

REVIEW

Western diets, gut dysbiosis, and metabolic diseases: Are they linked?

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ABSTRACT

Obesity afflicts 36.5% of the US population and 600 million individuals world-wide. Thus, it is imperative to understand the risk factors underlying metabolic disease including diet, activity level, sleep, and genetics. Another key contributory factor is the gut microbiota given its widely reported role in the development of metabolic disease. The gut microbiota, particularly its structure and function, is heavily influenced by Western style diets rich in a complex mixture of fats and high in simple sugars. In this review, the profound impact of obesity and Western diets on the gut microbiota will be illustrated, and the following research questions will be addressed: 1) to what extent do high fat diets (HFDs) alter community membership and function and does this depend upon the amount or type of fat consumed?, 2) how rapidly do dietary shifts alter gut microbial communities?, 3) are these alterations sustained or can the microbiome recover from dietary stress?, 4) how does diet drive host-microbe interactions leading to obesity?, and 5) what can be done to restore the detrimental impact of HFD on the gut microbiota? The goal of this review is to address these questions by parsing out the effects and underlying mechanisms of how Western diets impact the gut microbiota and host. By doing so, potential avenues for further exploration and strategies for microbiome-based interventions to prevent or treat diet-induced obesity may become more apparent.

ARTICLE HISTORY

Received 28 September 2016
Revised 11 November 2016
Accepted 5 December 2016

KEYWORDS

dietary fat; gut microbiota; gut microbiome; metabolism; obesity; probiotics; prebiotics; Western Diet

Introduction

Obesity and associated comorbidities, including diabetes, metabolic syndrome, and heart disease, adversely impact quality of life and result in substantial health care costs.^{1,2} Thus, it is imperative to understand the complex interplay between the many contributory factors underlying metabolic disease. These include diet, activity level, sleep, genetics, and the gut microbiota, among others. This review will focus specifically on the interaction between diet and the gut microbiota (i.e., the collection of bacteria, fungi, archaea, and viruses harbored in the gastrointestinal tract), with a specific emphasis on gut bacteria. The structure and function of the gut microbiota are heavily influenced by Western diets, rich in several types of fats and simple sugars, and the complex interaction between diet, host, and microbes may promote the development of obesity.

Research regarding the dietary impact on gut microbiota and host-microbe interactions has become an attractive area of study to better understand the etiology of obesity and to identify novel strategies to combat this world-wide epidemic. Not surprisingly,

the number of publications regarding the gut microbiota and human health has increased dramatically over the past 15–20 y.³ In a recent search conducted in 2015, 700 publications were found that provided associations between gut microbes and obesity, whereas the second leading number was 400 with associations with cancer.⁴ Studies utilizing mice raised in the complete absence of microbiota (germ-free mice) and fecal microbiota transplant (FMT) have provided the most compelling evidence establishing the causal link between gut microbes and increased adiposity.^{5–7} For example, several groups have shown that germ-free mice fed a high-fat, high-sugar diet are resistance to diet-induced obesity (DIO).^{5,8} Transplant of microbes induced by Western diets or obesity result in an increase in fat mass in germ-free mouse recipients.^{7,9} In contrast, transplant of gut microbiota from lean human individuals to those with metabolic syndrome has been shown to improve insulin sensitivity,⁶ suggesting that FMT may have therapeutic potential for metabolic disease. Taken together, these studies

illustrate the influence of gut microorganisms on host physiology and disease development.

The gut microbiota contains over 1 trillion cells belonging to thousands of different microbial species from bacteria, fungi, archaea, and protists, that collectively carry ≥ 100 times more genes than that of our own genome.¹⁰⁻¹² A recent estimate has shown that the ratio of bacterial to human cells is 1:1, revised from a previous overestimate of 10:1.¹² The gut microbiota is explained by compositional features (i.e., microbial diversity and relative abundance) and functional capacity (i.e., microbial fitness, metabolic capacity, and influence on the host). Microbial diversity is characterized by α and β diversity metrics, where α diversity explains the richness of the community or the number of different microbial species present and evenness meaning the relative abundance of microbial species in one sample. β diversity explains the difference in community membership and structure across samples as opposed to within one sample. While diversity embodies the presence/absence and distribution of bacteria, function refers to the activities of bacteria, such as formation of clades, establishment of niches and biofilms, response to environmental stimuli, metabolite production, and impact on the host. Each of these aspects of the gut microbiota is heavily influenced by dietary pressures particularly Western diets that are rich in saturated and unsaturated fats, simple sugars, and low in fiber. Studies investigating diet-mediated changes in microbial structure and function using 16S rRNA amplicon sequencing, metagenomics and metabolomics, respectively, will be discussed below.

In addition to illustrating the profound impact of obesity and Western diets on the gut microbiota, the following important research questions will be addressed: 1) to what extent do high fat diets (HFD) alter community membership and function and does this rely on the amount or type of fat and/or in what proportion they are consumed?, 2) how rapidly do dietary shifts alter our microbial communities and do these shifts precede the development of obesity?, 3) are these alterations sustained or can the microbiome recover from dietary stress?, 4) how does diet drive host-microbe interactions leading to obesity?, and 5) what can be done to restore the detrimental impact of HFD on the gut microbiota? The goal of this review is to answer these questions, thereby describing the deleterious impact of Western diets on the gut microbiota and current microbiota-centric strategies to ameliorate HFD-induced dysbiosis and metabolic disease.

Obesity and the gut microbiota

Obesity has been associated with the deleterious reorganization of the gut microbiota commonly referred to as dysbiosis. Dysbiosis in obesity is often characterized by decreased microbial diversity, changes in relative abundance of major phyla such as Firmicutes and Bacteroidetes, and/or a bloom of pathogenic microorganisms.¹³ However, a recent meta-analysis performed by Pat Schloss's group of 10 independent studies examining the gut microbiota in obese human subjects shows that the commonly used Firmicutes/Bacteroidetes ratio (F/B ratio) is an arbitrary metric.¹⁴ The F/B ratio is certainly an unreliable indicator of the functional properties of the gut microbiota and their impact on the host and can also be misleading because the relative abundance of certain members of Firmicutes like *Lactobacillus* have been shown to be decreased by HFDs. Nonetheless, the functional outcome of dysbiotic microbiota is clear given that transplant of dysbiotic communities to naïve germ-free mice increases adiposity, demonstrating a direct impact of microbes on promoting fat storage in the mammalian host.⁷

Conversely, weight loss or gastric bypass surgery in animals and humans can shift dysbiosis toward health-associated states.^{13,15,16} For example, Wang et al. observed that weight loss achieved through treatment with liraglutide, a GLP-1 receptor agonist, was associated with mild, yet significant changes in gut microbiota composition in mice, including a reduction in obesity-associated phylotypes and a concomitant increase in lean phylotypes.¹⁷ However, Wang et al. did not observe appreciable differences in the F/B ratio with liraglutide treatment. Surgical interventions in morbidly obese individuals who underwent gastric bypass displayed increased microbial diversity with altered microbial composition and function in under 3 months post-surgery prior to extensive weight loss, which was sustained for up to 1 y.¹⁸ Compositional changes seen after Roux-en-Y gastric bypass (RYGB) in mice include decreases in Firmicutes, and increases in both Bacteroidetes and Verrucomicrobia.¹⁹ While these findings were interesting, their functional significance was shown when mice conventionalized with RYGB microbiota exhibit reduced body weight and fasting triglyceride levels compared with sham controls.¹⁹ Together these data suggest that the microbiota following RYGB in humans and mouse models elicits a direct functional impact on host energy balance,

resulting in restoration of metabolic homeostasis and resistance to DIO.

In addition to the transfer of whole communities, delivery of single strains into germ-free mice, including *Enterobacter cloacae* B9,²⁰ *Clostridium ramosum*,²¹ and Lachnospiraceae strain AJ110941,²² led to an increased propensity for obesity. In contrast, introduction of *Akkermansia muciniphila*, belonging to the phylum Verrucomicrobia, to a complex community of gut microbiota, has been directly implicated in improving metabolic syndrome and atherosclerosis. This microbe is often underrepresented in obese subjects and increased after RYGB or during fasting.^{5,19,23-26} Additionally, administration of *A. muciniphila* to obese animals was shown to improve glucose tolerance.²³ Together these observations suggest certain microbes might regulate different and opposing aspects of host metabolic response to DIO, however further investigations are needed to determine direct causality and mechanism of action.

To what extent do high fat diets alter community membership and function and does this rely on the amount of fat or type of fat consumed?

Diet profoundly impacts microbial structure in animal models that closely resembles dysbiosis seen in obese humans as demonstrated by Peter Turnbaugh's group. Specifically, David et al. showed that high-fat diets consisting solely of animal-based foods such as meat and cheese dramatically and immediately shifted community structure of the gut microbiota in human participants.²⁷ Changes in β diversity were found after only two days of diet shift compared with baseline. The dominance of diet in altering microbial composition was further recapitulated in an animal study by Carmody et al. in which five different inbred mouse strains, four genetic knockout strains relevant to host-microbe interactions (e.g. *ob/ob*, *NOD2*, *MyD88*^{-/-}, and *Rag1*^{-/-}), and 200 outbred mice were placed on Western high-fat, high-sugar diet and compared mice fed diet rich in plant polysaccharides.²⁸ The Western diet profoundly modulated microbial community structure compared with the LF diet regardless of strain or genetic differences, suggesting that diet is the dominant driver in shaping the gut microbiota over host genetics.²⁸

An often over-looked component of diet-related studies such as those described above is the amount and type

of fat and/or in what proportion they are consumed to promote obesity and shifts in microbial structure. For instance, work from Eugene B. Chang's lab shows that dietary fats are not all the same in the way they affect both the host and how they shape gut microbial communities. Huang et al. showed that a diet rich in polyunsaturated (PUFA) omega-6 fatty acids from safflower oil resulted in altered microbial composition compared with diets rich in saturated milk fat (MF) or lard.²⁹ The PUFA diet was also associated with increased adipose tissue inflammation compared with all other high saturated fat diets. In a separate study by Devkota et al., it was found that the MF diet as opposed to the PUFA or lard diets promoted a bloom of bile-tolerant members of Deltaproteobacteria that was dependent upon production of taurocholate by the host.³⁰ This study clearly demonstrated a host-microbe interaction that ultimately contributed to the development of colitis in genetically susceptible *IL10*^{-/-} mice. Notably, the diets used in these studies were designed to mimic the current dietary intake of fat in the US population which at the time was ~37% kcal from fat (NHANES), whereas the typical amount of fat used in animal studies ranges from 45–60% kcal.

The amount and fatty acid composition of the fat source is quite important when considering study outcomes and impact on microbial composition and function versus host physiology. For example, one study comparing saturated fat (lard) to long-chain PUFA derived from fish oil detected significant differences in microbial composition and host adiposity.³¹ Here, a diet rich in fish oil prevented against negative shifts in gut microbes and resulted in a decrease in host obesity-associated inflammation. While this study provides proof-of-concept and compelling evidence that the source of dietary fat strongly influences microbial structure, the amount of fish oil provided in the study is not representative of the daily recommended intake or even therapeutic doses. In this study, fish oil contributed 45% total kcal content. In humans, this means that if all fat from a 2,000 kcal/day diet was coming from fish oil (2,000 kcal * .45 = 900 kcal from fat at 9kcal/g fat = 100 g), one would have to consume 100 g of fish oil. This is equivalent to 100 fish oil capsules or 33–100 servings of salmon per day depending on the type of salmon (given that 3 ounces of salmon contains 1–3 g omega-3 fatty acids).³² Therapeutic doses of fish oil for hyperlipidemia ranges from 3–4 g per day.³³ Therefore, the amount provided in the animal study is 25–33 times

more than recommended therapeutic doses. This level of omega-3s would not be achievable as most people consume < 2 g fish oil from the diet per day.³⁴ Despite these limitations and translatability, it was also demonstrated in this study that serum from lard diet-fed mice activated toll-like receptor (TLR)2 and TLR4 in HEK-Blue reporter cell lines over-expressing these TLRs as compared with serum from fish oil-fed mice.³¹ Additionally, lard-mediated weight gain and insulin resistance was lost in MyD88-deficient mice, thereby confirming the long-standing notion that bacterial products drive adiposity and insulin resistance in part through activation of TLRs. Germ-free mice receiving FMT of lard-induced microbiota experiences more weight and had elevated macrophage-2 staining in white adipose tissue (WAT) than the mice receiving fish oil-induced microbiota, despite all mice being maintained on a lard diet for 3 weeks.³¹

Despite the knowledge gained in this study, it would be interesting to know if the beneficial effect of the fish oil-induced microbiota was a result of direct impact of the diet on the microbial communities or if the effectiveness of fish oil-induced microbiota depended on the host cues as well. The former would allow for the production of potentially more effective probiotic therapies without the need for excessive fish oil consumption. However, in a separate study by Lam et al., it was found that supplementation with fish oil at 7% of total kcals (an amount achievable through diet or supplementation) or Resolvin D1 decreased HFD-mediated bloom in Deltaproteobacteria, increased HFD-mediated reduction in Bacteroides, as well as modestly impacted β diversity.³⁵ This group also compared diets rich in omega-3 vs omega-6 fatty acids and found differences in microbial communities after 8 weeks of diet feeding.³⁵ More studies are needed to determine amounts of fatty acids that are achievable either through supplementation or dietary consumption to elicit beneficial effects on the gut microbiota.

How rapidly do dietary shifts alter gut microbial communities?

The rapid shift of community membership and structure of the gut microbiota under HFD conditions has been extensively examined in several studies. First, in the study by Carmody et al. it was demonstrated that exposing mice to 3-day cycles of low-fat diet (LFD) or HFD led to consistent changes in community membership and structure.²⁸ Two cohorts of mice were initially

placed onto a LFD or HFD and each group switched to LF or HF every 3 d for several cycles. Particularly consistent was the inverse relationship of the relative abundance of Bacteroidales and Clostridiales under LFD or HFD conditions. Namely, Bacteroidales was increased during LFD cycles and Clostridiales was increased during HFD cycles. Observed changes in diversity and bacterial abundance were apparent within 1 day of diet shift and consistently reverted back after the introduction of the previous diet.²⁸ However, the functional importance of these shifts is unknown.

These rapid diet-induced changes were also observed in humans in the study conducted by David et al. Here, the study showed that humans given an animal-based diet, consisting of 69.5% kcal from fat, 30.1% kcal from protein and nearly 0 g of fiber, altered microbial structure in as little as 48 hours of diet initiation.²⁷ This is consistent with transit time being on average 2–3 d in humans.³⁶ Although this diet regimen is not representative of average intake of fat, protein, and fiber for most individuals,³⁷ the impact on the gut microbiota was rapid and profound. In addition to a significant change in β diversity of microbial communities from baseline, the animal-based diet also promoted blooms in bile-tolerant and pathogenic hydrogen-sulfide producing bacteria such as *Bilophila wadsworthia*.²⁷ This bacterium belongs to the phylum Deltaproteobacteria and has been implicated in the development of colitis and often associated with diets rich in saturated fat.^{30,35} While the animal-based diet significantly decreased short chain fatty acid (SCFA) levels, the plant-based diet increased the abundance of saccharolytic bacteria such as *Roseburia* and *Faecalibacterium prausnitzii*. RNA-seq analysis of the microbiome revealed that the plant-based and animal-based diet yielded different transcriptional responses, including increased expression of genes related to amino acid catabolism and the production of carcinogenic polycyclic aromatic hydrocarbons in the animal-based diet.²⁷ Taken together, this study showed that dietary shift and alterations in dietary fat source elicits significant changes in both the structure and function of the gut microbiome in as little as two days.

High fat diets also transform the metagenomes of the bacteriophage community, known as the “phageome.” Bacteriophage are small DNA viruses that depend on bacterial hosts for propagation through lytic or lysogenic cycles. The latter involves integration of the bacteriophage nucleic acid into the bacterial

host genome that can potentially impart changes to its functional properties. It was demonstrated by Howe and Ringus et al. that a diet high in saturated fat can shift the phageome within 24 hours in an independent manner from their bacterial host pattern. Notably, this shift was not reversible after washout, suggesting that diet-mediated changes in the phage community are persistent.³⁸ This concept raises an important concern as to whether or not microbial communities can be restored following dietary insult.

Are diet-mediated alterations sustained or can the microbiome recover from dietary stress?

Given the dramatic and rapid changes on microbial structure, it is of interest to know if the gut microbiome can recover from dietary stress. It was observed in the diet shift study by Carmody et al. that the abundance of several operational taxonomic units (OTUs) was dependent on previous dietary exposure. For example, several OTUs belonging to Clostridiales become more persistently abundant under high-fat diet conditions with each new cycle of HFD exposure.²⁸ This effect is known as hysteresis and has been demonstrated in other reports.³⁸⁻⁴⁰

Not only has a hysteresis effect been shown within one lifespan but also has been demonstrated across generations. Work by Justin Sonnenburg's group convincingly demonstrated that diets deficient in microbe-accessible carbohydrates (MAC) and high in simple sugars results in the generational loss of bacterial diversity.⁴⁰ Restoration of diversity and reappearance of specific microbes could only be achieved upon FMT but not from diet switching. This study sets the foundation for a theory whereby the rapid shift in Western diets consisting of processed high-fat and high simple carbohydrate foodstuffs may have led to the progressive loss of bacterial diversity over the past several decades. It is speculated that our bodies are not evolutionarily equipped to adapt to the sudden insult on our gut microbiota, thereby leading to excess fat storage and obesity.⁴⁰ Hence, the reintroduction of helpful microbes through FMT or probiotic supplementation may be an effective approach to combat HFD-induced dysbiosis and loss of bacterial diversity which is discussed below.

How do high fat diets drive host-microbe interactions that result in obesity?

In addition to compositional changes, differences are also observed in metabolic capacity of the microbial

community during obesity. Short chain fatty acids (SCFAs), for instance, have been shown to have a profound impact on host metabolism and energy balance. Previously, it was reported that an obese microbiome is enriched for metabolic pathways that are highly efficient at generating SCFAs, which are absorbed by the host via the portal vein resulting in hepatic gluconeogenesis and de novo lipogenesis. This alteration in energy harvest efficiency due to the microbiota was shown to have considerable influence on the regulation of peripheral fat deposition.^{41,42} Colonization of germ-free mice with gut microbes from obese humans or animals resulted in increased fermentation of carbohydrates within the distal gut leading to increased SCFA production and absorption, shifting host energy balance toward fat storage.^{16,41} Contrary to these reports, several more recent studies reveal that obese mice exhibit decreases in SCFA levels with concomitant increases in levels of hydrogen sulfide (H₂S).^{5,43} Several groups have revealed that SCFAs can elicit beneficial effects on glucose metabolism, and therefore the observed reduction of SCFAs in obesity is pertinent. For instance, both butyrate and propionate can induce intestinal gluconeogenesis, which has been shown to improve glucose tolerance in an animal model.⁴⁴ Additionally, SCFAs have been shown to activate G-protein coupled receptors (GPRs) specifically GPR41, GPR43, and GPR109A. Indeed, mice lacking GPR43 exhibit reduced β cell function and mass under HFD feeding conditions.⁴⁵

While decreases in SCFAs are observed in obesity, studies show that H₂S levels are elevated.⁵ Hydrogen sulfide can be produced endogenously by the host, however, levels in germ-free mice are 50–80% lower than in conventional animals in both peripheral plasma and the gastrointestinal tract, indicating gut microbes are significant contributors to the H₂S pool.¹⁵ As shown by Devkota et al., the increased H₂S production by gut microbes could, in part, be due to the direct impact of specific fat sources on host bile acid production.³⁰ Diets high in saturated fat elicit increased host production of taurine conjugated hepatic bile acids. This leads to the increased availability of organic sulfur to sulphite-reducing gut microbes within the intestine, including Deltaproteobacteria, allowing them to bloom. Whether microbially-derived vs. host-derived H₂S has a more dramatic impact on host metabolic function remains to be determined. Altogether, direct and indirect changes in microbial

function and metabolic byproducts elicited by HFDs can have a significant impact on metabolic health of the host.

Not only do the functions and metabolic byproducts of specific gut microbes impact host metabolism, but gut microbes themselves can directly alter the host metabolic landscape. For instance, angiotensin-like 4 (Angptl4) expression in the enterocyte is inhibited by gut microbiota, which results in increased activity of lipoprotein lipase (LPL), an enzyme that controls fatty acid uptake.⁴¹ Germ-free mice, which are resistant to diet-induced obesity, exhibit increased Angptl4 and decreased LPL activity.⁴¹ Furthermore, Angptl4-deficient mice have decreased fecal triglycerides and obese patients also exhibit decreased circulating plasma Angptl4 as compared with their lean counterparts.^{46,47} In addition to Angptl4, AMP-activated protein kinase (AMPK), a main metabolic fuel sensor in liver and skeletal muscle, is also impacted by gut microbiota. When activated, phosphorylated AMPK indicates increased levels of fatty acid oxidation. AMPK activity is increased in both skeletal muscle and the liver of germ-free mice as compared with conventionalized mice, suggesting that fat utilization is high in the germ-free mouse, protecting against HFD-induced obesity.⁴² Conversely, conventionally-raised mice fed a high fiber diet, which promotes microbial production of SCFAs, exhibited increased activation of AMPK.⁴⁸ This work suggests that the milieu of microbiota, coupled with specific dietary components can impact host molecular metabolism resulting in either protection from or induction of obesity.

Not only can diet-induced gut microbes impact underlying molecular metabolism, they can also increase host inflammation, which influences HFD-induced obesity. Previous work has demonstrated that innate immune adaptors including MyD88, a universal adaptor protein for the majority of Toll-like receptors (TLRs) or the loss of specific TLRs can protect against HFD-induced obesity. Indeed, TLRs, specifically TLR4 has been shown to be sensitive to saturated fatty acids.⁴⁹⁻⁵² Again, Caesar, et al. showed that the type of fat included in the HFD impacted diet-induced obesity.³¹ Here, mice fed lard diet high in saturated fat exhibited increased expression of TLR4 in WAT as well as increased circulating levels of LPS when compared with mice fed fish oil. MyD88-deficient mice were protected against these effects. Interestingly, these

changes appeared to be mediated by induction of monocyte chemoattractant protein, CCL2 in WAT by gut microbes promoted specifically by lard-based diet, but not fish oil.³¹ Together, this work demonstrates that microbes selected by high saturated fat diets can induce inflammation through innate immune signaling both within the gut and in peripheral tissues, including WAT, to promote the development of obesity. Several other mechanisms linking HFDs, gut microbiota, and obesity have been reported (i.e., lipid absorption, liver metabolism, and circadian rhythm) and beyond the scope of this review. Therefore, readers are referred elsewhere⁴³ for further insight into these mechanisms.

What can be done to restore the detrimental impact of high fat diet on the gut microbiota?

The host-microbiome field is moving toward improving metabolism and weight maintenance through modulating gut microbial communities using a variety of supplements including prebiotics and probiotics. Several studies have successfully employed prebiotic and probiotic therapies against HFD-induced obesity. Due to the lack of evidence regarding FMT, synbiotics, or postbiotics for DIO specifically, readers are referred elsewhere for a review on those therapies.⁴³

Prebiotics

It has been long appreciated that consumption of fiber is important for improved digestion and overall metabolic health by increasing satiety, slowing motility, increasing fecal bulk, and reducing lipid absorption.⁵³ Due to the boom in gut microbiota research, more is understood about the interaction between soluble fiber and saccharolytic bacteria and the mechanisms by which beneficial effects are imparted on the host. Prebiotics are foods or dietary supplements that encourage the growth of saccharolytic bacteria meaning those that metabolize non-digestible carbohydrates such as inulin and fructose-rich oligosaccharides (FOS). In order to be considered a prebiotic, the product must be resistant to gastric acidity, non-digestible by the host in the small intestine, fermentable by bacteria, and promote the abundance of beneficial bacteria.⁵⁴ Prebiotics have recently been shown to improve complications associated with HFD-induced metabolic disorders including obesity and insulin resistance.⁵⁵ For instance, bamboo shoot fiber was shown

to prevent HFD-induced weight gain and insulin resistance in mice, and concurrently altered microbial composition including increased relative abundance of Bacteroidetes and decreased abundance of Firmicutes.⁵⁶ Inclusion of 10% (w/w) short chain FOS in HFDs (60% kcal from fat) altered microbial composition and function as measured by changes in metabolic byproducts of the microbiome.⁵⁷ In another report, maize-derived feruloylated oligo- and polysaccharides (FOPS) included at 5% (w/w) of a HFD (60% kcal from fat) improved outcomes in a subset of mice that correlated with changes in microbial structure.⁵⁸

Mechanisms behind the beneficial effects of dietary fiber include SCFA production (discussed previously), stimulation of intestinal gluconeogenesis, increased epithelial integrity, release of hormones PYY and GLP1 to promote satiety and insulin sensitivity, increased expression of antimicrobial peptides, and alteration of gut microbial community structure (discussed previously).¹⁷ Fructooligosaccharide (FOS) treatment in mice fed a Western diet, exhibited improved glucose and insulin tolerance compared with controls. The therapeutic effect of FOS was lost in mice deficient in glucose-6-phosphatase catalytic subunit (G6Pc), thereby inhibiting intestinal gluconeogenesis. These findings implicate that intestinal gluconeogenesis is necessary for FOS-mediated glucose and insulin sensitivity.⁴⁴ Similar results have been shown in humans. For example, participants fed brown beans⁵⁹ or prebiotics containing wheat fiber and soluble⁶⁰ fiber displayed improved insulin sensitivity. Everard et al. found that oligofructose treatment (0.3 g per mouse per day – roughly 8.5% w/w assuming mouse eats 3.5 g per day) restored gene expression of the antimicrobial peptide, Regenerating islet derived 3 gamma (Reg3 γ), that was reduced after HFD (60% kcal from fat) feeding.²³ This was concurrent with a restoration of *Akkermansia*, *Bifidobacterium*, *Sutterella* and *Turicibacter*, which were not detectable under HFD treatment alone.²⁶ Oligofructose supplementation also increased intectin expression, which promotes epithelial cell turnover and maintenance.⁶¹ Collectively, these findings suggest that prebiotics work through several pathways and function as a safe and effective approach to improve metabolic health.

Probiotics

Several lines of evidence support the effective use of probiotics to thwart HFD-induced dysbiosis and metabolic disease. Probiotics are live microorganisms delivered individually or in combinations such as

VSL#3 that positively impact health outcomes in the host.⁵⁴ For example, VSL#3 contains 7 different strains belonging to the genera *Bifidobacterium* and *Lactobacillus* and has been shown to improve NAFLD in children.⁶² While mixed strain probiotics like VSL#3 or a synbiotic (the combination of a probiotic and prebiotic), may be more effective than single microbial isolates alone, it is difficult to parse the individual effectiveness of the microbes to identify the mechanisms through which they are impacting the host. Choosing a probiotic is further complicated by the sheer number of different formulations available on the market or used in research and the lack of regulatory oversight and quality control, and therefore it is difficult to discern the most effective probiotic therapy to fight HFD-induced obesity and also may need to be tailored to each individual.

Delivering a single strain vs. a combination of bacteria in probiotic formulations is an important consideration as each strain may have a different impact on microbial structure/function or on the host immune response. For example, in the same study, three strains of bacteria including *Lactobacillus paracasei* CNCM I-4270, *Lactobacillus rhamnosus* I-3690 and *Bifidobacterium animalis subsp lactis* CNCM I-2494 independently decreased body weight and improved glucose tolerance in mice albeit through distinct mechanisms⁵⁵ (as previously reviewed).⁴³ A thorough understanding of each strain cannot be fully understood unless they are analyzed separately in animal studies and randomized clinical trials (RCTs) in humans.

Additional strains that have been investigated for probiotic potential in rodent models of diet-induced obesity (DIO) include *Lactobacillus rhamnosus* *hsryfm* 1301,⁶³ *Bacteroides uniformis* CECT 7771,⁶⁴ *Bifidobacterium pseudocatenulatum* CECT 7765,^{65,66} *Lactobacillus casei* CRL 431 (Novotny et al. 2015). In studies conducted by Cano et al. (2012, 2013; *Bacteroides uniformis* and *Bifidobacterium* 7765, respectively) and Moya-Perez et al. (2015); *Bifidobacterium* 7765 strains were isolated from infant stool and selected based on anti-inflammatory potential as determined from preliminary screening assays examining IL10 and inflammatory cytokine production in macrophages.⁶⁴⁻⁶⁶ Daily gavage of each strain (5 \times 10⁸ to 1 \times 10⁹ CFUs) reduced body weight as well as serum and liver lipids, including cholesterol and triglyceride, and improved glucose tolerance after HFD (60% kcal from fat) feeding for 7–14 weeks. Another group found that *Lactobacillus rhamnosus* provided at 2 \times 10⁹

CFUs reduced serum and liver lipid levels in a rat model of diet-induced hyperlipidemia,⁶³ and *Lactobacillus casei* was reported to have anti-inflammatory effects in reducing infiltrating immune cells in the liver and monocyte chemoattractant protein in adipose tissue.⁶⁷ Collectively, these studies show promise for providing individual probiotic strains to improve metabolic outcomes in HFD-induced obesity, including increasing glucose tolerance, and decreasing hyperlipidemia and fatty liver. Notably, only one of these studies provided a thorough analysis of the gut microbiota using 454 pyrosequencing of the 16S rRNA gene and targeted qPCR, in order to show significant levels of the probiotic strain after supplementation.⁶⁶ Future research examining probiotics should thoroughly assess the colonization of microbes including their levels in the lumen vs mucosa, regional position throughout the GI tract, and how long they remain in the gut after supplementation ceases. This information could provide important information regarding the function of the probiotic, how it interacts with endogenous microbes, and most importantly the host.

Studies systemically determining the need to incorporate prebiotics to promote the growth of the probiotic are also needed. Fiber supplementation may aid in the growth, colonization, and function of the probiotic, especially given that enough information regarding the preferential fuel source of the probiotic is known. More stringent genomic, transcriptomic, and metabolomic analyses of specific probiotics could provide this information concerning the preferred fuel source and the most effective conditions for optimal growth and beneficial metabolite production for the host. Another important consideration is the level of inter-individual variability between humans that may have differential responses to probiotics. While dramatic shifts in HFDs are likely to induce similar shifts in the gut microbiota across a population, subtle changes due to the introduction of one microbe may not elicit changes to the same extent.

Many of the summarized studies present the utility of pre and probiotics in animal models. Further evidence highlighting the beneficial role and outcomes of the use of pre and probiotics in human subjects in the context of metabolic disease can be found in several recent reviews.⁶⁸⁻⁷⁰ Because probiotics are marketed as nutraceuticals (foodstuffs that provide health benefits in addition to their basic nutritional value) and not drugs under FDA purview, there is a concern for proper quality control and standardization. Unfortunately, the popularity

and marketing of probiotics have surpassed the rate at which adequate research can be conducted in humans to support claims of their beneficial effects. Therefore, consumers should be careful and research products they purchase before consuming probiotics on a daily basis. Altogether this body of research suggests that probiotics are a promising therapy, but more evidence is needed in humans, particularly in the form of randomized controlled trials, to aid in better understanding of which probiotics are effective, how much to take, how often, and how long to take them in order to promote lasting effects on metabolic health.

Summary

Given the many demonstrated effects of diet-induced dysbiosis on host metabolic functions and energy balance, modulating the structure and function of gut microorganisms may be an effective approach to fight diet-induced adiposity and its associated metabolic consequences. Important considerations for understanding how dietary fat-induced changes in the gut microbiota impact host metabolism and the development of obesity include: 1) the amount, type (e.g., unsaturated vs saturated), and mixture of dietary fats can dramatically shift gut microbial community membership and function, 2) the impact of HFDs on the gut microbiome can be rapid (occurring within 24–48 hours) and sustained if dietary habits persist, and may not be recoverable without change in diet, reintroduction of extinguished microbial strains, or dietary supplementation, 3) HFDs elicit complex interactions between microbes and the host owed to various underlying mechanisms, and 4) prebiotics and probiotics represent promising therapies for combating HFD-induced dysbiosis but more systematic animal studies and RCTs are warranted. Overall, more research, better study design and experimental models, increased awareness of the functional heterogeneity of diets, and better functional measures of gut microbiota are needed to understand the complex interactions of dietary components and their effects on gut microbes and host. However, with the growing interest and capabilities in host-microbe research, there is every reason to believe that significant gains will be made in our understanding of the microbial basis of human metabolism and in the development of novel and effective interventions for the prevention and management of obesity.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by the NIH NIDDK under grants DK42086 (DDRCC), DK097268, DK07074 and by the Gastrointestinal Research Foundation (GIRF).

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