Influence of dietary fat on intestinal microbes, inflammation, barrier function and metabolic outcomes

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Abstract

Recent studies using germ-free, gnotobiotic microbial transplantation/conventionalization or antibiotic treatment in rodent models have highlighted the critical role of intestinal microbes on gut health and metabolic functions of the host. Genetic and environmental factors influence the abundance and type of mutualistic vs. pathogenic bacteria, each of which has preferred substrates for growth and unique products of fermentation. Whereas some fermentation products or metabolites promote gut function and health, others impair gut function, leading to compromised nutrient digestion and barrier function that adversely impact the host. Such products may also influence food intake, energy harvest and expenditure, and insulin action, thereby influencing adiposity and related metabolic outcomes. Diet composition influences gut microbiota and subsequent fermentation products that impact the host, as demonstrated by prebiotic studies using oligosaccharides or other types of indigestible fiber. Recent studies also show that dietary lipids affect specific populations of gut microbes and their metabolic end products. This review will focus on studies examining the influence of dietary fat amount and type on the gut microbiome, intestinal health and positive and negative metabolic consequences. The protective role of omega-3-rich fatty acids on intestinal inflammation will also be examined.

Keywords: Dietary fat; Obesity; Microbiota; Inflammation; Barrier function

1. Introduction

Obesity is a global health issue with over 400 million people classified as obese and 1.6 billion overweight, including 20 million children under the age of 5 years [1]. In the United States, obesity is rapidly increasing among all age groups. Currently, all but two states have at least 20% of their population classified as obese [2]. Diets high in calories, especially from refined sugars, saturated and trans-fats, and sedentary lifestyles contribute to this obesity epidemic. The rapid rise in obesity is accompanied by a similar increase in cardiovascular disease, hypertension and insulin resistance or type 2 diabetes ([3], reviewed in Ref.[4]). An emerging feature of obesity and type 2 diabetes is their linkage with chronic inflammation that purportedly begins in white adipose tissue (WAT) and becomes systemic (reviewed in Ref.[5]; Fig. 1). Obese patients with type 2 diabetes have elevated levels of tumor necrosis factor (TNF)-α in their blood [6], WAT [7] and muscle [8]. Furthermore, impaired glucose disposal is positively correlated with TNF-α expression [6,8,9]. However, mechanisms that initiate WAT inflammation remain largely undefined.

1.1. Gut microbes and obesity

Studies using genetically obese mice were some of the first to provide evidence that gut microbes were associated with obesity (Fig. 2). Specifically, germ-free C57BL/6j mice inoculated with cecal microbes from normal mice presented increased adiposity and insulin resistance within 2 weeks compared to their germ-free cohorts [10]. Increased absorption of carbohydrates and elevated hepatic lipid synthesis were positively correlated with increased adiposity. Genetically obese ob/ob mice also exhibited a 50% reduction in numbers of Bacteroidetes and an increase in Firmicutes compared to their lean cohorts [11]. Similar increases in Firmicutes in diet-induced, obese mice were detected [12,13], suggesting that feeding an obesity-promoting, high-fat diet also impacts gut microbiota. Notably, transplantation of gut microbes from obese donor mice into germ-free mice increased adiposity compared to germ-free mice receiving gut microbes from lean mice [14]. Similar changes in microbial phyla reported in mice were detected in obese compared to lean human subjects, and feeding a low-calorie diet reversed these changes [15].

1.2. Gut microbes and metabolic dysfunction

The discovery that gut dysfunction and metabolic endotoxemia [e.g., elevated lipopolysaccharide (LPS) or peptidoglycan levels] were associated with obesity or insulin resistance in mice [16–18] and
Humans [19,20] provided new insights into a potential linkage between gut microbes, gut barrier function, WAT inflammation and diet, particularly with diet-induced obesity mediated by saturated fatty acids (Fig. 3). Indeed, gut microbes have been implicated in the development of diet-induced obesity [21], chronic inflammation [16,17] and insulin resistance (reviewed in Ref. [22]). Notably, changes in gut microbiota and intestinal inflammation precede weight gain and the metabolic consequences of obesity in several of these models. Furthermore, elimination of gut microbes [21,23,24] or inoculation with specific prebiotics or probiotics [22] attenuates obesity and metabolic dysfunction in several rodent models of obesity or diabetes. For example, germ-free C57BL/6j mice fed a Western diet (i.e., high fat; 41% kcal from fat; ½ beef tallow, ½ shortening) for 8 weeks had lower body mass and fat pad weights compared to conventionally raised mice [23]. This reduced adiposity in the germ-free mice was associated with increased phosphorylated AMP-activated protein kinase and carnitine palmitoyl transferase activity in gastrocnemius muscle or liver. Germ-free mice lacking fasting-activated protein kinase and carnitine palmitoyl transferase activity were protected against diet-induced obesity in high-fat-fed mice (45% kcal from fat; lard based) for 11 weeks weighed less, consumed less calories, excreted more fecal lipids and had improved insulin sensitivity and glucose tolerance compared to conventionally raised, high-fat-fed cohorts [24]. Collectively, these data show that when a high-fat, Western diet is fed, gut microbes can increase energy harvest and decrease energy expenditure in the host, causing a positive energy balance and obesity.

1.3. Prebiotics: microbial fermentation products and obesity

Products of microbial fermentation not only affect systemic inflammation, but impact satiety signals, energy harvest and expenditure, insulin action and immune function in the host as well (reviewed in Refs. [25,26], reviewed in Ref. [27]). For example, prebiotic treatment with oligofructose of ob/ob mice or diet-induced obese mice (60% kcal from fat; lard based) for 5–8 weeks decreased WAT mass and improved markers of the metabolic syndrome or endotoxaemia compared to non-prebiotic-treated mice [28,29]. In ob/ob mice, prebiotic treatment decreased Firmicutes and increased Bacteroidetes, along with alterations in over 100 other taxa, some by as much as 10-fold. Wheat-based prebiotic arabinoxylans prevented high-fat-diet (i.e., 60% kcal from fat; lard based) reductions in the abundance of several types of gut bacteria, including Bacteroides–Prevotella spp. and Roseburia spp., and increased cecal bifidobacteria content after 4 weeks of treatment [30]. These prebiotic-mediated changes in gut microbes were associated with reductions in markers of intestinal inflammation and markers of adipogenesis in WAT. Consistent with these data, arabinoxylans from wheat and chicory roots prevented high-fat-fed mice increased cecal and colon contents or tissue weights and Bifidobacterium spp., improved barrier function and reduced metabolic endotoxemia, systemic inflammation and WAT mass [31]. Chitin-glucan fiber [32], inulin-type fructans [33], tetrahydro isoflavones [34] and polyphenol extracts [35] are other types of prebiotics that alter gut microbes and improve metabolic dysfunction in high-fat-fed mice (45–60% kcal; lard based).

Proposed mechanisms by which these prebiotics improve gut health and reduce metabolic complications of obesity include the following: (a) increasing populations of Bifidobacterium spp. that are positively correlated with intestinal health and negatively correlated with obesity and diabetes; (b) reducing metabolic endotoxemia via enhanced gut barrier function; (c) increasing gut satiety signals such as peptide YY or reducing the orexogenic hormone ghrelin; (d) improving insulin action via increasing glucagon-like peptide (GLP)-1 synthesis; or (e) reducing lipogenesis or adipogenesis via
differential synthesis of the short-chain fatty acids acetate, propionate and butyrate (reviewed in Ref. [36]; Fig. 4). Relative to the latter mechanism, acetate and propionate may contribute to obesity and insulin resistance, because they are substrates for gluconeogenesis in the liver, or lipid synthesis in the liver or WAT, respectively (reviewed in Ref. [27]). Furthermore, acetate activates G-protein receptors (GPRs) 41 and 43 on intestinal epithelial cells, which, in turn, triggers mitogen-activated protein kinase (MAPK) signaling and cytokine and chemokine production [37]. In contrast, butyrate is metabolized by colonocytes for growth and has been reported to have anti-inflammatory properties in LPS-treated mice [38] and in cocultures of macrophages and adipocytes [39]. Notably, low millimolar levels of butyrate increased the expression and activity of angiopeptin-like protein-4 (ANGPTL4) in several human intestinal cell models [40]. Consistent with these data, butyrate provided by oral administration or colonization with Clostridium trybutyricum, a bacterium that selectively produces butyrate, increased the intestinal expression of ANGPTL4 in germ-free mice [40]. ANGPTL4 secretion decreased lipoprotein lipase activity and increased lipolysis in WAT [41], thereby decreasing triglyceride storage and increasing fatty acids available for oxidation. Lastly, supplementation of C57/B6 mice fed a high-fat diet (i.e., 60% kcal from fat; lard based) with a commercially available probiotic (http://shop.vsl3.com/vsl3-c2.aspx) successfully prevented and treated obesity and insulin resistance by altering gut microbial composition and increasing GLP-1 secretion [42]. Increased levels of butyrate stimulated GLP-1 release from intestinal L-cells, resulting in reduced food intake and improved glucose tolerance. Therefore, gut microbes that synthesize more butyrate relative to acetate or propionate may decrease food intake or energy harvest, thereby decreasing hepatic glucose production, de novo lipid synthesis and adipogenesis and increasing lipolysis in WAT, leading to reduced body fat. Collectively, these data provide strong evidence that gut microbiota and their fermentation products play an important role in regulating energy balance, adiposity and related comorbidities.

However, discrepancies in the literature exist concerning the extent to which obesity or insulin resistance results from or contribute to alterations in the gut microbiome [43]. Some of these discrepancies are most likely due to differences in the experimental models, source and variability in gut microbes, and methods employed. For example, genetically obese mice (ob/ob) are hyperphagic on most diets, whereas normal mice are hyperphagic primarily on high-fat, high-sucrose, or cafeteria diets. Thus, ob/ob mice over-consume calories regardless of the energy source, which may have distinct effects on microbial populations and their fermentation products compared to overconsumption of a specific type of dietary fat. Therefore, a number of unanswered questions remain concerning the relationship between diet, gut microbes and metabolic function. Specifically, is the type or the amount of dietary fat most important in regulating populations of mutualistic and pathogenic intestinal microbes? The following sections will explore the literature concerning evidence-based research to address this question.

2. Influence of high-fat diets made from lard or tallow rich in long-chain, saturated fatty acids on gut microbiota, inflammation, barrier function and metabolic outcomes

2.1. High-fat feeding and gut microbes

A number of studies have demonstrated that feeding a high-fat diet (e.g., 45%–60% kcal from fat) influences the types and amounts of gut microbes and adversely affects intestinal health. However, a direct connection between a specific type of gut microbe and metabolic dysfunction due to high-fat-feeding remains unclear. Most of these studies used a fat source rich in saturated fatty acids, particularly lard (i.e., pork fat; ≈40% saturated fatty acids, 45% monounsaturated fatty acids) or tallow (i.e., beef fat; ≈50% saturated fatty acids, 42% monounsaturated fatty acids) to accelerate metabolic dysfunction. Such diets are particularly rich in the long-chain, saturated

Fig. 4. Proposed mechanisms by which prebiotics improve gut health and prevent high-fat feeding-mediated obesity and its metabolic consequences. HF, high fat; GLP, glucagon-like peptide; PYY, polypeptide YY; SFA, saturated fatty acids.
fatty acids palmitate and stearate and are devoid of shorter-chain, saturated fatty acids.

For example, C57BL/6j mice fed a high-fat (94% kcal from fat; corn oil and lard based), carbohydrate-free diet for 4 weeks had lower expression levels of intestinal tight junction proteins and increased intestinal permeability that correlated with increased markers of WAT inflammation and body fat compared to low-fat-fed mice, which was abolished by antibiotic treatment [17]. Using a similar high-fat diet and the same mouse strain for 3 months, some high-fat-fed mice are more likely than others to develop insulin resistance, and those that do have a unique microbial signature (e.g., lower abundance of Clostridium leptum, Enterococcus spp., and Nitrospira spp.) associated with impaired gut barrier function, endotoxemia and increased adipocyte hyperplasia in visceral WAT [44]. Supplementation of high-fat diets with a prebiotic (i.e., gluco-oligosaccharide) also modulated the effects of the high-fat diet. The authors concluded that gut physiology and WAT influence the sensitivity of mice to high-fat feeding independent of genetic background or diet.

Conventionally raised, C57BL/6j mice fed a high-fat diet (i.e., 45% kcal from fat; lard based) for 2, 6 or 16 weeks presented with increased inflammatory signaling in intestinal epithelial, immune cells and endothelial cells prior to the development of obesity, dependent on the duration of treatment [21]. In contrast, germ-free mice fed the same high-fat diet were protected from increased gut inflammation and exhibited reduced adiposity compared to conventionally raised mice fed the high-fat diet [21]. Furthermore, colonization of germ-free mice fed a low-fat diet with fecal slurries from high-fat-fed, conventionally raised mice increased nuclear factor κB (NF-κB) expression [21], supporting the concept that high-fat feeding enhances populations of proinflammatory gut microbes. Consistent with these data, feeding a high-fat diet (i.e., 60% kcal from fat, of which 34% was saturated) to female C57BL/6j mice for 8 to 12 weeks increased gut markers of impaired barrier function and inflammation, reduced abundance of Lactobacillus, increased abundance of Oscillibacter in feces and increased markers of macrophage infiltration and inflammation in mesenteric WAT [45].

Two types of Sprague-Dawley rats [i.e., susceptible or resistant to diet-induced obesity when fed a high-fat diet (45% kcal from fat; lard based)] were used as models to determine the extent to which diet vs. obesity was most strongly correlated with changes in gut microbes and barrier function and endotoxemia [46]. Rats susceptible to diet-induced obesity had higher levels of markers of intestinal inflammation and endotoxemia and higher levels of Enterobacteriales, whereas susceptible and resistant rats had decreased total bacterial densities and relatively increased populations of Bacteroidales and Clostridiales orders. They concluded that diet-induced intestinal inflammation, as opposed to specific changes in gut microbes, was the most important predictor of hyperphagia and obesity in rats fed high-fat diets.

Resistin-like molecule 3 knockout mice, which are resistant to diet-induced obesity due to increased energy expenditure, and wild-type mice were fed a high-fat diet (i.e., 45% kcal from fat; lard based) for 21 weeks with or without antibiotics [47]. Regardless of genotype, high-fat-feeding decreased the abundance of Bacteroidetes sequences and increased Firmicutes and Proteobacteria. The authors concluded that their data indicate that high-fat feeding rather than obesity per se alters gut microbiota, and they emphasized the need to control diet composition when examining changes in gut microbiota.

Turbaba et al. [12] conducted a well-designed study comprising the following models to examine the relationship between diet, gut microbes, and energy balance: (a) germ-free mice colonized with a mixed microbial population and then fed a high-fat diet (i.e., 40% kcal from fat; ½ shortening and ½ beef tallow); (b) conventionally raised, wild-type mice fed a high-fat or high-carbohydrate diet; (c) conventionally raised mice fed a high-fat diet and then switched to either fat-restricted or carbohydrate-restricted diets; or (d) germ-free mice colonized with microbes from mice fed fat-restricted or carbohydrate-restricted diets. The high-fat diets fed for 14 days increased the abundance of the Mollicutes class of the Firmicutes phylum and reduced the abundance of Bacteroidetes, which were attenuated by diet restrictions that reduced weight gain. The upshift in Firmicutes was not division wide as observed in ob/ob mice microbiota due to increased numbers of a single class of Firmicutes—the Mollicutes. Mice receiving transplantation of microbes from high-fat-fed mice presented with increased adiposity compared to those receiving microbes from lean donors. Microbes from diet-restricted mice transplanted into germ-free, carbohydrate-fed recipients that had been made obese by feeding a high-fat diet resulted in reductions in WAT mass and the relative abundance of Mollicutes and increases in the relative abundance of Bacteroidetes. Collectively, these data highlight the important role that gut microbes and the host play in regulating net energy balance and the development of obesity. Specifically, feeding a Western diet rich in fat, particularly saturated fatty acids, and sugars triggers a robust increase in colonic Mollicutes at the expense of Bacteroidetes, thereby enhancing the metabolism of dietary sugars to short-chain fatty acids resulting in increased energy harvest, a positive energy balance and obesity.

2.2. Inverse relationship between high-fat feeding and Akkermansia muciniphila

A pioneering study by Everard et al. [48] demonstrated that A. muciniphila, a specific type of mucin-degrading bacteria that inhabits the mucus layer, plays a preventative role in the development of diet-induced obesity. These commensal bacteria normally represent 3–5% of the gut microbial population in humans. The abundance of these bacteria were 100-fold lower in colonic tissue of high-fat-fed mice (i.e., 60% kcal from fat; lard based) and were restored by oligofructose prebiotic treatment. This prebiotic-mediated increase in A. muciniphila was accompanied by decreased metabolic endotoxemia and expression of mRNA markers of inflammation in WAT. Furthermore, administration of A. muciniphila to C57BL6j mice fed a high-fat diet (i.e., 60% kcal from fat; lard based) for 4 weeks reduced adiposity, gut barrier dysfunction, metabolic endotoxemia, fasting hyperglycemia, insulin resistance and CD11c expression in WAT and also restored mucus thickness without affecting food intake compared to control mice. These probiotic effects were only modestly associated with improvements in epithelial signaling (e.g., increased expression of the Paneth cell product RegIIIγ), indicating that these bacteria do not improve gut barrier function via increasing the secretion of antibacterial peptides. Notably, administration of A. muciniphila increased intestinal levels of acetylglyceroil involved in glucose and intestinal homeostasis, suggesting a linkage with the gut endocannabinoid system. A. muciniphila levels were also lower in ob/ob mice, suggesting that leptin may play a role in controlling their abundance. Taken together, these data provide strong evidence that A. muciniphila enhances gut barrier function by stimulating the growth of the intestinal mucus layer, thereby preventing metabolic endotoxemia, systemic inflammation and obesity.

2.3. Linkage between obesity-promoting diets and inflammatory bowel disease

Although patients with inflammatory bowel disease are generally not obese, they share several common characteristics with obesity. One intriguing linkage is the effects of specific types of gut microbes on intestinal metabolism and inflammation. A linkage between these two inflammatory-related disorders was highlighted recently using a metagenomic systems biology approach [49]. Using shotgun metagenomic data in relation to community-level metabolic (enzymatic) networks, these authors suggest that inflammatory bowel disease and
obesity vary in the way the microbiome interacts with the gut environment instead of variation in core metabolic (enzymatic) process. Notably, the microbiome associated with inflammatory bowel disease and obesity shared several common features or biomarkers including regulation of enzymes involved in phosphotransferase systems required for sugar transport, particularly the up-regulation in members of the Firmicutes phylum during high-fat/sugar feeding. A similar positive correlation in the expression levels of genes in the nitrate reductase pathway in the microbiome from both disorders was found. This was important given the critical role of nitrate reductase in converting nitrate to nitrite and nitric oxide. Consistent with these data, elevated nitric oxide is associated with inflammatory bowel disease, inflammation and insulin resistance. However, a direct relationship between specific types of dietary lipids, gut microbiota and the development of inflammatory bowel disease and obesity was not examined.

2.4. Weight loss diets: effects of caloric content vs. type on gut microbes and health

Losing body weight may alter the types or abundance of gut microbes and their fermentation products. Such changes, however, may be dependent on the composition of the weight loss diet. For example, consuming a calorie-restricted diet rich in complex carbohydrates and fiber compared to consuming a diet with reduced fat calories will undoubtedly have differential effects on the types and abundance of gut microbes and overall health. To examine the independent effects of diet composition and weight loss by diet restriction, Ravussin et al. [50] conducted a study with the following four dietary treatments in C57BL/6j male mice: (a) a low-fat diet (i.e., 10% kcal from fat) for 35 weeks; (b) a high-fat diet (i.e., 60% kcal from fat; lard based) for 35 weeks; (c) a low-fat diet for 12 weeks followed by restricting intake of the low-fat diet for 23 weeks to maintain a 20% reduction in body weight; and (d) a high-fat diet for 12 weeks followed by restricting intake of the high-fat diet for 23 weeks to maintain a 20% reduction in body weight. Notably, weight-reduced, diet-induced obese mice exhibited the highest phylogenetic diversity and the low-fat, weight-reduced mice had the lowest phylogenetic diversity. In general, low-fat-fed mice had greater abundance of Firmicutes than high-fat-fed mice, due to the enrichment of two populations of microbes from the genus Allobaculum. However, high-fat-fed mice had a greater abundance of non-Allobaculum Firmicutes, Bacteroidetes and Deferribacteres (Mucispirillum) compared to the low-fat-fed mice, regardless of weight status. Changes in gut microbiota and circulating leptin levels in the obese, weight-reduced mice suggested that energy restriction per se may have its greatest impact on reducing body weight and improving gut health via increased leptin secretion, which has reported beneficial effects on several types of gut microbes (e.g., Mucispirillum) and goblet cell mucin secretion [51,52].

Zhang et al. [53] fed C57BL/6j mice a high-fat diet (35%, wt/wt; 60% kcal from fat; lard based) for 12 weeks to induce obesity and insulin resistance, followed by a normal chow diet for 10 weeks to examine the extent to which high-fat feeding alterations in gut microbial populations were reversible. Indeed, they demonstrated that the high-fat feeding induced significant changes in 77 key phyotypes, and these changes reverted back to control levels after 10 weeks of consuming the chow diet. Collectively, these data support the notion that diet-induced changes in populations of gut microbes are reversible and demonstrate the plasticity of the gut microbiota to dietary interventions.

2.5. Impact of the fat-to-fiber ratio on gut microbes

An important consideration when choosing a weight loss diet is its content of dietary fiber relative to fat, given the well-recognized, beneficial effects of fiber on gut health and satiety. For example, obese participants with at least one metabolic risk factor consumed one of two isocaloric, weight loss diets that differed in carbohydrate and fat content for 8 weeks [54]. Participants consuming the low-carbohydrate, high-fat diet had decreased defecation output and frequency and reduced fecal levels of butyrate and short-chain fatty acids compared to the high-carbohydrate, low-fat diet group. In participants consuming the high-carbohydrate, low-fat diet, the abundance of total anaerobes in fecal samples increased compared to baseline but was unchanged in the low-carbohydrate, high-fat diet group. In contrast, participants consuming the low-carbohydrate, high-fat diet had decreased abundance of bifidobacteria in fecal samples compared to baseline, whereas there was no difference in the high-carbohydrate, low-fat diet group. Thus, the fat-to-fiber ratio has a significant impact on the diversity of gut microbes and their metabolic end products.

2.6. Influence of overall energy intake on gut microbes

In a clinical study examining the acute effects of dietary calorie content on gut microbes among inpatients, lean and obese human participants were fed a low (2400 kcal/day) or high (3400 kcal/day) nutrient load with similar macronutrient profiles (i.e., 24%, 16% and 60% kcal from protein, fat and carbohydrate, respectively) [55]. Subsequent changes in fecal gut microbiota and calorie content were measured. Changes in gut microbes occurred within 3 days of ingestion of the test diets. Members of the Bacteroidetes and Firmicutes phyla in fecal samples represented 97% of the pyrosequencing reads of 16S rRNA genes, which was consistent with many previous animal and human studies. In general, the abundance of Firmicutes increased and Bacteroidetes decreased as calorie consumption (as a percentage of weight-maintaining energy needs) increased. In contrast, populations of Firmicutes decreased and Bacteroidetes increased as fecal calories (as a percentage of ingested calories) increased. These changes in gut microbes due to increased nutrient load were associated with ≥150-kcal increase in energy harvest, particularly in lean subjects. Consistent with these data, Turnbaugh et al. [56] demonstrated using a humanized mouse model (i.e., germ-free, C57BL/6j mice inoculated orally with human feces) that changing from a low-fat, complex carbohydrate diet to a high-fat, sugar-rich diet altered the structure of the microbiota and its metabolic pathways within 24 h. Collectively, these data indicate that acute caloric intake in excess of energy needs needs rapidly alters the gut microbiome and increases gut energy harvest, which may contribute to a positive energy balance and the subsequent development of obesity.

2.7. Linkage between dietary fat type and sulfate-reducing bacteria

A study by Zhang et al. [57] provided a new clue about how fat feeding may selectively increase the abundance of a specific type of bacteria linked to intestinal inflammation. Apoa-I knockout mice with impaired glucose tolerance and increased adiposity and wild-type controls were fed a high-fat diet (35% by weight, 60% kcal from fat; lard based) for 25 weeks [57]. Compared to low-fat-fed mice, both genotypes of mice fed the high-fat diet had dramatic alterations in gut microbiota, including loss of the gut barrier-protecting Bifidobacterium spp. Moreover, mice with impaired glucose tolerance due to high-fat feeding had increased abundance of Desulfovibrioaceae, a family of sulfate/sulfite-reducing bacteria that produce hydrogen sulfide, a genotoxic gas that causes barrier dysfunction and endotoxemia (reviewed in Ref. [58]). These data demonstrate that high-fat feeding increases the numbers of Desulfovibrioaceae, which may be an important mediator of impaired barrier function and systemic inflammation.
2.8. *Bilophila wadsworthia* produce hydrogen sulfide, a proinflammatory and genotoxic gas that disrupts barrier function

Hydrogen sulfide adversely affects epithelial intestinal cell integrity and viability via cytotoxic, genotoxic and proinflammatory effects, which contribute to the development of inflammatory bowel diseases and cancer (reviewed in Ref. [58]). Indeed, hydrogen sulfide release is elevated in feces from patients with ulcerative colitis compared to feces from control patients, and organic compounds such as mucin, cysteine and taurocholate increase its production [59]. For example, taurine from taurocholate can be used by *B. wadsworthia* as an electron acceptor for anaerobic respiration, resulting in the production of hydrogen sulfide (Fig. 5; [58]). Hydrogen sulfide is extremely toxic to colonocytes and adversely impacts their metabolic function, particularly by reducing butyrate oxidation [60–62]. Butyrate metabolism is critical for controlling colonocyte turnover [63]. Consistent with these data, patients with ulcerative colitis present with reduced colonic butyrate oxidation [62,64].

Another potential mechanism by which hydrogen sulfide diminishes epithelial integrity and increases intestine permeability is by increasing colonocyte turnover (reviewed in Ref. [65]). For example, sodium hydrogen sulfide treatment of mucosa rectum biopsies from patients with ulcerative colitis increased the proliferation of cells in the upper crypt, and co-supplementation with butyrate blocked this proliferative effect of hydrogen sulfide [63]. In agreement with these data, treatment of nontransformed intestinal epithelial cells (IEC-18) with sodium hydrogen sulfide increased IEC-18 proliferation, which was associated with altered redox status and increased activation of MAPKs [66].

2.9. Milk fat specifically increases *B. wadsworthia* via increasing hepatic taurocholic acid secretion

Feeding interleukin (IL)-10 knockout mice a milk fat-based diet at a level and with a composition similar to the Western diet (i.e., 37% kcal from fat; butter based; [67]) altered gut microbiota, decreasing gut barrier-protecting bacteria and increasing sulfate/sulfite-reducing bacteria, which correlated with the degree of gut inflammation and markers of inflammatory bowel disease [67]. Notably, milk fats including butter, whole and reduced milk, dairy desserts, cheese and pizza (cheese) constitute approximately 32% of the total fat intake of Americans [68]. Milk fats are rich in saturated, short-chain fatty acids (e.g., C4–14). The milk fat-based diet increased the abundance of *B. wadsworthia*, which was associated with a proinflammatory T helper type immune response and increased colitis in IL-10−/− mice [67]. Taurine-conjugated bile salts were responsible for these inflammatory responses, in part, due to their high sulfur content, which stimulates sulfite-reducing bacteria like *B. wadsworthia* (Fig. 6). Notably, milk fat was more inflammatory than beef tallow, suggesting the higher amounts of total saturated fatty acids (65% vs. 39%) and C4–C14 short-chain fatty acids (24% vs. 1%) in milk fat compared to lard contributed to increased hepatic taurocholic synthesis and secretion. Stearic and palmitic acid levels were equal in both groups and thus did not appear to affect the outcomes of this study.

Collectively, these data demonstrate that a diet rich in milk fat increases hepatic production of taurocholic acid. Taurocholic acid serves as a substrate for the growth of pathogenic *B. wadsworthia*, resulting in the production of the genotoxic gas hydrogen sulfide, causing adverse effects on gut health, at least in mice genetically susceptible to colitis (e.g., epithelial cell hyperplasia and transmural inflammation). However, there remains a huge gap in the literature regarding how specific types of dietary fat influence bile acid composition, and what this means not only in terms of taurine but also in terms of the generation of proinflammatory and genotoxic secondary bile acids.

3. Linkage between obesity, *Clostridium* cluster bacteria, deoxycholate and hepatocellular carcinoma

Genetic and diet-induced obesity increase the risk and incidence of liver cancer, although the specific mechanisms responsible for initiating or promoting obesity-mediated carcinogenesis are unclear. A recent study by Yoshimoto et al. [69] provides new insights concerning the role of gut microbes in mediating, in part, obesity-induced hepatocellular carcinoma. Notably, all dimethylbenz(a)anthracene (DMBA)-treated mice fed a high-fat diet (i.e., 60% kcal from fat; lard based) for 30 weeks developed hepatocellular carcinoma. In contrast, none of the DMBA-treated mice fed a normal diet developed hepatocellular carcinoma, although 5% developed lung cancer. Obese ob/ob mice treated with DMBA also developed liver cancer, suggesting that obesity, but not high-fat feeding, promoted liver cancer. DMBA-treated, obese mice had increased abdominal and hepatic stellate cell activation of the senescence-inducer gene p21\(^{WAF1/CIP1}\). Obese mice had increased abundance of bacteria from the Firmicutes phylum, cluster XI of the genus *Clostridium sordellii*, similar to research by Turnbaugh et al. [56] in a Westernized, humanized mouse model (*Clostridium innocuum*) and Ley et al. [11] in ob/ob mice (*Clostridium cluster XIVa*). Notably, obese mice exhibited increased microbial synthesis of deoxycholic acid. Consistent with these data, the expression of *bai*, a gene involved in the 7α-dehydroxylation of bile acids, was robustly increased in the feces of high-fat-fed mice and decreased by treatment with vancomycin. Decreasing deoxycholic acid levels in mice decreased the abundance of senescent hepatic stellate cells and hepatocellular carcinoma. Collectively, this study demonstrates a mechanism by which obesity promotes the development of hepatocellular carcinoma in mice, that is, increased populations of gut microbes (i.e., *Clostridium* cluster XI) that produce DNA-damaging, bile acid metabolites (i.e., deoxycholate) that trigger senescence in hepatic stellate cells and hepatocellular carcinoma. Given differences in bile acid metabolism in humans vs. rodents, clinical studies are needed to determine the extent to which this obesity-mediated mechanism exists in humans.

4. Protective role of n-3-rich diets on intestinal inflammation

Surprisingly, little has been reported on the effects of n-3 polyunsaturated fatty acids (PUFAs) on gut microbes, and hence, this section focuses on their protective role on intestinal inflammation.
inflammation. Dietary n-3 PUFAs have been reported to protect intestinal epithelial cells from proinflammatory insults and accelerate recovery from inflammation. For example, eicosapentanoic acid (EPA; C20:5n-3) and docosahexanoic acid (DHA; C22:6n-3) maintained the integrity of human intestinal epithelial cells exposed to IL-4 by enhancing epithelial resistance and membrane integrity [70]. Consumption of fish oil-rich diets containing 25%–30% EPA or DHA or perilla oil containing 55%–60% α-linolenic acid (C18:3n-3) relieved chronic ileitis in senescence-accelerated P1/Yit mice by inhibiting monocyte recruitment in inflamed intestinal tissue [71]. Supplementation of n-3 fatty acids protected against trinitrobenzenesulphonic acid-induced colitis in male Wistar rats [72] and dextran sodium sulfate (DSS)-induced colitis in male Sprague–Dawley rats [73] by reducing disease activity indices including colonic inflammation, myeloperoxidase activity, alkaline phosphatase activity and plasma endotoxin levels and enhancing the recovery from inflammatory bowel disease stimulated by DSS in pigs [74].

4.1. Mechanisms by which n-3 PUFA protect intestinal cells from inflammation

One potential mechanism by which n-3 PUFAs protect gut health is by reducing oxidative stress and NF-κB-mediated inflammation in immune and intestinal cells [75,76]. n-3 PUFA inhibited inducible nitric oxide synthase and nitric oxide production in RAW264.7 and mouse peritoneal macrophages [77,78], as well as NF-κB signaling in human THP-1 and asthmatic alveolar macrophages [79,80]. One possible mechanism by which n-3 PUFAs decrease NF-κB signaling is by activation of peroxisome proliferator-activated receptor (PPAR)-γ. Activation of PPAR-γ suppresses inflammatory gene expression by directly interfering with transcriptional activation of NF-κB and activator protein-1 (reviewed in Ref. [81]). For example, upon activation by ligand binding, PPAR-γ can be SUMOylated by binding to SUMO1, a small ubiquitin-like modifier. The SUMOylated PPAR-γ then binds to nuclear receptor corepressors, which interfere with clearance of the corepressor complex of NF-κB, thereby transrepressing LPS-mediated NF-κB activation [82]. Indeed, EPA treatment of murine C2C12 myotubes decreased inhibitory κB protein (IκB)-α phosphorylation, increased IκB-α level and decreased NF-κB DNA binding [83]. Notably, EPA-mediated suppression of NF-κB signaling was accompanied by increased expression of PPAR-γ, and PPAR-γ knockdown prevented EPA’s suppression of NF-κB signaling. Collectively, these data indicate that EPA suppresses NF-κB-mediated inflammation in a PPAR-γ-dependent manner, at least in murine myotubes. In contrast, EPA and DHA decreased NF-κB activity in dendritic cells derived from bone marrow of BALB/c mice, but this suppression was not dependent on PPAR-γ [84]. Lastly, supplementation with an equal mixture of EPA plus DHA or a PPAR-γ agonist (15d-prostaglandin J2) attenuated intestinal barrier dysfunction and decreased PPAR-γ levels caused by ischemia and reperfusion intestinal injury in a Sprague–Dawley rat model [85].

Given the proinflammatory actions of several MAPKs, particularly extracellular signal-related kinase (ERK1/2; P44/42) and c-Jun N-terminal kinase (JNK), suppression of specific MAPKs is a protective mechanism by which n-3 fatty acids block or attenuate gut inflammation. Consistent with this notion, ERK1/2 activates NF-κB (reviewed in Ref. [86]) and inactivates PPARγ via phosphorylation of serine-112, resulting in PPARγ ubiquination and proteasome degradation [87]. Intriguingly, EPA supplementation of LPS-stimulated RAW264.7 macrophages decreased the activation of P44/42 and activator protein-1, a proinflammatory transcription factor phosphorylated by JNK, and TNF-α gene expression [88].

Another probable mechanism by which n-3 fatty acids suppress inflammation is by activating GPR120, an n-3 fatty acid-activated receptor expressed in WAT and bone marrow-derived dendritic cells and macrophages. For example, DHA-mediated reductions in markers of inflammation in RAW264.7 cells and mice were dependent on GPR120 activation [89]. DHA blocked LPS- and TNF-α-mediated activation of transforming growth factor-β (TGF-β)–activated kinase 1 and subsequent downstream activation of JNK and NF-κB, which was dependent on GPR120 activation. Taken together, these data demonstrate that n-3 activation of GPR120 suppresses signaling by macrophages and dendritic cells found in inflamed intestinal tissue. n-3 fatty acids may also exert their anti-inflammatory actions through incorporation into the phospholipid membranes of plasma or immune cells or gut mucosal tissue as demonstrated in human and rodent models [90–92], reviewed in Refs. [93,94]). Alternatively, they may impact populations of immune cells and subsequent intestinal cytokine production [95,96]. For example, supplementation of n-3 fatty acids reduced synthesis of phospholipids derived from n-6 fatty acids, resulting in decreased production of arachidonic acid and its proinflammatory, cyclooxygenase- or lipoxygenase-derived prostaglandins, thromboxanes and leukotrienes [75], reviewed in Refs. [93,94]). Furthermore, membrane incorporation of n-3 fatty acids in human blood mononuclear cells directly regulated genes involved in inflammatory pathways including NF-κB signaling and eicosanoid synthesis [97].

Remarkably, Hudert et al. [98] developed a transgenic mouse model that expresses the Caenorhabditis elegans fat-1 gene encoding for an n-3 desaturase that endogenously synthesizes n-3 from n-6 PUFA, resulting in a low ratio of n-6 to n-3 PUFA. Fat-1 mice had 16-fold higher levels of colonic n-3 fatty acids and 17-fold lower ratio of n6 to n3 PUFA compared to wild-type mice. Notably, fat-1 mice treated with DSS had reduced symptoms of colitis and decreased colonic NF-κB activity, TNF-α levels and oxidative stress, as well as enhanced mucosal barrier function after the onset of colitis compared to DSS-treated, control mice [98]. Anti-inflammatory, n3-derived resolvins E1, resolvin D3 and neuroprotectin D1 levels along with prostaglandin E2 and leukotriene B4 were elevated in colonic tissue of the fat-1 mice, but not in wild-type mice. Taken together, these data demonstrate that increasing endogenous synthesis of n-3 fatty acids at the expense of n-6 fatty acids reduces gut inflammation and improves barrier function in DDS-treated mice. Long-term dietary n-3 PUFA supplementation of male adult Lewis rats altered cell populations of small intestinal intraepithelial lymphocytes by decreasing the number of CD4+CD8α–, TCRαβ+CD8α+CD103+ and CD28+ cells and increasing the number of TCRαβ+CD8α+CD103– and CD28– cells and increasing the number of TCRαβ+CD8α+CD103+ [95]. n-3 PUFA supplementation also reduced intestinal IL-15 expression and lymphocyte-mediated cytokine production, including TNF-α, interferon-γ, IL-4 and IL-10. Similar immunosuppressive effects of n-3 PUFAs have been observed in the colon of mice with inherent severe immunodeficiency after exposure to CD45RB T cells [96].
The combination of n-3 PUFA with several dietary nutrients has additive or synergistic therapeutic effects on gut inflammation. For example, liquid diets enriched with n-3 fatty acids alone decreased the levels of TNF-α and IL1-β in the colon of 2,4,6-trinitrobenzene sulphonic acid-treated male Wistar rats [72]. Supplementation with medium-chain triglycerides further reduced colon inflammation and inhibited the activity of myeloperoxidase, as well as reduced the pathological colitis score in these Wistar rats. Campos et al. [99] reported similar beneficial effects of n-3 PUFA plus medium-chain triglycerides on acute colitis in the same rat model. A 50:50 mix of conjugated linoleic acid and fish oil enhanced the healing process of inflammatory bowel disease in pigs, possibly by up-regulating colonic PPARα and keratinocyte growth factor [74], a growth factor that enhances the barrier integrity of epithelial cells [100]. A combination of n-3 PUFA with quercetin or olive oil reduced DSS-induced colonic myeloperoxidase, inflammation and oxidative stress in female Wistar rats [101,102].

Due to the proinflammatory and anti-inflammatory property of n-6 PUFA and n-3 PUFA, respectively, the current dietary recommended ratio of these two fatty acids is 4:1. In contrast, typical Western style diets have n-6 to n-3 PUFA ratios that exceed 16:1 (reviewed in Ref. [103]). Decreasing this ratio to at least 4.5:1 significantly attenuated the abundance of proinflammatory thromboxanes and prostaglandins in the colon in Wistar rats [99]. Having an n-6 to n-3 PUFA ratio of 3:1 or lower prevented damages of intestinal mucosal layer and resolved inflammatory colitis manifestations in both Sprague–Dawley and Wistar rats [73,99]. Intriguingly, a modified n-3 PUFA dietary intervention in clinical trials helped maintain remission of inflammatory bowel disease when the ratio of n-6/n-3 fatty acid in erythrocyte membrane phospholipid composition ratio was 1.5 [104].

Daily dietary n-3 PUFA intake recommended by the United States Department of Agriculture is 0.5 g/day for infants, 0.7–1.2 g/day for children under 8 years old and 1.1 and 1.6 g/day for female and male adults, respectively [105]. n-3 PUFA-rich foods include cold-water fatty fish, flaxseed oil, fish oil and, to a lesser extent, meats and eggs. The Tufts–New England Medical Center Evidence-Based Practice Center reported minor adverse events from 148 studies with n-3 PUFA supplementation, with dosages ranging from 0.3 to 8 g/day for 1 week and up to 7 years [106]. The main side effect of n-3 consumption was diarrhea, which occurred in less than 7% of the subjects. Currently, no life-threatening adverse side effects of n-3 PUFA consumption have been reported.

5. Conclusions and implications

Taken together, the studies reviewed here support the concept that gut microbes are influenced by the type and amount of dietary fat, which, in turn, may directly or indirectly impact the host. Feeding high amounts of lard or beef tallow increases populations of gut microbes that secrete proinflammatory products that impair gut barrier function, leading to systemic endotoxemia and inflammation as well as insulin resistance. These proinflammatory products may

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Fig. 7. Working models linking the consumption of a high-fat diet rich in saturated fatty acids (SFA) with pathways of microbial metabolism, particularly Clostridium clusters XI and XIVa and sulfate/sulfite-reducing bacteria, which potentially contribute to chronic intestinal and systemic inflammation (A), and n-3 fatty acids to decreased intestinal inflammation (B). 7ADB, 7α-dehydroxylating bacteria; AP1, activator protein-1; IFN, interferon; n-3 PUFA, omega-3 PUFAs; n-6, omega-6; SRB, sulfate/sulfite-reducing bacteria.
also enhance energy harvest, leading to a positive energy balance and obesity. Such metabolic changes to the host, may, in turn, further influence gut microbes, creating self-perpetuating cycles of gut and systemic dysfunction. Diets rich in long-chain, saturated fatty acids also decrease the abundance of A. muciniphila, a specific type of mucin-degrading bacteria that plays a preventative role in the development of diet-induced obesity. Data from several studies demonstrate that consuming a diet rich in short-chain, saturated fatty acids that are found in milk fat (and potentially coconut oil) preferentially selects for mucosal sulfate/sulfite-reducing bacteria (i.e., B. wadsworthia) that diminish epithelial integrity and increase intestine permeability through their production of the proinflammatory and genotoxic gas hydrogen sulfide. In turn, compromised barrier function leads to inflammation in the lamina propria, endotoxemia, systemic inflammation and related diseases.

These results contribute to a mechanistic model (Fig. 7A) linking the consumption of a high-fat diet rich in saturated fatty acids with pathways of microbial metabolism, particularly those of sulfate/sulfite-reducing bacteria and Clostridium cluster XI/XIVa bacteria, that potentially contribute to chronic intestinal and systemic inflammation. In contrast, studies reporting the impact of n-3 fatty acids on gut microbiota should help promote healthy populations of gut microbes, thereby improving intestinal health and reducing the risk of gut and systemic inflammation and related diseases.

References


