

Correctly identifying specific dietary components that effectively modulate the gut microbiota composition and function and lead to a predictable change in host physiology is confounded by numerous factors. This includes variability in the different diets (i.e. type, relative amount, length of interventional period, etc.) used in different

studies, thus making comparisons between studies difficult and yielding no definitive conclusions about the effects of a particular dietary compound. Furthermore, interindividual differences in the initial resident microbiota, particularly observed in human intervention studies, may substantially alter the response of a group of bacteria to a given dietary compound, which has not usually been considered. It has been observed that diet explains a much larger percentage of variation in mice (60%) compared to the same variation in humans (10%) due to the ability to control numerous factors (diet, environmental exposures, etc.) in mice compared to humans [130]. Therefore, conclusions regarding microbiota changes in mice in response to dietary changes need to be carefully weighed when applying them to human studies. Limitations inherent to experimental design (i.e. reduction in carbohydrate/fiber content at the expense of fat or protein in HF and HP experimental diets, respectively) have also precluded the identification of the dietary factor actually responsible for the observed changes in gut microbial composition and function. In addition, larger and more tightly controlled intervention trials, regarding dietary intakes and other confounding factors (e.g. physical activity, medication, etc.) are needed to be able to establish more robust cause-effect relationships in specific population groups. There is also a need to study both host-microbe interactions as well as microbe-microbe co-metabolic processes in order to truly understand what is taking place in the so-called 'black box' of the human gastrointestinal system.

Although current 16S rRNA gene amplicon surveys are providing valuable information on the bacterial taxonomic groups present in the mammalian gut during healthy and diseased states, there is a clear need to move beyond these studies to look at changes in functional genes using metagenomic and/or metatranscriptomic tools in order to better clarify the role that the gut microbiota plays in altering host physiology

and contributing to diet-related diseases. Predictive bioinformatic tools (e.g. PICRUSt) may also help to gain insight into the genes potentially present in these different ecosystems. Simultaneously, it is imperative that the functional annotation of many of the thousands of gut bacterial genes with unknown function be elucidated.

Unfortunately, this step requires often long, painstaking work with pure cultures and is the obvious bottleneck in all studies employing these molecular techniques. Previous reports indicate that a large percentage of human gut bacteria are able to be cultured (reviewed in [13]). However, certain bacterial taxonomic groups may not be able to be cultured due to unknown growth factors or growth conditions typically found in the human gut that favor their colonization. Improvements in microbial culturing of recalcitrant strains as well as better functional characterization of unknown genes from commonly isolated gut bacteria are a necessity to advance our current knowledge of the role of gut bacteria in diet-related diseases.

In summary, a mixture of cutting edge advanced ‘-omics’ techniques, as well as new gene discovery strategies applied to gut bacterial strains identified in well-design human studies, promises to reveal some of the mystery associated with this complicated ecosystem, which could be particularly relevant to improve the management of diet-related diseases.

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Table 1: Summary of recent dietary studies utilizing high fat (HF) diets that describe effects on gut microbiota. All dietary components are in percentages unless stated otherwise. Duration indicates the amount of time the dietary change/intervention was given. All subjects were adults unless specifically stated otherwise. The following is a list of abbreviations for subjects: HH (healthy human); M C57BL / 6NCrI mice (male C57BL / 6NCrI mice); Mice RELM (RELM β KO mice); F C57BL / 6NCrI mice (female C57BL / 6NCrI mice); SD rats (Sprague Dawley rats); Fout (Female outbred mice); M SD rats intestinal mucosa (intestinal mucosal samples from male Sprague Dawley rats); M Wist (male Wister rats). All samples were derived from fecal samples unless specifically stated otherwise. Methods for profiling are abbreviated as follows: Pyroseq (454 pyrosequencing); MiSeq (Illumina); MITChip (Mouse Intestinal Tract microarray chip).

Dietary change/ intervention	% dietary component (% carb / % prot / % fat)	Duration (weeks)	Subjects	Method for profiling	Effect on microbiota	Refs.
Fat						
Habitual long term diet	NA	NA	HH	Pyroseq	Protein and animal fat: ↑ <i>Bacteroides</i> Carbohydrates: ↑ <i>Prevotella</i>	[10]
Controlled HF/low-fiber and LF/high-fiber diets	HF/LFiber: 35 / 27 / 38 LF/HFiber: 69 / 18 / 13	1.5	HH	Pyroseq	Stable enterotype identity	[10]
Animal-based diet vs. Plant-based diet	NA	0.7	HH	MiSeq, qPCR	Animal: ↑ <i>Alistipes</i> , <i>Bilophila</i> and <i>Bacteroides</i> and ↓ Firmicutes (<i>Roseburia</i> , <i>Eubacterium rectale</i> and <i>Ruminococcus bromii</i>)	[9]
HFD vs. carbohydrate diet (CARB)	HFD: 21 / 19 / 60 CARB diet: 66 / 21 / 23	12	M C57BL / 6NCrl mice	MiSeq, FISH	HFD: ↑ <i>Rikenellaceae</i> and ↓ <i>Ruminococcaceae</i>	[29]
HFD vs. chow diet	HF diet: 35 / 20 / 45 Chow: 60 / 28 / 12	3	Mice RELM	qRT-PCR, Pyroseq	HFD: ↑ Firmicutes (Clostridiales) and Delta-proteobacteria. ↓ Bacteroidales.	[131]
HFD	26.3 / NA / 34.9	12	M C57BL / 6J mice	Pyroseq	HFD: ↑ Firmicutes (<i>Lachnospiraceae</i> , <i>Ruminococcaceae</i> , <i>Lactococcus</i>) and Proteobacteria. ↓ Bacteroidetes	[14]
Normal chow diet	61.3 / NA / 5.28	10			Chow: Addition of normal chow diet caused the HFD- induced microbiota taxonomic shifts to revert back to similar compositions found in control normal chow diet	
High saturated fat diet (HFD)	60% kcal from fat, 34% was saturated fat	12	F C57BL / 6J mice	Pyroseq	↑ Firmicutes and ↓ Bacteroidetes. At species level, ↓ <i>Lactobacillus</i> and ↑ <i>Oscillibacter</i> .	[34]
Purified HFD containing palm oil (HF-PO; P/S 0.4)	35 / 20 / 45	8	M C57Bl / 6J mice	MITChip	↑ <i>Clostridium</i> clusters XI, XVII, and XVIII	[30]
Purified HFD containing safflower oil	35 / 20 / 45	8	M C57Bl / 6J mice	MITChip	No significant changes	[30]
Purified HFD containing olive oil	35 / 20 / 45	8	M C57Bl / 6J mice	MITChip	No significant changes	[30]
HFD vs. Low-fat diet (LFD)	LFD: 70 / 20 / 10 HFD: 35 / 20 / 45	8-12	SD rats	qPCR	HFD: ↑ Bacteroidales and Clostridiales In DIO-P (obesity prone) rats: ↑ Enterobacteriales	[132]
HFD vs. chow diet (Chow)	HFD (pelleted): 40 / 17 / 43 Modified chow (saturated animal fat / condensed milk): 38 / 10 / 51 Control chow: 65 / 21 / 12	16	SD rats	Pyroseq	HFD: ↑ <i>Blautia producta</i> , <i>Morganella morgani</i> , <i>Phascolarctobacterium</i> , <i>B. fragilis</i> , <i>Parabacteroides</i> <i>distasonis</i> , <i>B. vulgatus</i> and groups <i>Bacteroidaceae</i> , <i>Lachnospiraceae</i> , <i>Enterobacteriaceae</i> , <i>Ruminococcaceae</i> , <i>Veillonellaceae</i> , <i>Porphyromonadaceae</i> and <i>Erysipelotrichaceae</i> . ↓ <i>Lactobacillaceae</i> (<i>Lactobacillus</i> <i>intestinalis</i>). CD: ↑ <i>L. intestinalis</i>	[133]

HFD not supplemented	NA	7	Four Mice	16S qPCR	↑ <i>Firmicutes</i> and the order <i>Enterobacteriales</i>	[134]
HFD with Oleic acid-derived compound	27.5 / 23.5 / 34.3 / Fiber 6.5	7	Four Mice	16S qPCR	↓ <i>Clostridium</i> cluster XIVa and <i>Enterobacteriales</i> ; ↑ <i>Bifidobacterium</i> spp. and <i>Bacteroidetes</i>	[134]
HFD with n-3 fatty acids (EPA and DHA)	27.5 / 23.5 / 34.3 / Fiber 6.5	7	Four Mice	16S qPCR	↑ Firmicutes and the group <i>Lactobacillus</i>	[134]
HFD/Energy diet	55.5 / 14.55 / 30	5.7	M SD rats intestinal mucosa	FISH	↑ <i>Lactobacillus/Enterococcus</i> ↓ <i>Bacteroides/Prevotella</i>	[135]

Table 2: Summary of dietary studies utilizing high fiber diets. All dietary components are in percentages unless stated otherwise. Duration indicates the amount of time the dietary change/intervention was given. All subjects were adults unless specifically stated otherwise. The following is a list of abbreviations for subjects: HH (healthy human); A w/MS (Adults with metabolic syndrome); IV (*in vitro* fecal samples); W (women); OW (obese women); H (D & EN) (underwent diarrhea and enteral nutrition); C W (constipated women); Over (overweight); Adoles girls (adolescent girls); OM (obese men); OH (obese human). All samples were derived from fecal samples unless specifically stated otherwise. Methods for profiling are abbreviated as follows: HITChip (Human Intestinal Tract Chip).

Dietary change/ intervention	% dietary component (% carb / % prot / % fat)	Duration (weeks)	Subjects	Method for profiling	Effect on microbiota	Refs.
Whole grain						
WG wheat	67.8 / 11.6 / 2.5 / 11.8 fiber	3	HH	FISH	↑ <i>Bifidobacterium</i> , lactobacilli	[52]
Wheat bran (WB)	48 / 14 / 3.5 / 27 fiber	3	HH	FISH	No significant changes	[52]
Maize-based WG	37.04 g / 2.09 g / 1.95 g 14.2 g fiber	3	HH	FISH	↑ <i>Bifidobacterium</i>	[136]
Non-WG	39.09 g / 1.63 / 1.68 / 0.81 g fiber	3	HH	FISH	No significant changes	[136]
WG barley	64.6 / 18.2 / 6.7 / 31.1 fiber	4	HH	Pyroseq	↑ <i>Blautia</i> ; Slight ↑ in <i>Roseburia</i> , <i>Bifidobacterium</i> , <i>Dialister</i>	[55]
Brown rice	80 / 8 / 3 / 7.3 fiber	4	HH	Pyroseq	↑ <i>Blautia</i>	[55]
WG rye bread (RB)	46 / 19 / 33 / 7-15 fiber	12	A w/MS	HITChip	No differences observed between groups	[54]
Amylase-pretreated wheat bran	NA	72 h at 37 °C	IV / HH	Pyroseq	↑ <i>Eubacterium xylanophilum</i> , <i>Butyrivibrio</i> spp. and <i>Roseburia</i> spp. (<i>Lachnospiraceae</i>). ↑ in butyrate-producers Firmicutes	[53]
Fructans (FOS and inulin)						
Inulin	Inulin or placebo 3 times/d (20 g/d)	4	W with low iron levels	qPCR	↑ bifidobacteria	[61]
Inulin + FOS	Fiber: 50% inulin and 50% fructo-oligosaccharide. Placebo: maltodextrin (6g)	4.1	W receiving radiotherapy	FISH	↑ <i>Lactobacillus</i> and <i>Bifidobacterium</i>	[60]
Formula milk + inulin + oligofructose	0.8 g/dL Orafti(®)Synergy1 (oligofructose-enriched inulin) supplemented infant formula	First 16 weeks of life	Infants	qPCR	Similar to microbiota of breastfed infants	[62]
Oligofructose/inulin fiber	7 g/d of oligofructose/inulin or placebo (maltodextrin)	1	H (D & EN)	FISH	Non-bifidogenic effect. ↓ <i>Faecalibacterium prautsnizii</i> and <i>Bacteroides/Prevotella</i>	[65]
Long-chain fructans / Short-chain fructans	Agave fructans with diff. degree of polymerization (DP) profiles. 5 g/kg b.w.	12 days	O mice	qPCR	Long-chain fructans: ↑ <i>Bifidobacterium</i> . Short-chain fructans: no bifidogenic effect	[137]
Agave inulin	0, 5.0, or 7.5 g agave inulin/d	3	HH	MiSeq	↑ <i>B. adolescentis</i> , <i>B. breve</i> , <i>B. longum</i> , <i>B. pseudolongum</i> . ↑ <i>Faecalibacterium</i> . ↓ <i>Desulfovibrio</i> .	[67]

Inulin/partially hydrolyzed guar gum mixture (I-PHGG)	15 g/d I-PHGG (fiber group) or maltodextrin (placebo group)	3	C W	qPCR	↓ <i>Clostridium</i> sp.	[138]
XOS, Inulin + XOS and maltodextrin (placebo)	5 g XOS, INU-XOS (3+1 g) or equivalent weight of placebo	4	HH	qPCR	XOS: ↑ <i>Bifidobacterium</i>	[139]
Inulin + oligofructose	Inulin/oligofructose 50/50 mix or placebo (maltodextrin) 16 g/day	12	OW	Microarray, qPCR	↑ <i>Bifidobacterium</i> and <i>Faecalibacterium prausnitzii</i> . ↓ <i>Bacteroides intestinalis</i> , <i>Bacteroides vulgatus</i> and <i>Propionibacterium</i>	[140]
Pea fiber + FOS	Formulated diets devoid or suppl with fiber (14 g/l)	2	HH	FISH	↑ bifidobacteria in fiber diet. ↓ <i>Faecalibacterium prausnitzii</i> and <i>Roseburia intestinalis</i> in both supplemented and fiber-free diet	[141]
Jerusalem artichoke inulin (JA)	55% inulin, 0.5% glucose, 2% fructose, 14% sucrose, 25% water, 3% minerals	3	HH	FISH	↑ bifidobacteria. ↓ <i>Bacteroides/Prevotella</i> , <i>Clostridium histolyticum</i> / <i>C. lituseburense</i> and <i>Clostridium coccooides/Eubacterium rectale</i> .	[142]
Chicory inulin (CH)	88% inulin, max. 10% free sugars and max. 0.3% minerals	3	HH	FISH	↑ bifidobacteria. ↓ <i>Bacteroides/Prevotella</i> , <i>Clostridium histolyticum</i> / <i>C. lituseburense</i> and <i>Clostridium coccooides/Eubacterium rectale</i> . ↑ <i>Enterobacteriaceae</i>	[142]
Inulin-oligofructose (5 g/day, twice)	Beneo; DKSH/Orafti Great Britain Ltd, Kent, UK	3	HH	qPCR, 16S clone libraries	No significant changes	[59]
Inulin and apple pectin	NA	1.7	IV / HH	MiSeq, qPCR	↑ <i>Bacteroides</i> , <i>Eubacterium eligens</i> , <i>F. prausnitzii</i> . ↓ <i>Bacteroides</i> spp.	[143]
GOS						
Trans-GOS mixture	5.5 g/d	12	Over H	FISH, ELISA	↑ bifidobacteria, ↓ <i>Clostridium histolyticum</i> , <i>Desulfovibrio</i> , <i>Bacteroides</i> spp.	[68]
GOS twice a day	0, 2.5 or 5 g GOS	3	Adoles girls	DGGE and qPCR	↑ <i>Bifidobacterium</i>	[144]
GOS supplemented formula milk	Suppl GOS (0.4 g/100 mL) formula	From day 15 of life	Infants	qPCR	↑ <i>Bifidobacterium</i> , <i>Lactobacillus</i> and ↓ <i>Clostridium</i>	[63]
GOS	Four increasing dosages: 0, 2.5, 5, and 10 g of GOS	12	HH	Pyroseq	↑ bifidobacteria. ↑ Firmicutes (some individuals). ↓ <i>Bacteroides</i>	[56]
Resistant starch						

RS (RS2)	Hi-Maize 260 (55.72 g/100g)	3	HH	Pyroseq, DGGE, qPCR	↑ <i>Ruminococcus bromii</i> and <i>Eubacterium rectale</i>	[72]
RS (RS4)	Fibersym (39.33 g/100g), water (18.36 g/100g), Midsol 50 native starch (16.39 g/100g)	3	HH	Pyoseq, DGGE, qPCR	↑ Actinobacteria and Bacteroidetes. ↓ Firmicutes. ↑ <i>Bifidobacterium adolescentis</i> and <i>Parabacteroides distasonis</i> .	[72]
RS (RS3)	434.1 g / 108.8 g / 126.5 g / 275.5 g starch / 25.56 g resistant starch	3	OM	16S clone libraries, qPCR, DGGE	↑ <i>Ruminococcus bromii</i> , <i>Eubacterium rectale</i> , <i>Roseburia</i> spp., <i>Oscillibacter</i> .	[7]
NSP (wheat bran)	427.3 g / 101.9 g / 135.9 g / 138.3 g starch / 2.33 g RS	3	OM	16S clone libraries, qPCR, DGGE	No significant changes	[7]
RS vs non-starch polysaccharides	52 / 13 / 35 / combined with 28 g RS or non-starch polysaccharides	3	OH	Phylogenetic microarray and qPCR	↑ <i>Ruminococcaceae</i> and ↑ <i>Lachnospiraceae</i> (respectively)	[130]

Table 3: Summary of dietary studies utilizing high protein (HP) diets. All dietary components are in percentages unless stated otherwise.

Duration indicates the amount of time the dietary change/intervention was given. All subjects were adults unless specifically stated otherwise.

The following is a list of abbreviations for subjects: Wist (Wister rats); OM (obese men); OH (obese human). All samples were derived from fecal samples unless specifically stated otherwise.

Dietary change/ intervention	% dietary component (% carb / % prot / % fat)	Duration (weeks)	Subjects	Method for profiling	Effect on microbiota	Refs.
Protein						
Hyperproteic-hypoglucidic isocaloric diet (HP); CH ₂ O not specified or controlled	(HP) 53% whole milk protein with 54% reduced sucrose and corn starch	2.1	Cecal & colonic content/ Wist M rats	qPCR, DGGE	↓ <i>Clostridium coccoides</i> , <i>C. leptum</i> , <i>Faecalibacterium prausnitzii</i> in cecum and colon. Microbiota diversity higher in cecum but lower in colon.	[109]
Normoproteic diet (NP)	(NP) 14% whole milk protein	2.1	Cecal & colonic content/ Wist M rats	qPCR, DGGE	No significant changes	[109]
HP and moderate-carbohydrate (HPMC) diet	181 / 139 g / 82 g	4	OM	FISH	No significant changes	[110]
HP and low-carbohydrate (HPLC) diet	22 g / 137 g / 143 g	4	OM	FISH	↓ <i>Roseburia</i> , <i>Eubacterium rectale</i> , <i>Bacteroides</i> spp. No changes in <i>Faecalibacterium prausnitzii</i> .	[110]
HP/Reduced carbohydrate weight loss diet	NA	3	OM	SSeq of 16S clone libraries, qPCR, DGGE	↓ <i>Collinsella aerofaciens</i> , <i>Eubacterium rectale</i> , and <i>Roseburia</i> spp.	[7]
HP and moderate-carbohydrate (HPMC) vs HP and low-carbohydrate (HPLC) diet	HPMC: 35 / 30 / 35 HPLC: 4 / 30 / 66	4	OH	FISH	↓ <i>Bifidobacterium</i> , <i>Roseburia</i> spp. and <i>Eubacterium rectale</i> . No differences in <i>Bacteroides</i> or other <i>Clostridium</i> clusters (XIVa, IX, IV).	[28]
Weight loss (WL) diet (high protein-medium carbohydrate)	50 / 35 / 15	3	OH	Phylogenetic microarray and qPCR	↓ bifidobacteria	[130]

