REVIEW

Microbiome-Mediated Effects of the Mediterranean Diet on Inflammation

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ABSTRACT

The Mediterranean diet pattern is increasingly associated with improved metabolic health. Two mechanisms by which consuming a Mediterranean diet pattern may contribute to improved metabolic health are modulation of the gastrointestinal (GI) microbiota and reduction of metabolic endotoxemia. Metabolic endotoxemia, defined as a 2- to 3-fold increase in circulating levels of bacterial endotoxin, has been proposed as a cause of inflammation during metabolic dysfunction. As the largest source of endotoxins in the human body, the GI microbiota represents a crucial area for research on strategies for reducing endotoxemia. Diets high in saturated fat and low in fiber contribute to metabolic endotoxemia through several mechanisms, including changes in the GI microbiome and bacterial fermentation end products, intestinal physiology and barrier function, and enterohepatic circulation of bile acids. Thus, the Mediterranean diet pattern, rich in unsaturated fats and fiber, may be one dietary strategy to reduce metabolic endotoxemia. Preclinical studies have demonstrated the differential effects of dietary saturated and unsaturated fats on the microbiota and metabolic health, but human studies are lacking. The role of dietary fiber and the GI microbiome in metabolic endotoxemia is underinvestigated. Clinical research on the effects of different types of dietary fat and fiber on the GI microbiota and GI and systemic inflammation is necessary to determine efficacious dietary strategies for reducing metabolic endotoxemia, inflammation, and subsequent metabolic disease. *Adv Nutr* 2018;9:193–206.

Keywords: fiber, fatty acids, intestinal microbiota, obesity, metabolic syndrome

Introduction

Obesity rates continue to rise, with 35.0% of adult men and 40.4% of adult women affected in the United States (1). Obesity-related morbidity represents a deepening crisis with diseases such as type 2 diabetes mellitus and cardiovascular disease afflicting 9.4% (2) and 28.5% (3) of the US population, respectively, and contributing $209.7 billion in excess annual healthcare-associated costs (4). Dietary strategies for the prevention and treatment of obesity and its comorbidities are an important research area. The Mediterranean diet pattern, characterized by high intake of fruits, vegetables, legumes, whole grains, and nuts; moderate consumption of seafood and red wine; and the use of olive oil as the main source of fat (5) (**Table 1**) is one dietary strategy recommended in the USDA's 2015–2020 Dietary Guidelines for Americans as a way to promote health and prevent chronic disease (6). This is in comparison to the Western diet pattern, widely consumed in the United States, which is characterized by high consumption of red and processed meats; high consumption of refined grains and sugar-sweetened beverages; and low consumption of fresh fruits, vegetables, and legumes (7–9) (Table 1). Epidemiologic studies have revealed that consumption of a Mediterranean diet pattern is associated with lower all-cause mortality (HR, 0.77; 95% CI: 0.68, 0.88) (10), coronary heart disease (HR, 0.61; 95% CI: 0.43, 0.88) (10), cardiovascular disease (HR, 0.71, 95% CI: 0.58, 0.88) (10), type 2 diabetes (HR, 0.84; 95% CI: 0.62, 1.14) (11), and cancer (HR 0.81; 95% CI: 0.51, 1.28) (11). However, clinical trials are limited (**Table 2**) and intervention trials often lack an explanation of the underlying mechanisms.

Metabolic diseases, such as obesity (12, 13), type 2 diabetes (14, 15), cardiovascular disease (16), and nonalcoholic fatty liver disease (17), are characterized by chronic subclinical inflammation with increased serum concentrations of pro-inflammatory cytokines like C-reactive protein (CRP), IL-6, and others. In an analysis of the 1988 to 1994 NHANES, 20.2% of obese women had clinically elevated CRP concentrations (>1.0 mg/dL) compared to 4.0% of healthy weight

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Abbreviations used: CD, cluster of differentiation; CRP, C-reactive protein; GI, gastrointestinal; LAL, *Limulus* amebocyte lysate; TLR, toll-like receptor.

TABLE 1 Comparison of Mediterranean and Western diet patterns

Foods	Level of consumption	
	Mediterranean (5)	Western (7–9)
Fresh fruits and vegetables	High	Low
Whole grains	High	Low
Refined grains	Low	High
Legumes	High	Low
Nuts	High	No specification
Seafood	Moderate	No specification
Red and processed meats	Low	High
Sugar-sweetened beverages	Low	High
Red wine	Moderate	No specification

women (18). In a case-cohort study, those in the highest quartile of serum IL-6 concentrations (>2.96 pg/mL) had a 65% higher chance of developing diabetes compared with the lowest quartile (15). In addition, 1 prospective study reported that men in the highest tertile of serum CRP concentrations (>2.4 mg/L) had an OR for coronary heart disease of 2.13 compared with those in the lowest tertile (<0.9 mg/L) after adjusting for known vascular risk factors (19). The cause of this inflammation is a web of complex associations amongst diet, the gastrointestinal (GI) microbiome, and the immune system, which leads to a state of metabolic endotoxemia defined as a 2- to 3-fold increase in circulating levels of bacterial LPS (20). Preclinical research provides evidence that microbial, immune, and dietary factors are collectively necessary for the development of metabolic endotoxemia as gnotobiotic mice (21, 22), mice with nonfunctional toll-like receptor (TLR) signaling pathways (23), and mice fed diets low in saturated fat (23, 24) do not exhibit the inflammation and metabolic disease that occur when all these factors are combined.

LPSs, also known as endotoxins, are structures present on the outer membrane of gram-negative bacteria, consisting of a polysaccharide O-antigen portion connected to a membrane-dwelling lipid-A portion. It is the lipid-A portion that exerts most of the immunogenic effects of LPSs, including the activation of TLR4, an immune receptor that upregulates the transcription of pro-inflammatory cytokines (25). The O-antigen portion activates components of the adaptive immune system, which results in the production of antibodies. Both the lipid-A portion and O-antigen portion vary structurally and immunogenically (26), although this variation and its effects on metabolic endotoxemia are poorly understood. As the primary source of LPSs in the body, changes in the composition of the GI microbiota and/or production of microbial metabolites may alter the pool of pathogen-associated molecular patterns that encounter the intestinal epithelium and enter circulation. Increasingly, diet has been shown to be the primary mediator of the composition and function of the GI microbiota (27). Fat and fiber, 2 important components of the Mediterranean diet pattern, have differential effects on the GI microbiota. Diets high in saturated fat may exacerbate endotoxemia and inflammation by increasing transport of LPSs (24), by increasing the amount of LPSs available for transport by expanding the proportion of gram-negative bacteria in the gut (20), or by the ability of saturated fats to stimulate TLR4 receptors directly (28, 29). Conversely, high-fiber diets may decrease the proportion of gram-negative bacteria (30) and improve gut barrier function, preventing endotoxemia (31) and subsequent metabolic disease.

The aim of this review is to summarize the current state of knowledge about diet, the GI microbiome, and inflammation; elucidate gaps in knowledge; propose future areas of study; and suggest dietary strategies to address metabolic endotoxemia.

Dietary fatty acids

Staples of the Mediterranean diet pattern include nuts, seafood, and olive oil, which are rich in unsaturated fatty acids and low in SFAs. Consumption of these foods is lower than recommended for most age groups in the United States (6). This is problematic as overconsumption of saturated fats contributes to the development of cardiovascular disease (32) and diabetes (33). The current average saturated fat intake in the United States is 11% of energy, which exceeds the recommended limit set by the USDA of 10%, and it is estimated that only 29% of the population meets this recommendation (6). In 1 clinical cross-over trial, participants consumed dietary patterns rich in SFAs or PUFAs for 5 wk each by substituting the respective fat sources, e.g., substituting butter with a PUFA-based spread and cooking oils and vice versa. At the end of the high-PUFA and reduced saturated fat period, participants had a 20% increase in insulin sensitivity and a 19 mg/dL decrease in LDL cholesterol (34). A systematic review of 102 clinical trials reported that replacement of saturated fat with PUFAs resulted in decreased fasting glucose concentrations (0.04 mmol/L reduction), fasting insulin concentrations (0.5 pmol/L reduction), and insulin resistance (4% reduction) (35). However, a meta-analysis of 8 randomized controlled trials enrolling 663 participants reported that there was not strong evidence that replacement of dietary saturated fat with unsaturated fat improved blood lipid profiles (36), indicating a need for more high-quality studies on the topic.

LPS translocation

There are 2 main purported mechanisms by which bacterial LPSs from the GI tract may enter systemic circulation: transcellularly, with the absorption of dietary fat; and paracellularly, through compromised enterocyte tight junctions (37, 38). Preclinical studies have revealed that dietary fat absorption facilitates the translocation of LPSs across the intestinal epithelium (20, 24, 39), and there is increasing evidence that these relations also translate to clinical populations (37, 40–42) (**Table 3**). During digestion, TGs, the predominant form of dietary fat, are broken down into free fatty acids that enter enterocytes by free diffusion through the cellular membrane and by specific fatty acid transporters (43). These free fatty acids are then transported to the endoplasmic reticulum by fatty acid binding proteins where they

TABLE 2 Summary of clinical studies of the Mediterranean diet pattern

Outcome	Study type	Sample size	Treatment	Reference
A Mediterranean diet pattern may be effective in reducing the prevalence of metabolic syndrome	Interventional	180 adults with metabolic syndrome	Control—received advice to eat generalized healthful diet Treatment—received personalized advice to consume a Mediterranean diet pattern	Esposito et al. (77)
Consumption of a plant-based diet, consistent with high-level adherence to the Mediterranean diet pattern, is associated with beneficial microbiome-related metabolomic profiles	Cross-sectional	153 individuals following omnivore, vegetarian, or vegan diet	NA[1]	De Filippis et al. (81)
Adherence to a Mediterranean diet pattern was negatively associated with plasma endotoxin concentrations (evaluated by ELISA) and major adverse cardiovascular events; however, no inflammatory markers were included	Prospective	912 patients with atrial fibrillation	NA	Pastori et al. (96)

[1] NA, not available.

are repackaged, along with cholesterol, fat-soluble vitamins, and LPSs, into prechylomicrons (43, 44). The prechylomicron, stabilized by apolipoprotein B, is transported through the Golgi apparatus, preparing it for secretion from the cell into the lymphatic and eventually cardiovascular circulation (44).

Whereas dietary lipids are absorbed mainly in the proximal small intestine, the majority of the GI microbiota, the largest source of LPSs in the body, is resident in the distal small intestine and colon. One possible explanation for this contradiction is that endotoxemia may be the result of small intestinal bacterial overgrowth, which has been reported to be 17.1% more likely in those with obesity (45). One clinical study of patients with nonalcoholic steatohepatitis reported that small intestinal bacterial overgrowth was present in 50% of participants. Intestinal permeability, plasma LPS concentrations [measured by *Limulus* amebocyte lysate (LAL) assay], and serum TNF concentrations were also measured. Whereas intestinal permeability and plasma LPS concentrations were not significantly different between healthy patients and patients with nonalcoholic steatohepatitis, serum TNF concentrations were lower in healthy patients (14.2 pg/mL and 7.5 pg/mL, respectively) (46). In another cross-sectional study, small intestinal bacterial overgrowth, defined as $\geq$105 total colony forming units/mL of jejunal secretions, was present in 59% of patients with liver cirrhosis. Endotoxemia (measured by LAL assay) was associated with small intestinal bacterial overgrowth, although no inflammatory markers were reported (47). Taking these studies into account, measures of small intestinal

TABLE 3 Summary of clinical interventional trials of metabolic endotoxemia[1]

Outcome	Sample size	Treatment(s) and control	Endotoxin	Inflammatory markers included	Reference
A high-fat meal increased plasma endotoxin concentrations and endotoxin-neutralization capacity	12 healthy adult men	Control—no meal Treatments—~900 kcal serving of toast with butter with or without 3 cigarettes	↑[2]	TNF↔	Erridge et al. (40)
A high-fat, high-carbohydrate meal increased plasma endotoxin concentrations and pro-inflammatory activity	20 healthy adults	Control—oatmeal, milk, orange juice, raisins, peanut butter, and English muffin Treatment—egg muffin and sausage muffin sandwiches and 2 hash browns	↑[2]	TNF↔, CRP↔, NFκB↑	Ghanim et al. (41)
A high-fat meal increased plasma endotoxin concentrations and these concentrations were positively correlated with triglyceridemia	40 morbidly obese adults	Treatment—50 g fat (10 g SFA, 29.46 g MUFA, and 10.625 g PUFA)	↑[2]	CRP↔	Clemente-Postigo et al. (42)
A high–saturated fat meal increased plasma endotoxin concentrations and inflammation compared to a high–unsaturated fat or low-fat meal	20 healthy adults	Control—porridge made with olive oil (20% fat) Treatment—porridge made with fish oil, grapeseed oil, or coconut oil (35% fat)	↑[2]	IL-6↔, IL-8↔, TNF↔	Lyte (37)

[1] CRP, C-reactive protein. ↑, increase; ↓, decrease; ↔, even
[2] Measured using the *Limulus* amebocyte lysate assay.

TABLE 4 Summary of preclinical studies of saturated fat and endotoxemia[1]

Outcome	Model	Treatment(s) and control	Endotoxin	Inflammatory markers included	Reference
High-saturated fat diet increased proportion of gram-negative gut microbiomes and increased endotoxemia	C57BL/6J mice	Control—standard murine diet Treatment—72% energy from fat (corn oil and lard), 28% energy from protein, and >1% energy from carbohydrate	↑[2]	TNF↑, IL-1↑, IL-6↑, plasminogen activator inhibitor↑	Cani et al. (20)
Chronic high-saturated fat diet increased metabolic endotoxemia	C57BL/6, Myd88$^{-/-}$, Trif$^{-/-}$, Tlr4$^{-/-}$, and Tlr2$^{-/-}$ mice	Treatments—45% of energy from fish oil or lard	↑[2]	TNF↑	Caesar et al. (23)
Saturated fat increased postprandial endotoxemia and treatment of ileal epithelium increased permeability to LPS	Pig	Control—porridge made with 50 mL water Treatments—porridge made with 50 mL of fish oil, vegetable oil, or coconut oil	↑[3]	None	Mani et al. (24)
Arabinoxylan supplementation resulted in less fat mass development, better insulin sensitivity, greater gut integrity, less endotoxemia, less inflammation, a bloom of *Bifidobacterium*, cecal and colon enlargement, and an increase in colonic expression of genes related to SCFA response	C57BL/6J mice	Control—high-fat (60% of energy) Treatment—high-fat diet supplemented with 7.5% (wt:wt) arabinoxylan extract	↓[2]	TNF↔, IL-1↔, IL-6↓, monocyte chemoattractant protein↔	Neyrinck et al. (93)

[1] LAL, *Limulus* amebocyte lysate; ↑, increase; ↓, decrease; ↔, even.
[2] Measured using the LAL assay.
[3] Assessed using the PyroGene rFC Assay, Lonza, Switzerland.

bacterial overgrowth may be prudent additions to clinical studies of metabolic endotoxemia.

The differential effects of dietary fats on metabolic endotoxemia have been investigated in several preclinical studies (**Table 4**). One murine study compared mice fed diets supplemented with SFAs in the form of lard or unsaturated fatty acids in the form of fish oil. Circulating LPS levels (measured by LAL assay) were similar between groups at 3 wk of feeding, but were higher in the lard-fed group at 11 wk. Acutely, both saturated fat and unsaturated fat facilitate LPS translocation; however, chronically, metabolic endotoxemia was more pronounced in the high-saturated fat group, which may be due to long-term changes in the GI microbiota including an increase of *Akkermansia muciniphila*, which was only enriched in the fish oil-fed mice at 11 wk (23). In mice, *Akkermansia* is routinely reduced following periods of high-fat feeding and is inversely associated with obesity and inflammation (48, 49), whereas reconstitution with *A. muciniphila* may improve insulin signaling and glucose tolerance (50).

Other murine studies that compare the immunomodulatory effects of dietary fat types have similarly demonstrated the deleterious effects of diets high in saturated fats. Mice fed a high-MUFA (canola oil) diet or a high-saturated fat (palm oil) diet had higher plasma LPS concentrations (measured by LAL assay) in the canola oil condition, but higher plasma concentrations of the pro-inflammatory cytokines IL-6 and IL-1β in the palm oil condition. In an accompanying in vitro experiment, adipocytes stimulated with LPSs and incubated with palm oil had higher expression of the endotoxin-sensing TLR4 and cluster of differentiation (CD)14 compared with adipocytes incubated with canola oil (high n–3 PUFA), sunflower oil (high n–6 PUFA), or milk fat (high saturated fat) (**Table 5**). This suggests that whereas both high saturated and unsaturated fats may induce endotoxemia, high-saturated fat diets generally lead to more inflammation than unsaturated fats in mice (39).

The differential effects of postprandial saturated and unsaturated fat exposure on gut permeability and endotoxemia were further demonstrated in a porcine model, whereby postprandial serum LPS concentrations (measured by PyroGene rFC Assay, Lonza, Switzerland) were increased with saturated fat (coconut oil) consumption as compared with n–3 PUFAs (fish oil). In addition, ex vivo treatment of ileal epithelium with fish oil and cod liver oil reduced gut permeability, whereas treatment with coconut oil increased permeability. The authors attributed this increase in permeability to the tendency of saturated fats to form lipid rafts in the cellular membranes of enterocytes, thereby decreasing membrane fluidity. Saturated fat–induced endotoxemia was attenuated by administration of a compound that causes dissociation of lipid rafts (24). This preclinical evidence (23, 24, 39) demonstrates how diets high in saturated fat may induce metabolic endotoxemia and systemic inflammation through increasing intestinal permeability and altering the GI microbiota composition.

TABLE 5 Lipid composition of fats and oil[1]

	Fish oil[2]	Canola oil	Sunflower oil[3]	Olive oil	Palm oil	Coconut oil	Milk fat (39)	Lard
% PUFAs	37	29	11	7	10	7	10	12
% MUFAs	29	64	81	79	39	7	49	47
% Saturated	33	7	7	14	52	86	41	41
n–6/n–3 PUFAs	0.07	2.08	NA	12.83	45.5	88.58	NA	10.2

[1] Based on reference 130.
[2] Menhaden.
[3] Med-oleic.

Fat and the GI microbiota

Diets high in saturated fat may alter the GI microbiome and intestinal physiology in ways that contribute to metabolic dysfunction. Using a murine model, 1 study demonstrated that a high-fat (45% energy) palm oil diet decreased fat absorption and increased fecal fat concentrations compared with a high-fat olive oil or high-fat safflower oil diet, with the 3 groups presenting daily fecal fat concentrations of 27.7 mg/day, 16.8 mg/day, and 17.6 mg/day for the palm oil, olive oil, and safflower groups, respectively. Increased fecal fat concentrations in the palm oil group were accompanied by a reduction in microbial diversity, an increased Firmicutes:Bacteroidetes ratio, and an increase in mucosal expression of lipid-related genes. The authors hypothesized that the presence of higher amounts of fatty acids in the distal intestine had an antimicrobial effect that produced a loss of overall diversity (51), considered to be a sign of dysbiosis.

Another murine study demonstrated the deleterious effects of an exceptionally high fat diet (72% energy), reporting an increased proportion of gram-negative gut microbes; a 2- to 3-fold increase in circulating levels of LPSs; increased liver, adipose, and skeletal muscle expression of proinflammatory cytokines (TNF, IL-1, IL-6, and plasminogen activator inhibitor); poorer glucose tolerance; and increased hepatic TG. Interestingly, mice deficient in the LPS receptor protein, CD14, were spared the negative effects of the high-fat diet, exhibiting hypersensitivity to insulin and normal liver TG content, suggesting the LPS/CD14 system was driving the metabolic effects of the high-fat diet in the wild-type mice (20). Although these 2 preclinical studies utilized diets higher in fat (45% and 72% of energy for these 2 studies, respectively) than the American average of 35% (52), they draw attention to the need for further clinical investigation of dietary fats, the GI microbiota, and endotoxemia.

Fat and fiber have differential effects on the GI microbiome. Preclinical research has demonstrated that high-fat feeding may increase the proportion of gram-negative bacteria in the gut while decreasing the abundance of some gram-positive bacteria like the *Eubacterium rectale/Clostridium coccoides* group and *Bifidobacterium* (20). This may be because gram-negative bacteria are generally more bile-tolerant than gram-positive bacteria for reasons that are still poorly understood, but which may include the protective qualities of LPSs (53), as bacteria lacking the O-antigen portion of the LPS structure have reduced bile acid–tolerance (54). However, not all gram-positive bacteria respond poorly to a fat challenge and some gram-negative bacteria flourish during high-fiber feeding. For example, the gram-negative genus *Prevotella* is fiber-degrading and enriched in high-fiber diets (55), whereas the gram-positive Clostridia class are bile-resistant and increased during high-fat feeding (56). In addition, a cross-sectional study of children consuming a traditional rural African diet in Burkina Faso compared with children consuming a Western diet in Italy revealed that children from Burkina Faso had greater (58.5%) abundance of gram-negative bacteria, particularly Bacteroidetes, whereas European children had greater (70.4%) abundance of gram-positive bacteria, particularly Firmicutes. The diets of these 2 groups varied greatly with the African children consuming more fiber and fewer calories (1–2 y of age: 10 g fiber, 672.2 kcal; 2–6 y of age: 14.2 g fiber, 996 kcal) than the European children (1–2 y of age: 5.6 g fiber, 1068.7 kcal; 2–6 y of age: 8.4 g fiber, 1512.7 kcal) (55). Clinical studies utilizing complete feeding interventions have demonstrated that the addition of walnuts or almonds (high in unsaturated fats and fiber) to the diet facilitates increases in several genera of gram-positive bacteria in the Clostridia class including *Lachnospira*, *Roseburia*, *Oscillospira* (57), *Faecalibacterium*, and *Clostridium* (58), and decreases in others, including *Ruminococcus*, *Dorea*, and *Bifidobacterium* (member of class Actinobacteria) (58).

One reason a simple gram-negative/gram-positive divide may not be possible is the variation in immunogenicity of LPSs between species of gram-negative bacteria, so that it may not be the abundance of gram-negative bacteria that predicts inflammation, but rather the abundance of highly immunogenic LPS-producing gram-negative bacteria. It has been demonstrated that GI microbial dysbiosis can result in inappropriate immune activity. One study comparing type 1 diabetes incidence and differences in microbiome structure in children reported a high abundance of *Bacteroides* and low abundance of *Escherichia coli* in children from Finland and Estonia, where type 1 diabetes rates are high, and a high abundance of *E. coli* and low abundance of *Bacteroides* in children from Russia, where type 1 diabetes rates are low. The authors further demonstrated that differences in the LPS structure between the *Bacteroides* species and *E. coli* may account for the improper education of the immune system in children with type 1 diabetes (59). These results were complemented by a study that demonstrated that mice with nonfunctional TLR4 pathways do not develop type 1 diabetes,

further implicating the LPS-TLR4 system in inappropriate immune education (60). This type of work has not been replicated for metabolic endotoxemia in adults, but would be of great value in the elucidation of the mechanisms by which microbiome structure affects inflammation and metabolic disease.

Endotoxemia and inflammatory markers

Clinical studies of metabolic endotoxemia often use a method of LPS quantification called the LAL assay (Table 3). The LAL is an extract of blood cells from the North American horseshoe crab, which coagulates when in contact with LPSs. However, this reagent cannot be used for LPS detection in human blood due to proteins and other factors in human blood which interfere with the enzymatic cascade that results in LAL coagulation (61). Indeed, the LAL assay fails to provide specific, sensitive, and reproducible results for the quantification of endotoxin in human blood, plasma, and sera samples (62). To improve reproducibility, some have introduced a method which involves diluting and heating the plasma or sera samples before the introduction of the LAL, which denatures the interfering proteins (63). Although this does improve reproducibility, this method fails to produce results that are reflective of the total amount of circulating LPSs (63).

As plasma LPS is difficult to measure by direct assay, indirect methods may be more accurate, such as LPS-binding protein assay (64), or proxy measures of endotoxemia like inflammatory cytokines (IL-6, TNF, and CRP). A comparison of 2 clinical trials assessing LPSs and other inflammatory cytokines exemplifies the inconsistencies among measures. One clinical study of the effects of a high-fat meal (~900 kcal serving of toast with butter) on endotoxemia reported a 50% increase in circulating endotoxin concentrations compared with baseline (measured by LAL assay) (40). However, neither plasma CRP nor TNF concentrations were increased after the high-fat challenge (40). In another clinical trial, participants given a high-fat, high-carbohydrate meal (egg muffin and sausage muffin sandwiches and 2 hash browns) had a 47% increase in plasma LPS concentrations (measured by LAL assay) 3 h postprandially compared with baseline. This increase in LPSs was accompanied by a 72% increase in DNA binding by NFκB, a transcription factor which upregulates the expression of pro-inflammatory cytokines, in mononuclear cells over baseline, as well as increased expression of TLR2 and TLR4 in these cells (41). These additional inflammatory measures provide valuable insight into the physiologic postprandial state that is not possible to attain with LPS measurements alone.

Bile acids

Bile acids are another mechanism by which dietary fats and fibers affect the GI microbiota and intestinal physiology. Bile acids are increasingly implicated in the pathogenesis of human colitis and colorectal cancer and this may be due to their interactions with the microbiome and immune system (65). Exposure to secondary bile acids alters gene expression in enterocytes and microbiota. Cholic acid and chenodeoxycholic acid, the primary bile acids produced in the human liver (66), are deconjugated and dehydroxylated by gut bacteria to produce the secondary bile acids deoxycholic acid and lithocholic acid (67), which have both been associated with localized gut inflammation in mice (68). Elevated levels of deoxycholic acid in the serum and feces have been reported in humans with colon cancer (69) and in murine studies, deoxycholic acid exposure modifies the expression of genes involved in inflammation, apoptosis, cell proliferation, DNA repair, and angiogenesis (70). Fecal concentrations of deoxycholic acid at levels associated with a high-fat diet have been associated with disruption of gut barrier function in mice, whereas fecal concentrations of deoxycholic acid associated with low-fat diets are not associated with changes in gut barrier integrity (71). Furthermore, mice fed deoxycholic acid had a 1.5-fold increase in intestinal permeability compared with control mice. High-fat diets are also associated with decreased fecal concentrations of ursodeoxycholic acid, which may be cytoprotective as these decreased fecal concentrations of ursodeoxycholic acid are also associated with gut barrier dysfunction in mice (71).

Changes in the intestinal bile acid pool during periods of high saturated fat intake may alter the microbiome composition and increase intestinal inflammation. One murine study investigating the effects of dietary fat saturation on bile acids and the gut microbiota reported a bloom in the sulfite-reducing bacterium *Bilophila wadsworthia*, a pathobiont not normally abundant in healthy individuals, and the development of colitis in genetically susceptible mice during a high-saturated fat diet. After feeding mice a diet with 37% of energy from fat in the form of milk fat, lard, or safflower oil, or a low-fat diet, the milk fat group exhibited an expansion of *B. wadsworthia*, which the authors attribute to an increase in bioavailable sulfur in the form of taurine-conjugated bile acids. This effect was reported to be a direct effect of the dietary milk fat. The increase in sulfur-containing bile acids was not found in the lard, safflower, or low-fat groups, but was replicated in a low-fat group with taurocholic acid supplementation (72). A strength of this study is that the composition of this diet (37% of energy from fat, including 15% from saturated fat) is similar to the Western diet (35% of energy from fat, including 11% from saturated fat) (52).

Bile acids have profound effects on the structure of the microbial community in the GI tract, both stimulating the growth of microbes that utilize bile as an energy source and repressing the growth of microbes that are intolerant to its detergent effects (56). Increased concentrations of bile acids, which may result from a high-fat diet, favor the expansion of gram-positive members of the Firmicutes phylum, including some members of the Clostridia class capable of secondary bile acid production like *Blautia* and *Rumminococcaceae* (56). Secondary bile acid production allows certain microbes to decrease competition for nutrients because these bile acids act as electron carriers for cellular metabolism and as antimicrobial agents (56). In a murine model, the addition of cholic acid to the diet increased the Firmicutes:Bacteroides

ratio, decreased bacterial diversity, and increased deoxycholic acid production (73). The authors noted that dietary cholic acid mimicked the effects of a high-fat diet and also resulted in a decrease in serum adiponectin concentrations (73), which is associated with obesity and its related comorbidities (74).

Dietary fiber

Much like dietary fats, dietary fibers are a highly heterogeneous group of molecules that vary in solubility (the ability to dissolve in aqueous solutions), viscosity (the ability to form gels in aqueous solutions), and fermentability (the ability to be anaerobically metabolized by GI microbes) (75). Using these 3 characteristics, dietary fiber can be divided into 4 clinically significant designations: insoluble; soluble, nonviscous, and readily fermentable; soluble, viscous, and readily fermentable; and soluble, viscous, and nonfermentable (75). Other recent reviews provide additional insight on the differential effects of these 4 designations of fiber on the structure and function of the GI microbiota (76) and clinical implications of fiber ingestion (75).

Americans fall short of the 14 g fiber/1000 kcal recommendation with the average adult consuming only 8 g fiber $\cdot$ 1000 kcal^{-1} $\cdot$ day^{-1} (52). The current level of intake is related to deficits in the consumption of legumes, whole grains, fruits, and vegetables (6). Indeed, the majority of fiber in the US diet is from foods made of refined grains, which have up to 80% of the fiber removed during processing (6). The Mediterranean diet pattern, abundant in plant foods, may be one way of increasing fiber intake. In a 2-y randomized controlled trial, those following a Mediterranean diet pattern increased fiber intake by 18 g/day—at baseline, participants were consuming 14 $\pm$ 1.2 g/day, which mirrors the average intake in the United States, whereas at the 2-y time point, participants were consuming an average of 32 $\pm$ 2.8 g/day (77). Evidence supporting the health benefits of a high-fiber diet is abundant and continues to grow with 1 review comparing metabolic disease risk in high and low fiber consumers reporting reductions in the relative risks of developing obesity (30%), coronary artery disease (29%), and diabetes (18%) (78). In addition, a recent meta-analysis of 12 randomized controlled trials, including 609 adult participants with overweight and obesity, reported that isolated soluble fiber intake reduced BMI (kg/m^2) by 0.84, body weight by 2.52 kg, body fat by 0.41%, fasting glucose by 0.17 mmol/L, and fasting insulin by 15.88 pg/mL compared with the effects of placebo treatments (79).

Fiber and the GI microbiota

Knowledge of the associations between the GI microbiota and health has expanded greatly in recent decades. It is now known that altered microbiota composition is associated with many disease states including obesity, nonalcoholic fatty liver disease, and inflammatory bowel disease (80). Diet, and dietary fiber especially, has a profound impact on the GI microbiota. Fiber-rich, plant-based diets have been associated with greater microbial richness in terms of both taxonomy and gene expression (81). Richness is a marker of microbial community health and perhaps host health as well. Low microbial richness has been associated with greater adiposity, insulin resistance, dyslipidemia, and inflammation (82).

Humans rely on microbes to metabolize the indigestible components of food like dietary fibers (83). SCFAs are the end products of microbial fiber fermentation and our understanding of their effects on human health is still unfolding. An observational study of high-level adherence to a Mediterranean diet pattern found significant associations between plant-based diets and microbiome structure and function (81). Those consuming vegetarian and vegan diets had enriched fiber-degrading bacteria *Prevotella* and *Lachnospira*, compared with those following an omnivorous diet. In addition, SCFA concentrations were positively correlated with adherence to the Mediterranean diet pattern regardless of diet type. The authors also reported that those with high animal protein and fat intake had higher urinary levels of phenolic and indolic compounds derived from the deamination of aromatic amino acids and methylamines by the GI microbiota. Of specific concern is the microbial-derived compound trimethylamine oxide, which has been linked to the development of atherosclerosis (81).

Fecal SCFA concentrations are indicative of intestinal health and are an important measure in clinical research where more direct sampling of the intestinal environment is not always feasible. The most abundant fecal SCFA is acetate (40–70 mM), followed by propionate and butyrate (10–30 mM). SCFAs are readily absorbed from the intestinal lumen into the circulation with acetate, of the 3, reaching the highest concentration in serum. Acetate is mainly utilized by peripheral tissues, propionate by the liver, and butyrate by the colonic mucosal cells (84). In 1 study of the effects of butyrate on colonic gene expression, healthy adult volunteers received enemas containing butyrate in concentrations achievable through a high-fiber diet or a placebo enema containing no butyrate. Analysis of human colon tissue biopsies revealed an increase in expression of genes involved in energy metabolism, fatty acid metabolism, and oxidative stress in the butyrate group. In addition, butyrate was shown to differentially regulate genes involved in gut integrity and apoptosis (85), which may be one mechanism by which butyrate decreases gut inflammation. Indeed, colonic expression of NFκB inhibitor α, an inhibitor of the pro-inflammatory transcription factor NFκB, was upregulated in the butyrate group (85).

Butyrogenic bacteria and bacteria that cross-feed with butyrogenic bacteria are common targets of prebiotic fiber interventions. Cross-feeding occurs when one species produces substrates that are utilized by another species. For example, 2 molecules of acetate can be converted into butyrate via microbes that possess the enzyme butyryl-CoA (86). *Bifidobacterium* is a main GI symbiont of interest because of its interaction with the innate immune system and production of acetate and lactate, which has been shown in vitro to be a substrate for cross-feeding with

butyrogenic species like *Faecalibacterium prausnitzii*, *Anaerostipes* spp., *Eubacterium* spp., and *Roseburia* spp. that possess the butyryl-CoA enzyme (86). In vivo, a study in healthy adults consuming ≤7.5 g agave inulin/day reported a bloom in *Bifidobacterium* and a positive correlation between increasing dietary fiber and butyrate concentration, as well as positive relations between *Faecalibacterium* and butyrate concentrations. As *Faecalebacterium* possess the butyryl-CoA enzyme, the authors purported that these relations between butyrate and increasing dietary fiber may be in part related to the increased *Bifidobacterium* that resulted from inulin consumption (87). Indeed, in in vivo studies of prebiotic supplementation which report an increase in *Lactobacillus* and/or *Bifidobacteria* and butyrate, cross-feeding is likely the mechanism responsible for an increase in butyrate as neither *Lactobacillus* nor *Bifidobacteria* are capable of producing butyrate.

LPS translocation

Dietary fibers are the second nutrient of greatest interest in preventing metabolic endotoxemia owing to their ability to block both transcellular and paracellular routes of LPS translocation. As stated earlier, dietary fiber can be divided into 4 clinically significant classifications described by McRorie and Fahey (75). Those fibers that are soluble and viscous like pectin, β-glucan, and psyllium, reduce lipid digestion, which may also contribute to reduced transcellular translocation of LPSs. Pectins specifically have been shown to interact with bile acids and phospholipids, reducing the amount of surface-active components, and reducing the solubility of dietary fats (88). Another study investigating the effects of cereal fiber on fecal composition reported an increase in fecal fat concentrations when dietary fiber consumption was increased from 17 to 45 g/day (89). This reduced fat absorption may also lead to reduced LPS translocation.

Through interactions with the GI microbiota, the presence of fermentable dietary fibers in the diet may strengthen gut barrier function, thus preventing paracellular translocation of LPSs. In a study of fermentable compared with nonfermentable fiber supplementation on a high-fat diet, mice fed 10% short-chain fructooligosaccharide or 10% inulin fiber (both fermentable fibers) had greater cecal crypt depth (short-chain fructooligosaccharide: 141 μm; inulin: 145 μm) than mice fed cellulose (nonfermentable fiber) (5% and 10%: 109 μm). In addition, inulin-fed mice had greater cecal transmural resistance (101 $\Omega \times cm^2$) than 5% cellulose-fed mice (45 $\Omega \times cm^2$) (90). Butyrate has been shown to upregulate the expression of tight junction proteins and increase the rate of tight junction assembly, buttressing gut barrier integrity. An in vitro study of rat intestinal cells found that butyrate increases the expression of tight junction claudin-1, an important component of barrier function (31). Similarly, an in vitro study of human colonic epithelial cells showed that butyrate also increases the activity of adenosine monophosphate-activated protein kinase, increasing the rate of tight junction assembly (91). This type of mechanistic research can be difficult to replicate in humans, but an increasing number of clinical studies investigate fecal SCFA concentrations. A recent systematic review and meta-analysis of 68 clinical studies of SCFAs and systemic inflammation reported a significant decrease in ≥1 marker of systemic inflammation (CRP, TNF, IL-6) in 14 of the 29 included prebiotic studies and 13 of the 26 synbiotic studies and this association was stronger in studies that supplemented oligosaccharides. The meta-analyses revealed a reduction in CRP with prebiotic consumption and a reduction in CRP and TNF with synbiotic consumption although there was a high degree of heterogeneity amongst the included studies indicating a need for further research (92). In a study measuring high-level adherence to the Mediterranean diet pattern, those with the greatest level of adherence to the diet pattern had the highest fecal SCFA concentrations (81); however, no measures of endotoxemia or inflammation were collected.

Using a murine model, 1 preclinical trial demonstrated the effects of prebiotic (arabinoxylan oligosaccharide) supplementation on metabolic endotoxemia and inflammation. Obese mice fed a high-fat (60% of energy) diet supplemented with 7.5% (wt:wt) arabinoxylan extract exhibited less fat mass development, better insulin sensitivity, upregulation of intestinal tight junction proteins, lower plasma endotoxin concentrations (measured by LAL assay), and significantly lower circulating levels of IL-6 than mice fed the same high-fat diet without arabinoxylan supplementation (93). These improvements in metabolic health and measures of metabolic endotoxemia may be explained by changes in the GI microbiota. Arabinoxylan supplementation resulted in a 2-log increase in cecal *Bifidobacterium*, cecal and colon enlargement, and an increase in colonic expression of genes related to SCFA response (93). This thorough experiment, which provides measures of GI microbial structure, gut integrity, circulating LPSs, and inflammation, creates a detailed picture of how diet-induced changes in the microbiome affect the translocation of LPSs and development of endotoxemia.

Inflammation and metabolic disease

A reduction in endotoxemia and inflammation may be one mechanism by which increased consumption of dietary fiber improves metabolic health. An analysis of data from the 1999–2000 NHANES reported an inverse relation between dietary fiber intake and the risk of having elevated CRP, defined by the American Heart Association as a serum concentration >3.0 mg/L, and a modest direct association of saturated dietary fat intake and the risk of elevated serum CRP, a potent pro-inflammatory cytokine (94). The protective effects of dietary fiber were seen in the third and fourth highest quartiles of intake defined as consuming 13.3–19.5 and ≥19.5 g/day, respectively, which both fall short of the 28 g/day suggested by the 2015–2020 Dietary Guidelines for Americans (6). Those in the highest quartile of saturated fat intake were consuming >35.1 g/day (94). Although these analyses are almost 2 decades old, this study maintains its relevance as fiber consumption has remained similar from 1999–2014. Median fiber consumption reported

in 1999–2000 NHANES data was 6.5 g/1000 kcal compared with 8 g/1000 kcal in the 2013–2014 NHANES data. This is one illustration of the pro-inflammatory effects of saturated fat and anti-inflammatory effects of dietary fiber. However, when the effects of a Mediterranean diet pattern were tested clinically in a cohort of healthy male university students there was no change in CRP levels (95), which may be due to the difficulty of measuring CRP in healthy individuals (94).

A recent large prospective single-center cohort study examined the relation between consumption of a Mediterranean diet pattern and endotoxemia—the authors reported that plasma LPS concentrations (measured by ELISA) were negatively associated with adherence to the Mediterranean diet pattern (measured by validated questionnaire) and with fruit and legume intake in adults with atrial fibrillation (96). Plasma LPS concentrations were also positively associated with plasma LDL cholesterol and major adverse cardiovascular events (96). This study provides a valuable report on the links between consumption of a Mediterranean diet pattern and improved cardiometabolic health through decreased metabolic endotoxemia. However, interventional trials in a more general population should be conducted to determine whether these links are causative. It should also be noted that use of ELISA for the quantification of plasma LPS concentrations is regarded with similar skepticism as the LAL assay (97). Unfortunately, no other inflammatory measures were reported in this article.

SCFAs and particularly butyrate have further immunoregulatory effects and are increasingly recognized for their ability to disrupt the development and progression of metabolic disease. SCFAs have a profound ability to regulate the activity of leukocytes including recruitment to sites of inflammation and the production of cytokines like TNF, IL-2, IL-6, and IL-10, eicosanoids, and chemokines (98). In vitro, butyrate appears to have a greater influence on leukocyte behavior than acetate or propionate and an overall anti-inflammatory effect (99). This may be one mechanism by which butyrate prevents the development of metabolic dysfunction. One murine study demonstrated the ability of butyrate to prevent insulin resistance in obese mice fed a high-fat diet supplemented with 5% wt:wt butyrate (100). Another set of murine studies has demonstrated that supplementation of sodium butyrate (101) and a butyrate-producing probiotic, *Clostridium butyricum* (102), hinders the progression of nonalcoholic fatty liver disease to nonalcoholic steatohepatitis.

There have been many clinical trials that have investigated the effects of a Mediterranean diet pattern on metabolic syndrome and 1 meta-analysis, reporting on 8 studies with >10,000 participants, found 5 studies which reported a beneficial effect of a Mediterranean diet pattern. Some of these benefits included reduced waist circumference (−0.42 cm), increased serum HDL cholesterol (1.17 mg/dL), decreased serum TGs (−6.14 mg/dL), decreased systolic (−2.35 mm Hg) and diastolic (−1.58 mm Hg) blood pressure, and decreased blood glucose (−3.89 mg/dL) in participants instructed to consume a Mediterranean diet pattern compared with those who were not given instructions to change their diet (103). Although it has been demonstrated that a Mediterranean diet pattern can improve metabolic health, interventional studies investigating the mechanism of metabolic endotoxemia are needed.

Phytochemicals

In addition to dietary fiber, high consumption of whole plant foods as part of the Mediterranean diet pattern provides an abundance of phytochemicals that also affect the GI microbiome, inflammation, and the development of metabolic disease. Phytochemicals, which include polyphenols, phenolic acids, flavonoids, carotenoids, and lignans (104, 105), are not defined as essential nutrients but have been shown to decrease the risk of coronary heart disease (104, 106), diabetes (107), and nonalcoholic fatty liver disease (108). This reduction in risk may be due to interactions with the GI microbiome and immune system. Phytochemicals that escape absorption in the proximal small intestine may be transformed by the microbiota in the distal small intestine, increasing phytonutrient availability and altering the structure of the microbial community (109, 110). One in vitro study of the effects of phenolic compounds in tea reported repression of pathogenic microbes such as *Clostridium perfringens, Clostridium difficile*, and *Bacteroides* spp. without significantly affecting concentrations of commensal microbes such as *Clostridium* spp., *Bifidobacterium* spp., and *Lactobacillus* spp. (111). Proanthocyanidins have a similar effect on the microbiome, stimulating the growth of *Lactobacillus* spp., *Bifidobacterium* spp., and some butyrate-producing bacteria while decreasing colonic inflammation (112).

Preclinical trials have demonstrated the ability of polyphenols to improve gut barrier function in mice (113), ameliorate metabolic endotoxemia in mice and rats (113, 114), prevent LPS-induced liver damage on a high-fat diet in rats (114), and aid in the prevention of type 2 diabetes in humans (115). One clinical trial in which participants were given a meal with grape extract or a placebo reported lower postprandial endotoxemia (measured by LAL assay) in the grape extract group (116). Unfortunately, the Western diet pattern, which is characterized by low consumption of fruits, vegetables, legumes, and whole grains, provides a dearth of phytochemicals. There is a large gap between the 2 cup-equivalents/d of fruits and 2.5 cup-equivalents/d of vegetables recommended by the 2015–2020 Dietary Guidelines for Americans (6) and the 1.1 cup-equivalents/d of fruits and 1.6 cup-equivalents/d of vegetables consumed by Americans (117). However, a 2-y randomized controlled trial studying the effects of the Mediterranean diet pattern on adults with metabolic syndrome reported fruit, vegetable, legume, and nut consumption of 487 g/day at the end of the trial compared with a baseline intake of 198 g/day (increase of 289 g/day) (77), illustrating the ability of the Mediterranean diet pattern to promote plant food consumption, thereby increasing dietary intake of the phytochemicals that may prevent metabolic disease.

Conclusions and future directions

The complexity of the associations between diet, the GI microbiome, and inflammation is vast, but there are many high-quality studies on the topic. Animal studies have demonstrated differential effects of saturated and unsaturated fats on intestinal permeability and endotoxemia, but large-scale human trials are lacking. One potential obstacle for clinical research is the inability to easily access intestinal tissue for testing, but peripheral markers of intestinal permeability may be able to partially overcome this. Many murine and human studies only differentiate between high- and low-fat diets or high-saturated and high-unsaturated fat diets, but additional studies on specific dietary fats and their effects on the microbiome, bile acids, and inflammation would be greatly beneficial as some researchers have found differential physiologic effects of saturated fat from different food sources (i.e., milk fat compared with palm oil) (118). Many murine studies also use supra-physiologic doses of dietary fat with low amounts of carbohydrates and fiber, making them of questionable translational relevance (20, 51). In addition, owing to the TLR4-stimulating effects of some fatty acids, it is unclear in some studies of postprandial endotoxemia whether LPSs or dietary fats are the source of inflammation. It should also be mentioned that saturated fats vary in their pro-inflammatory ability. Preclinical research has demonstrated that medium-chain TG supplementation may ameliorate the obesogenic, diabetogenic, and pro-inflammatory effects of a high-fat diet (119). Similarly, a recent review demonstrates evidence that medium-chain TG intake may be related to the maintenance of metabolic health during obesity through mechanisms that involve the GI microbiota (120).

Research of fiber and health is also plagued by a lack of specificity with many studies measuring total grams of dietary fiber with no further differentiation (i.e., soluble compared with insoluble or fermentable compared with non-fermentable). Dietary fibers vary greatly in their impact on digestion, the GI microbiota, and health depending on chemical composition, degree of polymerization, and biological or synthetic origin (76). Of specific interest is how fibers of differing solubility and fermentability affect bile acids, microbiota composition and function, and inflammation. Preclinical studies on fiber have found that high-fiber diets do improve barrier function and decrease endotoxemia during a high-fat diet, but the ability to extrapolate these results to clinically significant reductions in metabolic disease risk has yet to be demonstrated.

What clinical interventional studies exist for metabolic endotoxemia are scant (Table 3) and most use the LAL assay, which is an unreliable measure of plasma LPS concentration (63). Use of ELISA for LPS quantification should be regarded with similar skepticism (97). As the reliability and accuracy of measuring direct serum LPS concentrations are limited, researchers are encouraged to measure and report proxy measures like LPS-binding protein, inflammatory markers, and/or use cell-based methods (mononuclear cell stimulation with LPSs) for a more accurate investigation of metabolic endotoxemia.

Research on the microbiome is rapidly expanding owing to technological and computational advances that make DNA sequencing cheaper and data analysis easier. However, current research is focused more on individual taxa and their contributions to the microbiome rather than the metabolic potential of the microbial community. As the major source of endotoxins in the body, the importance of characterizing diet-induced changes in the GI microbiota cannot be understated. Additional studies utilizing shotgun sequencing and multi-omic approaches will help answer the questions of which bacteria are present, what functional capacities they possess, and what their metabolic outputs are. The bacterial metabolic processes of fermentation and bile acid modifications are of specific interest in investigating endotoxemia and inflammation.

The area of inflammation and metabolic disease is better understood. Much has been done to characterize the inflammatory profiles of those with obesity and metabolic diseases. In addition, there is accumulating evidence on the beneficial effects of the Mediterranean diet pattern on metabolic syndrome (103, 121), diabetes (122–124), cardiovascular disease (5, 125–127), and nonalcoholic fatty liver disease (128, 129). Now is the time to further investigate mechanisms that inform dietary recommendations for the treatment and prevention of inflammatory metabolic disorders.

Although the crisis of obesity and its related comorbidities continues to deepen, there is a wealth of high-quality research occurring. Evidence of the efficacy of the Mediterranean diet in treating and preventing metabolic disease is abundant. Moving away from a Western diet pattern rich in red and processed meats, refined grains, and solid cooking fats toward a Mediterranean pattern rich in MUFAs and PUFAs from foods like nuts, avocados, salmon, and olive oil could help Americans meet dietary recommendations and improve their metabolic health. Research on the mechanisms by which a Mediterranean diet pattern improves health, including modulation of the GI microbiota, endotoxemia, and inflammation, is minimal and there is a need for large-scale comprehensive clinical trials.

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