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# Obesity-associated inflammation countered by a Mediterranean diet: the role of gut-derived metabolites

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The prevalence of obesity has increased dramatically worldwide and has become a critical public health priority. Obesity is associated with many co-morbid conditions, including hypertension, diabetes, and cardiovascular disease. Although the physiology of obesity is complex, a healthy diet and sufficient exercise are two elements known to be critical to combating this condition. Years of research on the Mediterranean diet, which is high in fresh fruits and vegetables, nuts, fish, and olive oil, have demonstrated a reduction in numerous non-communicable chronic diseases associated with this diet. There is strong evidence to support an anti-inflammatory effect of the diet, and inflammation is a key driver of obesity. Changes in diet alter the gut microbiota which are intricately intertwined with human physiology, as gut microbiota-derived metabolites play a key role in biological pathways throughout the body. This review will summarize recent published studies that examine the potential role of gut metabolites, including short-chain fatty acids, bile acids, trimethylamine-N-oxide, and lipopolysaccharide, in modulating inflammation after consumption of a Mediterranean-like diet. These metabolites modulate pathways of inflammation through the NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome, toll-like receptor 4 signaling, and macrophage driven effects in adipocytes, among other mechanisms.

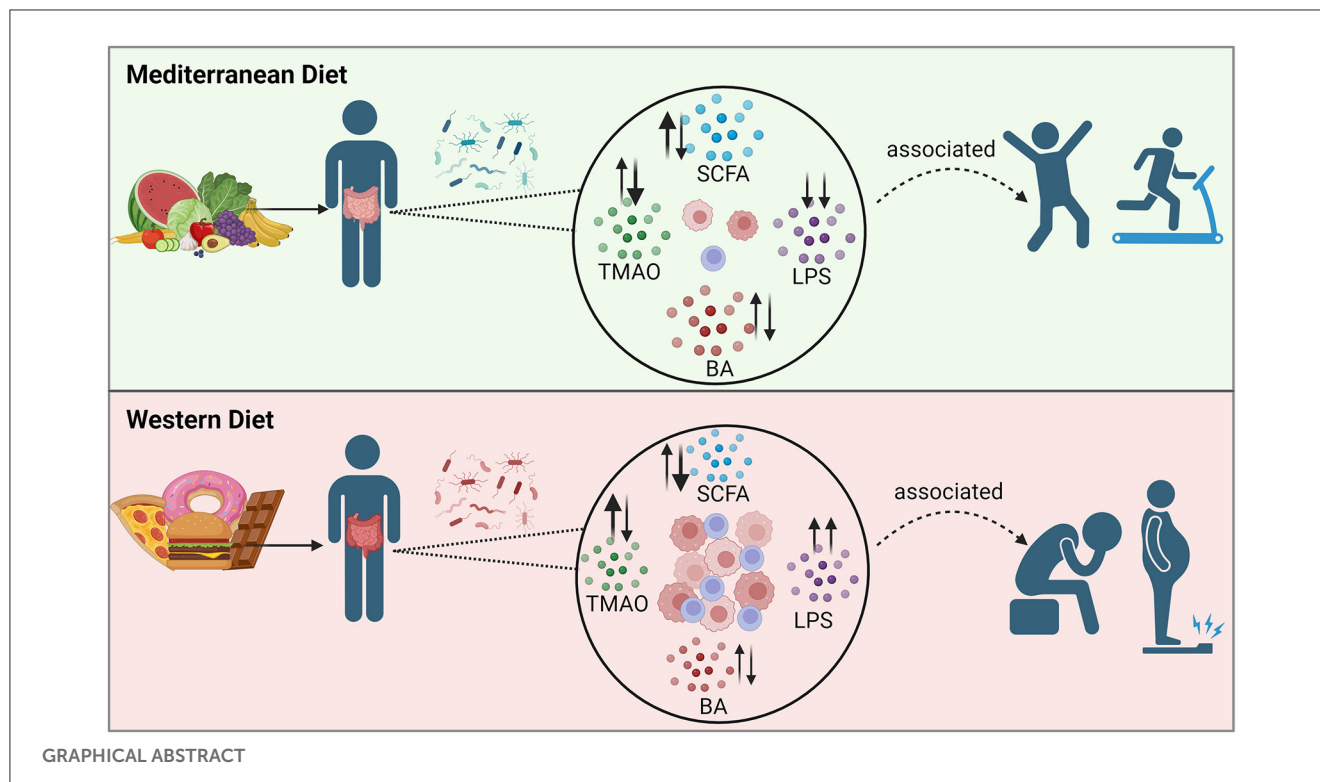
## KEYWORDS

**Mediterranean, obesity, microbiome, metabolites, inflammation, short-chain fatty acids, trimethylamine N-oxide, bile acids**

## 1 Introduction

The World Health Organization (WHO) estimates that over 1 billion individuals worldwide now grapple with overweight/obesity<sup>1</sup>. Obesity is associated with numerous comorbid conditions, notably non-communicable diseases including hypertension, type 2 diabetes (T2D), and cardiovascular disease (CVD) which contributed to a staggering five million deaths globally in 2019 (1–3). These comorbid conditions present a significant health burden in individuals with overweight/obesity and make combating obesity a public health priority. Complex factors influence the prevalence of obesity, including genetics, physical activity levels, dietary pattern, caloric intake, medical conditions

1 World Health Organization. Available online at: <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight> (accessed on January 11, 2024).



and their treatments, socioeconomic status, sleep habits, stress, and environmental chemicals (4). Inflammation, particularly chronic low-grade inflammation, has been implicated in numerous non-communicable diseases including obesity, metabolic syndrome, T2D, CVDs, and certain cancers (5). Research suggests that inflammation is a key driver of obesity (6) and that excess adipose tissue and dysfunctional adipocytes contribute to increased inflammation (7). Obese individuals have higher circulating inflammatory markers than lean individuals, and those markers are lowered following weight loss (8). Increasing inflammation in rodent models induces weight gain (6), and treatment with the anti-inflammatory cytokine interleukin (IL)-10 alleviated high-fat diet (HFD)-induced obesity (9). The aim of this review is to discuss recent studies that examine the influence of a Mediterranean diet (MedDiet) on inflammation and obesity. Specifically, we are interested in the observed effects of MedDiet adherence on gut-derived metabolites and their role in the physiology of obesity. There are multiple pieces of evidence required to connect specific dietary elements to conditions such as obesity and heart disease: (1) how digested food affects the gut microbiota composition, (2) which/how specific gut microbes in the host environment affect which gut metabolites that are present and in what quantities, (3) which/how gut metabolites influence cellular functions and biological pathways, and (4) which pathways are part of the physiology of healthy or diseased states. There have been a number of excellent reviews on some of the topics within this review, such as reviews on the MedDiet and inflammation (5) the MedDiet and the gut microbiome (10), or the role of some of the gut metabolites in obesity (11–14). We present a review that highlights the most recent literature and discusses all these topics: the MedDiet, obesity, inflammation, and gut metabolites, with a focus on the updates for four of the gut-derived metabolites that have been the focus of

multiple recent investigations. Each individual scientific study may focus on only one of the four elements. In this review, we introduce the associations between obesity and inflammation, then we focus in more detail on the evidence for biological roles of specific metabolites. We focus on the most recent research results related to the pathways that include four gut metabolites (short-chain fatty acids, bile acids, trimethylamine N-oxide, and lipopolysaccharide) and discuss gaps in our understanding.

## 2 Method

For this narrative review, most articles included were chosen from searches in PubMed and Google Scholar. The online searches were conducted from September 2023–May 2024 using the keywords: Mediterranean, diet, food, Western, microbiome, bacteria, microbiota, obesity, obese, short-chain fatty acid (SCFA), lipopolysaccharide (LPS), bile acid (BA), trimethylamine N-oxide (TMAO), inflammation, inflammatory, immune, immunity, gut, metabolites, metabolomics, pathophysiology, pathway, chronic disease, and combinations thereof. Additional relevant publications were found in the citations of the articles found in our literature search. We included original research articles, reviews, meta-analyses, and clinical trials. Publications were restricted to the English language, selected on a discretionary basis by a consensus of the four authors, and we prioritized articles published within the last 4 years, though other older relevant articles were included. We focused on a subset of human studies of recently published original research reports that investigate the association between MedDiet adherence, inflammation, obesity, and gut metabolites, but also included studies in animal models that investigated biological pathways relevant to gut metabolites and inflammation.

### 3 Dietary contribution to inflammation and obesity

The escalating health burden of obesity has prompted research into its causes and possible preventive measures, particularly in modifiable lifestyle factors such as diet. In addition to energy intake, diet may also mediate other determinates of obesity such as inflammation, and there are multiple studies examining the role of nutrition in low-grade inflammation. Unraveling causality and defining pathways that connect nutrition and inflammation has proven very challenging due to the multifaceted nature of inflammatory pathways (15, 16). Pathways identified as important in inflammation, as related to diet and obesity, include the NLRP3 inflammasome, macrophage-mediated chronic low-grade inflammation in adipose tissue, and the toll-like receptor 4 (TLR4) signaling pathway that is activated by saturated fatty acids. C-reactive protein (CRP), adipocyte-derived metabolites, and inflammatory cytokines [such as tumor necrosis factor alpha (TNF- $\alpha$ ), IL-1 $\beta$ , and IL-6] have been shown to play a role in inflammation associated with obesity, and in the development of insulin resistance (7, 15, 17–20). Indices like the Dietary Inflammatory Index (DII) have been developed to assess the inflammatory potential of a diet (21). A large study of more than 27,000 individuals over a period of about a decade found an association of overall obesity and abdominal obesity with a poor quality, pro-inflammatory diet. The authors used three indices, the Alternative Healthy Eating Index (AHEI), DII, and MedDiet Score, and found the AHEI to provide the best assessment of obesogenic potential of a diet, though the three indices have generally similar items in their assessment (22).

Several excellent reviews have been published that outline the connections between obesity, inflammation, and immunity. The review by May and den Hartigh focused on the impact of SCFAs on adipose tissue metabolism (23). A comprehensive review of the association of diet and gastrointestinal immunity itemized specific physiological effects associated with particular dietary macromolecules (24). A review of recent advances in our understanding of intestinal immunometabolism and microbiology provided a description of physiological differences between lean and obese states (25). Grosso et al. effectively summarized the proposed role of specific dietary elements, including macronutrients and phytochemicals, in the regulation of inflammation and immunity as relates to obesity (15). A review of ten meta-analyses summarized the evidence connecting the MedDiet with reduced dyslipidemia and decreased inflammatory mediators through modulation of the gut microbiota (10). Given the extensive data associating obesity and inflammation, combined with the data associating dietary changes with inflammation, dietary changes are justifiably proposed as one critical component of the treatment for obesity.

#### 3.1 MedDiet

The MedDiet, originating from the traditional practices of people in the Mediterranean basin, has captured researchers' attention due to its reported health benefits, and is included as a

healthy dietary pattern in the 2020–2025 Dietary Guidelines for Americans (26–28). The MedDiet promotes daily consumption of whole grains, nuts, vegetables, and fruit, with olive oil as the primary fat, moderate intake of fish, poultry, and wine, and rare intake of red meat and sweets (26, 29, 30). Anti-inflammatory effects have been attributed to multiple specific elements of the MedDiet, investigated alone in controlled studies, including olive oil, nuts, fatty fish, legumes, fruit, vegetables, and a reduction of red meat and refined foods (5). Although there is a general consensus regarding the characteristics of the MedDiet, criteria for calculating a “MedDiet score” vary considerably between studies (22, 29, 31). Regardless of the details of the MedDiet score, there is an abundance of data on the benefits of a MedDiet. A systematic review of 84 studies concluded that there is strong evidence to support an association of the MedDiet with fewer chronic diseases, including neurological diseases, CVD, cancer, T2D, liver disease, and renal disease. The MedDiet was also associated with reduced obesity-related metabolic features, inflammation, and lower mortality (31).

The Western diet, in contrast to the MedDiet, is a dietary pattern prevalent across many industrialized nations. Key components of the Western diet include high consumption of refined grains, red meat, and sugar sweetened beverages, which are associated with weight gain and obesity risk (10, 15, 22, 24, 32, 33). The Western diet can also include 50% or more of the calories from foods that are classified as ultra-processed, meaning they contain formulations of ingredients assembled in industrial processes as opposed to whole foods. Studies have associated ultra-processed foods (UPF) with low-grade inflammation and multiple chronic diseases (32, 34). Our review is not primarily focused on UPFs; however, individuals following a MedDiet, or other similar healthy dietary patterns, tend to consume fewer UPFs and would be spared the inflammatory response, and consequence of the inflammation, that may be associated with them.

#### 3.2 Effects of a MedDiet vs. a Western diet on obesity and inflammation

The benefits of the MedDiet have been evaluated in many observational and intervention studies of obesity and its comorbidities, suggesting that the MedDiet can ameliorate obesity across various populations. Although there are numerous investigations of dietary patterns, or specific dietary components, and the health consequences, we will focus on a subset of studies: recently published original research reports that investigate the association between MedDiet adherence, inflammation, and obesity.

##### 3.2.1 Observational studies of the effect of the MedDiet on obesity and inflammation

The results of recent observational studies provide supporting data for the association of the MedDiet with weight loss and reduced inflammation. Dietary intervention studies have shown that the MedDiet, with or without caloric restriction, may induce weight loss in individuals with overweight and obesity (35–37).

A study of self-selected diets by individuals with obesity found the MedDiet resulted in an average weight loss of 2.8 kg after 12 months. The weight loss induced by the other diets evaluated, Paleo and intermittent fasting, showed similar results to the MedDiet in this study (38).

Beyond examination of weight changes, studies have reported changes in inflammatory cytokines and a decrease in comorbidities in association with a MedDiet, even without weight loss. A multi-year study of over 39,000 individuals who were included in the Melbourne Collaborative Cohort found an association between the development of T2D and a higher DII, as well as a lower AHEI, but they did not find an association with the MedDiet score in this study (39). A study of 238 individuals who had non-alcoholic fatty liver disease, now called metabolic dysfunction-associated steatotic liver disease (MASLD) (40) showed that adherence to a MedDiet, as assessed by questionnaire, correlated with lower oxidative stress and inflammation (41). Monitoring of 612 subjects during a year-long study found an association between adherence to a MedDiet and lower inflammatory markers, CRP and IL-17. Additionally, changes in the gut microbiome seen with MedDiet adherence correlated with lower frailty, improved cognition, and reduced inflammation (42). In the observational study of 307 male participants as part of the Health Professionals Follow-up study that involved broad examination of sequence data, food logs, and blood biomarkers, long-term adherence to the MedDiet was associated with a change in the gut microbiome and their associated metabolic pathways, including SCFAs, secondary BA production, and fiber metabolism. They did not find an association of the MedDiet with the abundance of *Prevotella copri*, but they did find an association between the presence of *P. copri* with reduced risk of CVD, allowing for hypotheses of the pathways of this species that contribute to the observed phenotype (43).

A study of 1,040 individuals, as a subset of the Hellenic National Nutrition and Health Survey, found a significant association between adherence to the MedDiet, lower weight, and reduced hypertension (44). A cross-sectional study of 65 individuals examined the association of diet and inflammation using food diaries, hyperinsulinemic-euglycemic clamps, intravenous glucose tolerance test, dual-energy X-ray absorptiometry, cytokine levels, and adipokine levels. Adherence to a MedDiet was associated with greater insulin sensitivity and decreased inflammatory markers in adults with overweight/obesity (45). One study showed that women with obesity with higher adherence to the MedDiet had lower incidence of MASLD (46). High adherence to the MedDiet was observed to lower the risk of developing an unhealthy metabolic phenotype in individuals with and without obesity (47). Women with obesity and polycystic ovary syndrome who had higher adherence to the MedDiet also had lower cardiometabolic risk factors, including reduced levels of CRP, insulin resistance, and fatty liver index (48). Whole grain consumption, a component of the MedDiet, is also associated with decreased inflammation, in contrast to consumption of refined grains, in part due to its increased amount of dietary fiber (49). The MedDiet also discourages the consumption of red meat which has been consistently associated with inflammation, in favor of poultry or fish, the latter of which are high in omega-3 polyunsaturated fatty acids (50–52).

One strength of observational studies is that they can be quite large with thousands of participants, creating the potential for a statistically very well-powered study. Limitations of observational studies include the uncontrolled variables of each study that are outside of the diet being examined, such as physical activity, sleep habits, stress from injury or other medical conditions, all of which can affect the inflammatory state of the participants. The level of detail of the diets is less than can be obtained in a controlled trial for which food is provided. The range of what is considered a MedDiet might include those scoring anywhere from 10 to 17, out of 17 total points that describe a “fully-compliant” MedDiet on a PREDIMED score for example, so the food consumed by all of the participants in the “MedDiet” group could be quite variable, affecting the results of one study as compared to another. An additional limitation of these studies is that they cannot directly examine specific biological pathways. Despite the limitations of these studies, the strength of the collective evidence supports the role of the MedDiet in reducing obesity-associated inflammation and comorbidities.

### 3.2.2 Randomized controlled trials of the effect of the MedDiet on obesity and inflammation

Observational studies frequently include large cohorts for statistical power but randomized controlled trials (RCTs) add a layer of rigor and control to the results, moving us closer to determining the cause of the investigated effect. A randomized dietary intervention study of individuals with obesity and features of metabolic syndrome compared 128 genes expressed in abdominal subcutaneous adipose tissue for those on a Nordic diet, which is a Nordic alternative to the MedDiet, and those on a control diet. The authors concluded that the Nordic diet was associated with a decrease in inflammatory gene expression (53). A randomized controlled trial of 82 subjects with overweight/obesity comparing the MedDiet to a control diet demonstrated significant changes in the endocannabinoid system, along with an increase in *Akkermansia muciniphila* on the MedDiet. The change in the oleoylethanolamide/palmitoylethanolamide (OEA/PEA) endocannabinoid ratio following the MedDiet also diminished the homeostatic model assessment of insulin resistance index and decreased serum high-sensitive CRP, a measure of systemic inflammation. Their results support a role for the MedDiet in ameliorating insulin sensitivity and inflammation (54). A randomized controlled trial involving 28 adults with quiescent ulcerative colitis found that a MedDiet reduced levels of fecal calprotectin, a measure of intestinal inflammation (55). Higher adherence to the MedDiet is associated with lower inflammatory biomarkers, including multiple interleukins, interferon gamma (IFN- $\gamma$ ), TNF- $\alpha$ , and CRP (5, 55).

An evaluation of over 7,000 subjects in the PREDIMED (Prevention with Mediterranean Diet) trial, conducted over a median time of 4.8 years, demonstrated an association between weight gain and increased consumption of refined grains, red meat, potatoes, alcohol, processed meat, white bread, and sweets. Increased waist circumference was associated with increased consumption of snacks, fast-food and pre-prepared dishes, processed meat, alcohol, and sweets (56). Individuals with obesity instructed to follow an energy-restricted MedDiet



in the PREDIMED-Plus cohort lost more weight on average than individuals on a standard MedDiet after 1 year (57). A cross-sectional study of 62 individuals with overweight or obesity reported an association of better cardiorespiratory fitness and adherence to a MedDiet with lower blood pressure and lower body fat composition (58). For individuals with genetic risk factors for obesity, those with higher adherence to the MedDiet were less likely to develop obesity in 7–15 years of follow-up (59). A sub-study of the PREDIMED trial examining changes in inflammatory markers after 3 years of MedDiet intervention found reduced plasma levels of several inflammatory cytokines (IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , IFN- $\gamma$ , hs-CRP, MCP-1, MIP-1 $\beta$ , RANTES, and ENA78), but these did not reflect at the gene level (60).

RCT MedDiet intervention studies consistently show lower TNF- $\alpha$ , IFN- $\gamma$  (60–62), and fecal calprotectin (55, 63). Cannabinoids as drugs, particularly those targeting the CB<sub>2</sub> receptors, have been associated with relief for a number of inflammatory disorders (64). Bourdeau-Julien et al. (65) and Forteza et al. (66) both detected increased endocannabinoids (OEA and EPEA) following MedDiet intervention in healthy volunteers of normal weight. In contrast, Tagliamonte et al. found that plasma arachidonylethanolamide (AEA) was decreased following MedDiet intervention in individuals with overweight/obesity, which increased the oleoylethanolamide/arachidonylethanolamide (OEA/AEA) ratio concomitantly with reduced cholesterol (54).

Olive oil, as well as other components of the MedDiet, such as fresh fruits and vegetables, contain polyphenols that have anti-inflammatory properties. In a study of multiple types of olive oil, individuals eating a diet supplemented with olive oil that contained high amounts of polyphenols had significantly improved plasma inflammatory biomarkers (decreased IL-8 and TNF- $\alpha$ ) (67), and another study reported a connection between olive oil and reduced body weight, waist circumference, and hepatic steatosis, in subjects with metabolic syndrome. The anti-inflammatory cytokine IL-10 increased, while pro-inflammatory cytokines decreased (IL-6, IL-17, TNF- $\alpha$ , and IL-1 $\beta$ ) (68).

Strengths of these randomized controlled trials include that they can control for variables that are not controlled in observational studies. For example, the PREDIMED and PREDIMED-Plus trials each compared two versions of a MedDiet: MedDiet with olive oil vs. MedDiet with nuts, or energy-restricted MedDiet vs. non-energy-restricted MedDiet (57, 60). Researchers are able to collect health information that may not be available in large observational studies, such as information on alcohol consumption, physical activity, and medication/supplement use, which can be used as exclusion criteria or taken to account in statistical analyses (55, 65, 66). Additionally, when studies provide the food for the participants, the content is known in detail and is much better controlled than when participants prepare their own food. Bourdeau-Julien et al. (65) and Forteza et al. (66) provided food to their volunteers, so they could exactly track the nutrient intake and compliance of their volunteers. Limitations of the RCTs include that most often there are a low number of participants: the studies described here had fewer than 100 study subjects, with the exception of the PREDIMED trial studies. Another limitation is that the food consumed is determined from records that are not seven days per week, so extrapolation is

required to interpret the information as the individual's whole diet, and data are dependent on the accuracy and adherence of the study subjects. Most of the studies described here have a narrowly defined inclusion criteria, such as those with a specific disorder, so the results may not translate to healthy individuals or individuals with other medical conditions. Most of the studies examine the effect of the intervention over a short period of time, often weeks to several months, raising the question of whether the intervention had time to establish an effect, and whether an effect would be sustainable. However, the PREDIMED trial, which is the exception and covered a long period of time, ended after 4.8 median years of follow-up, and showed strong evidence of the benefit of the MedDiet in many areas, resulting in over 350 publications so far according to their website (69). Overall, the results of the RCTs are consistent with the results of the observational studies and the evidence supports the role of the MedDiet in modulating inflammation and obesity.

### 3.2.3 Mechanisms of dietary effects on inflammation

Meta-analyses of multiple studies provide support for the conclusion that the MedDiet reduces the risk of obesity. For example, a meta-analysis of 15 RCTs of MedDiet interventions that measured obesity parameters in children and adolescents reported that the interventions had a significant effect on reducing BMI and obesity in this population (70). A systematic review of ten RCTs found that diets such as the MedDiet, and other similar dietary patterns, were associated with a significant reduction of CRP and an increase in adiponectin, both indicators of reduced inflammation (71). A systematic review of 20 RCTs reported the following changes in biomarkers in association with a MedDiet: decreased pro-inflammatory cytokines IL-1 $\alpha$ , IL-1 $\beta$ , IL-5, IL-6, IL-7, IL-8, IL-18, IFN- $\gamma$ , TNF- $\alpha$ , CRP, high-sensitivity CRP and increased anti-inflammatory cytokines IL-4 and IL-10 (5). A meta-analysis of 32 studies concluded that omega-3 polyunsaturated fatty acid dietary supplementation had anti-inflammatory effects, as shown by a decrease in CRP and TNF- $\alpha$  (72). Therefore, the anti-inflammatory effects of the MedDiet as a whole, as well as of the individual dietary components, contribute to its status as a healthy diet that may combat obesity. Data that associate the MedDiet with reduced inflammation are abundant but obtaining an understanding of the detailed pathophysiology is a more challenging goal. Some recent studies delving into the mechanisms of dietary effects on inflammation are reviewed below.

To define biological pathways affected by components of the diet, studies using murine models and *in vitro* cultures can be quite valuable (23, 73, 74), as specific mechanistic hypotheses can be generated from such studies. A study of 952 individuals using genome-wide genotyping, gut metagenomic sequence data, and fecal SCFA levels, reported that increased butyrate production was associated with impaired insulin response and that abnormal production or absorption of propionate was associated with T2D risk (75). In Section 5.1, we will discuss the evidence that SCFAs are increased in response to the MedDiet and this excellent study by Sanna et al., combined with the other literature, allow us to associate the MedDiet to SCFA changes to an impaired insulin response and obesity.

Cross-sectional studies examining adherence to the MedDiet and CRP concentrations found these to be inversely correlated (76, 77). This was also observed in a large population-based study (78). A recent study attempting to better define specific physiologic connections between obesity and inflammation used a mouse model with a CRP transgene. The investigators provided evidence that CRP is not merely a marker of inflammation, but instead has a causal role in the development of obesity (6).

As part of the CORDIOPREV (CORonary Diet Intervention with Olive oil and cardiovascular PREvention) prospective RCT, researchers suggest that the genetic variant of the NLRP3 inflammasome may modulate the benefits of the MedDiet (79). Murine and human brain cells treated with virgin olive oil reduced activation of the inflammatory TLR4/NLRP3 axis (80). Deficiency of NLRP3 attenuated systemic inflammation, especially with a HFD, caused changes in the plasma metabolome, metabolites in the liver and myocardium, and gut microbiota compared to wild-type mice (81). The saturated fats common in the Western diet are also associated with increases in inflammation by the nuclear factor kappa B (NF- $\kappa$ B) pathway and NLRP3 inflammasome, possibly contributing to obesity, as reviewed by Las Heras et al. (24). Even occasional consumption of Western diet patterns increased inflammation and insulin resistance in a rodent study (82). The effects of dietary patterns on health are complex and understanding their mechanisms will be necessary to use diet for the treatment of obesity and other health conditions.

## 4 MedDiet and the gut microbiome

Dietary patterns such as the Western diet and UPF consumption likely contribute to obesity partially through their impact on the gut microbiome. The gut microbiome is highly modifiable by diet and multiple studies have shown alterations to the microbiome from dietary patterns like the MedDiet (65, 83–85). Due to the complexity of the microbiome and the variation in how MedDiet is characterized between studies, it is difficult to define one consistent microbiome signature associated with the MedDiet (86). Clear changes in the microbiome have not been found during all MedDiet interventions, especially when the starting microbiome of the individual had high diversity, as the diverse microbiome was somewhat more resistant to changes (65, 87). When trying to assimilate all of the available literature on a topic, it is our view that if a meta-analysis reveals striking differences in results between various studies, this does not negate the results of each individual well-controlled study, but instead the meta-analysis demonstrates that generic conclusions about the MedDiet may not apply to every population and disease state. The discrepancies highlight our lack of understanding regarding which of the key variables in each study are most contributory to the outcome. The MedDiet can also have a considerable impact on microbial metabolites, even without a significant corresponding change to microbiome composition. Regardless of our limited understanding of the complex gut microbial communities, and their individual or overlapping roles, there are data to support beneficial changes to the microbiome from MedDiet intervention.

Several studies have investigated the role of diet in SCFA metabolism. For example, a MedDiet intervention in women with

obesity was able to reverse features of dysbiosis by increasing microbiome biodiversity and SCFA-producing taxa (88). MedDiet adherence in both individuals with obesity and normal weight was positively correlated with SCFA-producing taxa such as *Bifidobacterium animalis* (89). MedDiet intervention has been reported to increase fecal SCFAs (90), and the abundance of butyrate-producing microbes (87).

Some studies have focused on changes in BAs. An 8-week RCT of 82 individuals with overweight and obesity reported that increased adherence to a MedDiet resulted in a reduction of plasma cholesterol and fecal BAs. Gut microbiome analysis revealed an increase in *Faecalibacterium prausnitzii* and decrease in *Ruminococcus gnavus*. Furthermore, there were increased urinary urolithins, fecal BAs degradation, and insulin sensitivity in subjects on the MedDiet, which correlated with specific microbial taxa (91).

Fiber is known to be a critical component of the MedDiet. Dietary fiber originates primarily from whole grains and vegetables, foods that can serve as a prebiotic for bacterial growth, but different types of fiber may have different effects. Healthy adults supplemented with resistant potato starch had increased bifidobacteria and butyrate production in their gut, while supplementation with fiber from maize and chicory root did not show a statistically significant difference. Among individuals whose microbiome changed, the highest butyrate concentrations were correlated with *Ruminococcus bromii* or *Clostridium chartatabidum* increases (92). The effect of fiber supplementation on the microbiome and SCFA production varies between individuals. The authors report that some individuals are limited in their capacity to produce SCFA from fiber supplementation, and this may be driven by their microbiome (92, 93). Another dietary intervention showed that fiber from a mixture of fruits and vegetables resulted in increased bifidobacteria but no increases in SCFAs over a short 2-week period (94).

There have been numerous studies of the MedDiet component olive oil. Mice supplemented with olive oil had microbial changes associated with reduced inflammation and the prevention of colorectal cancer compared to mice fed other fat types. Interestingly, the olive oil diet in the mice increased the Firmicutes/Bacteroidetes ratio, which correlated with lower colorectal cancer risk but higher risk of obesity in this study (95). Olive oil consumption, particularly oil enriched with phenolic compounds, was also associated with increased bifidobacteria in a RCT in individuals with high cholesterol (96). Olive oil is an important source of flavonoids, and microbial metabolism is required to make flavonoids biologically available (97).

There are a few common patterns to the changes to the microbiome that have been reported repeatedly, either in studies comparing the MedDiet to a Western diet, or in studies comparing individuals with obesity to lean controls. A study of 92 individuals found an association of overweight/obesity with specific gut microbiota patterns when compared to those of normal weight: Bacteroidetes taxa were decreased and several Firmicutes taxa were increased (98). The Western diet is associated with decreased beneficial bacteria such as bifidobacteria and eubacteria in the human gut (99) and, in rodents, decreased *Akkermansia* spp., species that are associated with a number of human diseases (100). Lean mice receiving fecal transplants from mice with obesity gain weight (101, 102) and individuals with obesity receiving

transplants from lean individuals had improved metabolic disease symptoms (103). These studies show the combined value of animal and human studies. The studies make associations between the microbiome, obesity, and metabolic syndrome. The data supporting an association of the MedDiet with a reduction in inflammation and obesity from Section 3 of this review, combined with studies in Section 4 that investigated the microbiome and obesity, serve to connect the MedDiet to inflammation, obesity, and the gut microbiome. Every study does not prove direct causation, but the results allow the development of a larger hypothesis for definitive testing. Human fecal microbiome transplants have successfully altered the microbiomes of individuals with obesity to resemble lean donors, however no change in BMI occurred over the 12-week study. The time required to significantly change the BMI may be longer than the time to alter the microbial community (104). Individuals with obesity have distinct microbial communities, often characterized by having an increased ratio of Firmicutes to Bacteroides compared to lean individuals and decreased microbial diversity (98, 102) although these results are not consistent across all studies (105).

Gut permeability and energy efficiency are two other elements that have been examined closely. The microbiomes of individuals with obesity may result in increased energy absorption from food. Increases in Firmicutes relative to Bacteroides elevate levels of alpha amylases and amylomalases for more efficient energy extraction from foods, which increases the number of calories absorbed (102). An imbalanced microbiome can also contribute to obesity through its role in inflammation. The dysbiosis of obesity can lead to increased gut permeability and allow proinflammatory molecules to enter systemic circulation. The microbiome of humans and mice with obesity reduced the expression of the zonula occludens-1 tight junction protein, weakening the gut barrier (106). Individuals with obesity also have increased Gram-negative bacterial taxa of the *Enterobacteriaceae* family in their microbiome, resulting in elevated levels of LPS which can leak from the gut (98). LPS is proinflammatory and promotes low grade inflammation which promotes the storage of excess lipids (107). Further discussion of LPS as it relates to the MedDiet is included in Section 5.4 of this review.

By combining all of the findings from the many investigations discussed above, a positive role of the MedDiet on obesity and inflammation seems quite clear. We have yet to obtain a detailed understanding of the pathophysiology of obesity, but recent work has started to dissect the role of specific gut microbial metabolites in these pathways.

## 5 Interplay of obesity, the MedDiet, and gut-derived metabolites

Obesity is associated with changes in the composition of the gut microbiota, and in the amounts and types of microbial metabolites that are formed. Two groups of metabolites of demonstrated importance in obesity physiology are SCFAs and BAs. An increase in a third gut-derived metabolite, TMAO, has been associated with obesity and inflammation; however, its effects are proposed to be context-dependent (108, 109). A fourth metabolite

associated with inflammation and obesity is LPS. Obesity has been associated with increased intestinal permeability, which allows the movement of bacteria and bacterial products, like LPS, into the bloodstream with an associated increase in inflammation (110). The interactions between the obese gut microbiota, gut-derived metabolites, and the effects on its host are quite complex and multifactorial.

Previous reviews indicate that adults with obesity have been shown to have increased total concentrations of fecal SCFAs (11, 12) and BAs (13, 14), likely due to dysregulated metabolism and absorption. However, analysis of the gut microbiota of over 1,900 individuals in the METS-microbiome study showed an association of obesity with a reduction of fecal SCFA concentrations, gut microbial diversity, and of the bacteria that synthesize SCFAs, while the country of origin for the study subjects was the most important variable. Using predictive modeling, SCFA concentrations could not predict obesity status, suggesting the relationship between SCFAs and obesity is still unclear (111). Many of the studies examining SCFAs in populations with overweight/obesity have been cross-sectional analysis, with or without disease comorbidities and/or medications, and using different biospecimen types (fecal vs. blood), making it difficult to draw definitive conclusions (112–114). Meanwhile, the clinical controlled trials measuring SCFAs in populations with obesity also apply various pre/probiotic, dietary, or weight-loss interventions which make comparing studies difficult (115–117). Several variables such as diet and physical activity can affect SCFA production, and the direction of change for individual SCFAs (i.e., acetate vs. butyrate vs. propionate, etc.) likely differ, as is observed in Table 2, and should be considered when comparing data between studies.

Few studies have examined SCFA levels in children with obesity, however, within the last 5 years, two studies showed increased fecal SCFA concentrations (118, 119), while one study showed fecal SCFAs were reduced (120) in children with obesity. The differences in study results may be due to study design and inclusion criteria, as Wei et al. and Gyarmati et al. excluded volunteers who had received antibiotic, prebiotic, or probiotic treatments within the last 3 months before the studies, while the study by Slizewska et al. did not (118–120).

In Tables 1–3 we have summarized some of the recent human studies that have investigated changes in gut metabolites in association with a MedDiet compared to other diets. Below, we discuss the effects of MedDiet on SCFAs, BAs, TMAO, and LPS, and the mechanisms by which the MedDiet could potentially alter the gut microbiota to combat obesity.

### 5.1 Short-chain fatty acids

SCFAs are derived from the fermentation of non-digestible dietary fiber by gut bacteria, and they play a critical role in intestinal physiology. Acetate, propionate, and butyrate account for 95% of the SCFAs in the intestinal tract. In a healthy individual, <5% of SCFAs are excreted in feces, as most are absorbed through the gut mucosa and utilized in the gut, while some enters the

TABLE 1 Characteristics of recent clinical studies investigating the effects of the MedDiet on gut-derived metabolites.

References	Country	Study design (cohort)	Study population	MedDiet intervention	Control	Sample size (n)	Sex (n)	Age (y: mean $\pm$ SD, or CI, and/or range)	BMI (kg/m <sup>2</sup> : mean $\pm$ SD, or CI, and/or range)	Duration	MedDiet score
André et al. (121)	France	Cross-sectional (Alienor Study, subsample of 3C Study)	French older community-dwelling adults	N/A	Traditional dietary pattern	698	266 M; 432 F	73.1 $\pm$ 4.4	26.3	N/A	8-Item Study specific score from FFQ
Baratta et al. (41)	Italy	Observational cohort study (PLINIO Study)	Patients with NAFLD (now MASLD)	N/A	N/A	238	135 M; 103 F	53.1 $\pm$ 12.4	31.2 $\pm$ 5.4 sNox2-dp tertile I; 30.3 $\pm$ 4.1 II; 29.4 $\pm$ 4.2 III	N/A	9-item Mediterranean-diet questionnaire (122)
Barber et al. (87)	Spain	Randomized cross-over (N/A)	Healthy men	Fiber-enriched MedDiet	Western-type diet	20	20 M; 0 F	18–38	19.2–25.5	2 Mo (2 W each diet)	Food was provided
Barrea et al., (123)	Italy	Cross-sectional (N/A)	Healthy adults	N/A	N/A	144	67 M; 77 F	31.55 $\pm$ 6.19	22.84 $\pm$ 1.51	N/A	14-point Mediterranean Diet Adherence Screener (MEDAS) from PREDIMED Study (124)
Barrea et al. (125)	Italy	Cross-sectional (OPERA Project)	Healthy Caucasian adults	N/A	N/A	247	100 M; 147 F	36.6 $\pm$ 11.0	28.8 $\pm$ 9.1; 19–59	N/A	14-point Mediterranean Diet Adherence Screener (MEDAS) from PREDIMED Study (126)
Barrea et al. (127)	Italy	Case-control, cross-sectional (OPERA Project)	Patients with Hidradenitis Suppurativa (HS) and healthy controls	N/A	N/A	70	22 M; 48 F	25.37 $\pm$ 8.36 HS; 26.14 $\pm$ 7.28 healthy	29.26 $\pm$ 5.33 HS; 29.22 $\pm$ 5.62 healthy	N/A	14-point Mediterranean Diet Adherence Screener (MEDAS) from PREDIMED Study (126)
Bourdeau-Julien et al. (65)	Canada	Fixed-sequence (N/A)	Healthy adults	MedDiet	CanDiet	21	10 M; 11 F	20–29 M; 20–34 F	20.4–25 M; 20.1–24.1 F	19 D (3 D MedDiet, then 13 D CanDiet, then 3 D MedDiet)	Food was provided
De Filippis et al. (128)	Italy	Cross-sectional (N/A)	Healthy adults	N/A	Omnivore = Western diet	153	64 M; 89 F	39 $\pm$ 9 vegetarian; 37 $\pm$ 10 vegan; 37 $\pm$ 9 omnivore	21.9 $\pm$ 2.5 vegetarian; 21.3 $\pm$ 2.2 vegan; 22.1 $\pm$ 2.0 omnivore	N/A	11-unit dietary score based on tertiles (129)

(Continued)



TABLE 1 (Continued)

References	Country	Study design (cohort)	Study population	MedDiet intervention	Control	Sample size (n)	Sex (n)	Age (y: mean $\pm$ SD, or CI, and/or range)	BMI (kg/m <sup>2</sup> : mean $\pm$ SD, or CI, and/or range)	Duration	MedDiet score
Forteza et al. (66)	Canada	Randomized Cross-over (N/A)	Healthy, physically-active women	MedDiet	CanDiet (Western-type)	7	0 M; 7 F	25 $\pm$ 5; 19–32	22.52 $\pm$ 1.57; 19.50–24.49	35 D (7 D per diet)	Food was provided
Galie et al. (130)	Spain	Randomized cross-over (METADIET)	Adults with overweight/obesity and metabolic syndrome	MedDiet plus mixed nuts (50 g/day)	Habitual diet supplemented with nuts (50 g/day)	44	NR	25–60	25–35	5 Mo (2 Mo each diet + 1 Mo washout)	17-point MedDiet score used in PREDIMED-Plus (131)
Garcia-Mantrana et al. (132)	Spain	Cross-sectional (N/A)	Healthy adults	N/A	N/A	27	11 M; 16 F	39.5 $\pm$ 7.3	25.29 $\pm$ 2.76 M; 21.95 $\pm$ 2.72 F	N/A	14-point Mediterranean Diet Adherence Screener (MEDAS) from PREDIMED Study (124)
Ghosh et al. (42)	UK, France, Netherlands, Italy & Poland	Randomized parallel (NU-AGE Study)	Elderly non-frail adults	MedDiet tailored for elderly (Nu-AGE diet)	Habitual diet	612	286 M; 326 F	65–79	18.5–46	12 Mo	Adherence scores to the MedDiet calculated based on the NU-AGE Food Based Dietary Guidelines (FBDG) (133)
Griffin et al. (134)	USA	Randomized parallel (Healthy Eating Study for Colon Cancer Prevention)	Healthy adults at increased risk for colon cancer	MedDiet	Healthy Eating diet	115	32 M; 83 F	52 $\pm$ 12	27.0 $\pm$ 3.7	6 Mo	7-item Self-Efficacy score (not specific to MedDiet) (135)
Guasch-Ferre et al. (136)	Spain	Randomized parallel (PREDIMED Study)	Community-dwelling adults at high risk for CVD	MedDiet + EVOO or MedDiet + mixed nuts	Control diet (reduce intake of all types of fat)	980	442 M; 538 F	67.5 $\pm$ 10.9	29.6 $\pm$ 3.6	12 Mo	Not provided
Gutierrez-Diaz et al. (137)	Spain	Cross-sectional (N/A)	Healthy adults	N/A	N/A	31	8 M; 23 F	42.1 $\pm$ 10.9	26.3 $\pm$ 4.7 MDS $\geq$ 4; 26.2 $\pm$ 5.0 MDS < 4	N/A	8 point Mediterranean diet score (138, 139)
Haskey et al. (55)	Canada	Randomized parallel (N/A)	Adults with ulcerative colitis (UC)	MedDiet	Habitual CanDiet	28	10 M; 18 F	18–65 MedDiet; 25–64 CanDiet	17–30 MedDiet; 19–29 CanDiet	3 Mo	24 Point Mediterranean Diet Serving Score (140)
Krishnan et al. (141)	USA	Randomized cross-over (N/A)	Adults with overweight/obesity	MedDiet + 200 g red meat/week	MedDiet + 500 g red meat/week	39	12 M; 77 F	30–69	30.5 $\pm$ 0.3; 25–37	14 W (5 W per diet)	Food was provided

(Continued)

TABLE 1 (Continued)

References	Country	Study design (cohort)	Study population	MedDiet intervention	Control	Sample size (n)	Sex (n)	Age (y: mean $\pm$ SD, or CI, and/or range)	BMI (kg/m <sup>2</sup> : mean $\pm$ SD, or CI, and/or range)	Duration	MedDiet score
Maldonado-Contreras et al. (142)	USA	Cross-sectional (N/A)	Caribbean Latino older adults	N/A	N/A	20	6 M; 14 F	62.7 $\pm$ 8.1	28.9 $\pm$ 4.9	N/A	9-point MedDiet score (MDS) modified from (143)
Meslier et al. (91)	Italy	Randomized parallel (N/A)	Healthy adults with overweight/obesity and sedentary lifestyle	MedDiet tailored to individual energy intake	Volunteers who maintained their regular diets	82	39 M; 34 F	43 $\pm$ 12	31.1 $\pm$ 4.5	2 Mo	11-item Italian Mediterranean Index (129)
Mitsou et al. (144)	Greece	Cross-sectional (N/A)	Healthy adults	N/A	Low MedDiet score (assumed Western diet)	120	61 M; 55 F	41.27 $\pm$ 13.33	27.29 $\pm$ 4.48	N/A	11-item MedDiet score (145)
Nagpal et al. (146)	USA	Randomized cross-over (N/A)	Older adults with mild cognitive impairment and cognitively normal controls	Modified Mediterranean-Ketogenic diet (MMKD)	American Heart Association Diet (AHAD)	17	5 M; 12 F	64.6 $\pm$ 6.4	NR	18 W (6 W each diet + 6 W washout)	Extra virgin olive oil was supplied to volunteers and ketones were measured weekly
Pagliai et al. (90)	Italy	Randomized cross-over (CARDIVEG Study)	Healthy adult Caucasian omnivores with overweight/obesity and low-to-moderate cardiovascular risk	Hypocaloric MedDiet	Hypocaloric vegetarian diet	23	7 M; 16 F	58.6 $\pm$ 9.8	31.06 $\pm$ 0.67 MedDiet; 30.10 $\pm$ 0.61 vegetarian	6 Mo (3 Mo per diet)	9-item MedDiet Adherence Score in CARDIVEG Study (147)
Park et al. (148)	USA	Post-hoc analysis of randomized cross-over (N/A)	Healthy adults	Moderate fat MedDiet (South Beach)	High fat (Atkins), low fat (Ornish)	14	NR	30.6 $\pm$ 9.6	22.6 $\pm$ 3	20 W (4 W per diet)	N/A
Pastori et al. (149)	Italy	Prospective (N/A)	Adults with atrial fibrillation	N/A	N/A	912	521 M; 391 F	73.5 $\pm$ 8.3	27.5 $\pm$ 4.7	Median follow-up 40.0 (20.5-68.0) Mo	A 9-item MedDiet validated survey (122)
Pastori et al. (150)	Italy	Post-hoc analysis of a prospective study (N/A)	Adults with atrial fibrillation	N/A	N/A	907	516 M; 391 F	73.5 $\pm$ 8.2	NR	Median follow-up 40.5 Mo	A 9-item MedDiet validated survey (122)

(Continued)

TABLE 1 (Continued)

References	Country	Study design (cohort)	Study population	MedDiet intervention	Control	Sample size (n)	Sex (n)	Age (y: mean $\pm$ SD, or CI, and/or range)	BMI (kg/m <sup>2</sup> : mean $\pm$ SD, or CI, and/or range)	Duration	MedDiet score
Pignaneli et al. (151)	Canada	Cross-sectional (N/A)	Adults with atherosclerosis	Educated about MedDiet	N/A	276	164 M; 112 F	66.87 $\pm$ 10.45	28.49 $\pm$ 6.08	N/A	8-point Mediterranean (aMED) diet scores from the FFQ (143, 152)
Quercia et al. (153)	Italy	<i>Post-hoc</i> of randomized Parallel (N/A)	Adults with reactive hypoglycemia (RH) and healthy adults	MedDiet and Ma-Pi 2 diet designed for hypoglycemia	Free MedDiet consumed by healthy controls	19	NR	27–65 RH; 25–36 healthy	21.7–37.4 RH; 20–23.4 healthy	3 D	Food was provided
Ruiz-Saavedra et al. (154)	Spain	Cross-sectional (N/A)	Healthy older adults	N/A	N/A	73	20 M; 53 F	56–95	19.9–37.5	N/A	Mediterranean adapted Diet Quality Index-International (DQI-I) (155) Modified Mediterranean Diet Score (MMDS) (156) Relative Mediterranean Diet Score (rMED) (157) All calculated from FFQ
Seethaler et al. (158)	Germany	Randomized parallel (LIBRE Study)	Women with BRCA1 and/or BRCA2 gene mutations and intestinal barrier impairment	MedDiet	Standard diet	260	0 M; 260 F	43.9 (CI: 42, 46) MedDiet; 44.8 (CI: 43, 46) control	25.0 (CI: 24, 26) MedDiet; 25.0 (CI: 24, 26) control	3 Mo	14-point Mediterranean Diet Adherence Screener (MEDAS) from PREDIMED Study (159) translated into German and re-validated (160) FFQ MedDiet score provided using the adapted Mediterranean Diet Score (MedD-Score) according to Trichopoulou et al. (143)
Seethaler et al. (161)	Germany	Randomized parallel (LIBRE Study)	Women with BRCA1 and/or BRCA2 gene mutations	MedDiet	Standard diet	68	0 M; 68 F	42 (CI: 35, 49) MedDiet; 41 (CI: 35, 50) control	23 (20, 27) MedDiet; 24 (CI: 21, 28) control	12 Mo	14-point Mediterranean Diet Adherence Screener (MEDAS) from PREDIMED Study (159) translated into German and re-validated (160) FFQ MedDiet score provided using the adapted Mediterranean Diet Score (MedD-Score) according to Trichopoulou et al. (143)

(Continued)

TABLE 1 (Continued)

References	Country	Study design (cohort)	Study population	MedDiet intervention	Control	Sample size (n)	Sex (n)	Age (y: mean $\pm$ SD, or CI, and/or range)	BMI (kg/m <sup>2</sup> : mean $\pm$ SD, or CI, and/or range)	Duration	MedDiet score
Shankar et al. (162)	USA & Egypt	Cross-sectional (N/A)	Healthy preadolescent and adolescent males	N/A	U.S. teenagers consuming Western diet	42	42 M; 0 F	13.9 $\pm$ 0.6 Egyptian; 12.9 $\pm$ 2.8 American	18.9 $\pm$ 2.5 Egyptian; 21.2 $\pm$ 3.4 American	N/A	N/A
Shoer et al. (163)	Israel	Randomized parallel (N/A)	Pre-diabetic individuals	Personalized postprandial glucose-targeting (PPT) diet	MedDiet	200	87 M; 113 F	50.92 $\pm$ 8.03 MedDiet; 50.37 $\pm$ 7.86 PPT	30.86 $\pm$ 6.01 MedDiet; 30.68 $\pm$ 5.23 PPT	6 Mo intervention + 6 Mo follow-up	N/A
Strauss et al. (63)	Canada	Post-hoc analysis of randomized controlled clinical trial (N/A)	Patients with ulcerative colitis (UC)	MedDiet	Habitual diet	40	21 M; 19 F	21–80	19–32	2 Mo	Modified 14-question Mediterranean Diet Adherence Screener (MEDAS) for the PREDIMED study (164)
Tanaka et al. (165)	USA	Cross-sectional (BLSA cohort)	Community-dwelling older adults, who reside primarily in the Washington DC–Baltimore area	N/A	N/A	806	391 M; 415 F	73.3 $\pm$ 7.1	NR	N/A	9-item MedDiet score (143) Mediterranean–DASH Diet Intervention for Neurodegenerative Delay (MIND) score (166)
Vitale et al. (167)	Italy	Randomized parallel (N/A)	Healthy adults with overweight/obesity	Isoenergetic MedDiet	Western-type diet (habitual control diet)	29	14 M; 15 F	41.6 $\pm$ 12.3 MedDiet; 45.9 $\pm$ 13.0 control	28.9 $\pm$ 2.3 MedDiet; 29.3 $\pm$ 3.5 control	2 Mo	Main foods provided
Zhu et al. (168)	USA	Randomized cross-over (N/A)	Healthy young adults	MedDiet	Fast-food diet	10	NR	22.1 $\pm$ 2.33	24.39 $\pm$ 3.71	12 D (4 D each diet)	Food was provided

MedDiet, Mediterranean diet; WD, Western diet; CanDiet, Canadian diet; BMI, body mass index; FFQ, food frequency questionnaire; NAFLD, nonalcoholic fatty liver disease; MASLD, Metabolic Dysfunction-Associated Steatotic Liver Disease; MEDAS, Mediterranean Diet Adherence Screener; MMDS, Modified Mediterranean Diet Score; CVD, cardiovascular disease; UC, ulcerative colitis; MDS, Mediterranean Diet Score; rMED, relative Mediterranean Diet Score; PPT, Personalized Postprandial Glucose-Targeting; FBGD, Food Based Dietary Guidelines; DQI-I, Diet Quality Index-International; PPGR, Postprandial Glycemic Response; MACE, Major Adverse Cardiovascular Event; DASH, Dietary Approaches to Stop Hypertension; MIND, Mediterranean-DASH Intervention for Neurodegenerative Delay; HS, Hidradenitis Suppurativa; EVOO, extra virgin olive oil; RH, reactive hypoglycemia; HS, Hidradenitis Suppurativa; D, days; W, weeks; Mo, months; M, male; F, female; CI, 95% confidence interval; MMKD, Mediterranean-Ketogenic diet.



TABLE 2 Results summary of metabolite changes in recent clinical studies investigating associations between gut-derived metabolites and the MedDiet.

References	Specimen type	Direction of change in metabolites relative to Med-like diet adherence				
		SCFA/BCFA	BA	TMAO	LPS	Other metabolites
André et al. (121)	Blood (plasma)	—	—	—	↓ MedDiet ( $p = 0.03$ ); ↓ Prudent diet ( $p = 0.01$ ); ↑ Traditional diet ( $p = 0.04$ ); ↔ Complex Carbohydrate diet ( $p = 0.41$ )	—
Baratta et al. (41)	Blood (serum)	—	—	—	↑ with ↓ MedDiet by association; ↑ LPS = ↑ sNox2-dp (tertile III, $p = 0.002$ )	↑ sNox2-dp (tertile III) = ↓ wine ( $p = 0.046$ ) and ↓ fish ( $p = 0.030$ ) according to MedDiet score
Barber et al. (87)	Urine	—	—	↑ (1.5-fold) after MedDiet	—	↑ deoxycholate glucuronide (2.1-fold), 5-hydroxyindole (2-fold), L-aspartyl-L-phenylalanine (2.4-fold) after MedDiet
Barrea et al., (123)	Blood (serum)	—	—	↓ ( $p < 0.001$ M; $p = 0.002$ F) with MedDiet adherence	—	—
Barrea et al. (125)	Blood (plasma)	—	—	↓ ( $p < 0.001$ ) with MedDiet adherence	—	—
Barrea et al. (127)	Blood (serum)	—	—	↓ by association with MedDiet adherence	—	—
Bourdeau-Julien et al. (65)	Blood (serum)	↑ valerate after CanDiet vs. first MedDiet ( $p < 0.01$ ); ↓ valerate after second MedDiet vs. CanDiet ( $p < 0.05$ ); ↑ BCFAs isobutyrate & isovalerate after CanDiet vs. first MedDiet ( $p < 0.05$ ); ↓ BCFAs isobutyrate & isovalerate after second MedDiet vs. CanDiet (both $p < 0.05$ )	—	—	—	↑ ECs after first MedDiet vs. baseline (DHEA, $p < 0.01$ ; EPEA, $p < 0.05$ ; 2-DHG, $p < 0.01$ ; 2-EPG, $p < 0.01$ ); ↓ ECs after CanDiet vs. first MedDiet (DHEA, $p < 0.001$ ; EPEA, $p < 0.01$ ; OEA, $p < 0.05$ ; 2-DHG, $p < 0.001$ ; 2-EPG, $p < 0.001$ ; 2-OG, $p < 0.01$ ); ↑ ECs after second MedDiet vs. CanDiet (DHEA, $p < 0.001$ ; EPEA, $p < 0.05$ ; OEA, $p < 0.01$ ; 2-DHG, $p < 0.001$ ; 2-EPG, $p < 0.01$ ; 2-OG, $p < 0.05$ )
De Filippis et al. (128)	Feces (SCFA), urine (TMAO)	↑ butyrate, propionate, acetate ( $p < 0.01$ ), and ↓ valerate ( $p < 0.05$ ), with high MedDiet adherence vs. low MedDiet adherence	—	↓ TMAO in vegetarian and vegan diets compared to omnivores	—	Several significant metabolites in Table S3 of original article
Forteza et al. (66)	Blood (plasma)	↑ acetic acid and ↓ isovaleric acid after MedDiet before aerobic exercise ( $p < 0.05$ )	—	—	—	↑ EC OEA after MedDiet before and during exercise; ↑ ECs AEA ( $p < 0.05$ ) and EPEA ( $p < 0.001$ ) after MedDiet immediately after exercise
Galie et al. (130)	Blood (plasma)	—	TLCA and GUDCA positively associated with MedDiet; TCA negatively associated with MedDiet	TMA positively associated with MedDiet	—	See Table 2 in original article for all 65 metabolite results

(Continued)

TABLE 2 (Continued)

References	Specimen type	Direction of change in metabolites relative to Med-like diet adherence				
		SCFA/BCFA	BA	TMAO	LPS	Other metabolites
Garcia-Mantrana et al. (132)	Feces	↑ acetate + propionate + butyrate ( $p = 0.023$ ) with MedDiet; ↑ acetate ( $p = 0.006$ ; $p = 0.001$ ), propionate ( $p = 0.016$ ; $p = 0.004$ ), and total SCFA ( $p = 0.020$ ; $p = 0.003$ ) with vegetal proteins and polysaccharides, respectively	—	—	—	—
Ghosh et al. (42)	Blood (plasma)	↑ SCFAs & BCFAs inferred with positive microbiome changes	↑ CA ( $p < 0.006$ ), GCDCA ( $p < 0.006$ ) and ↓ CDCA ( $p < 0.03$ ) with MedDiet OTUs	—	—	—
Griffin et al. (134)	Blood (serum)	—	—	↔ TMAO, choline, carnitine, betaine, $\gamma$ -butyrobetaine after MedDiet and healthy eating	LPB positively associated with TMAO	—
Guasch-Ferre et al. (136)	Blood (plasma)	—	—	↑ after MedDiet + EVOO; ↔ after MedDiet + Nuts	—	—
Gutierrez-Diaz et al. (137)	Feces	↑ butyrate ( $p = 0.018$ ) & propionate ( $p = 0.034$ ), in MDS $\geq 4$ vs. MDS $< 4$	—	—	—	—
Haskey et al. (55)	Feces	↑ total SCFAs ( $p = 0.01$ ), acetic acid ( $p = 0.03$ ), butyric acid ( $p = 0.03$ ), and valeric acid ( $p = 0.008$ ) after MedDiet vs. CanDiet	—	—	—	↓ FCP after MedDiet vs. CanDiet ( $p = 0.01$ ); ↑ fecal sIgA after MedDiet vs. baseline ( $p = 0.004$ )
Krishnan et al. (141)	Blood (serum)	—	—	↑ after MedDiet + 500 g red meat vs. MedDiet + 200 g red meat ( $p < 0.001$ ), but ↔ choline, betaine, and carnitine	—	—
Maldonado-Contreras et al. (142)	Feces	↓ acetate ( $p = 0.08$ ) and butyrate ( $p = 0.08$ ) with ↑ MedDiet score	—	—	—	—
Meslier et al. (91)	Feces (SCFA, BAs), blood/plasma (TMAO, carnitine, choline, creatinine, betaine), urine (TMAO, carnitine, choline, creatinine, betaine)	↔ acetate, butyrate, and propionate after MedDiet; ↓ BCFAs at 4 weeks (valerate, $p = 0.04$ ; 2-methylbutyrate, $p = 0.003$ ) or 8 weeks (isovalerate, $p = 0.004$ ; isobutyrate, $p = 0.007$ ) after MedDiet	↓ total BAs ( $p = 0.0001$ ), total 1st BAs ( $p = 0.04$ ), total 2nd BAs ( $p = 0.0009$ ) DCA, and LCA after 8 weeks of MedDiet	↓ carnitine after MedDiet ( $p < 0.001$ )	—	↑ Total urolithins ( $p = 0.033$ ) and urolithin-A-glucuronide ( $p = 0.025$ ) after MedDiet

(Continued)

TABLE 2 (Continued)

References	Specimen type	Direction of change in metabolites relative to Med-like diet adherence				
		SCFA/BCFA	BA	TMAO	LPS	Other metabolites
Mitsou et al. (144)	Feces	↑ acetate ( $p = 0.009$ ) and ↓ valerate ( $p = 0.014$ ) with high MedDiet adherence	—	—	—	—
Nagpal et al. (146)	Feces	↑ butyrate ( $p < 0.05$ ) after MMKD	—	—	—	—
Pagliai et al. (90)	Feces	↑ propionic acid ( $p = 0.034$ ) in MedDiet vs. vegetarian diet	—	—	—	Propionate negatively correlated with IP-10, IL-12 ( $p < 0.05$ ), and VEGF ( $p < 0.01$ ); acetic acid negatively correlated with IP-10, IL-10, IL-17 ( $p < 0.05$ ), VEGF, and IL-12 ( $p < 0.01$ ); butyric acid negatively correlated with VEGF, MCP-1 ( $p < 0.05$ ), IL-12 and IL-17 ( $p < 0.01$ ); isovalerate with IL-1RA ( $p < 0.05$ ); isobutyric acid with IL-1RA and MCP-1 ( $p < 0.05$ ) after MedDiet
Park et al. (148)	Plasma	—	—	↔ after Med-like vs. baseline or high fat diet	—	↓ AA valine ( $p \leq 0.05$ ) in Med-like diet compared to high fat diet; ↑ valine ( $p = 0.004$ ) and leucine ( $p = 0.01$ ) with high fat diet vs. baseline
Pastori et al. (149)	Blood (serum)	—	—	—	MedDiet score predictor for log-LPS ( $p < 0.001$ ); ↓ LPS with ↑ fruit ( $p = 0.009$ ), ↑ legumes ( $p = 0.005$ ) and ↓ trend meat (0.085)	↑ TxB2 with ↑ MACE ( $p < 0.001$ ); log-LPS ( $p < 0.001$ ) and MedDiet score ( $p < 0.001$ ) associated with TxB2
Pastori et al. (150)	Blood (plasma)	—	—	—	↑ with ↓ MedDiet adherence by association	↑ PCSK9 with ↓ MedDiet adherence ( $p = 0.001$ ), especially ↓ EVOO ( $p = 0.001$ ) and ↓ moderate wine consumption ( $p = 0.007$ )
Pignanelli et al. (151)	Plasma	—	—	—	—	p-cresyl sulfate, hippuric acid, indoxyl sulfate, p-cresyl glucuronidate, phenyl acetyl glutamine, and phenyl sulfate did not correlate with MedDiet
Quercia et al. (153)	Feces	↔ butyrate ( $p = 0.2$ ), propionate ( $p = 0.5$ ), or acetate ( $p = 0.5$ ) with MedDiet vs. baseline; ↑ butyrate, propionate, and acetate with vegan diet vs. baseline (all $p < 0.01$ )	—	—	—	—
Ruiz-Saavedra et al. (154)	Feces	butyric acid ( $p < 0.012$ ), propionic acid ( $p = 0.001$ ), and acetic acid ( $p < 0.001$ ) positively associated with MMDS	—	—	—	↑ IL-8 with ↑ scores on MedDiet indices (rMed, $p = 0.018$ ; MMDS, $p = 0.017$ )

(Continued)

TABLE 2 (Continued)

References	Specimen type	Direction of change in metabolites relative to Med-like diet adherence				
		SCFA/BCFA	BA	TMAO	LPS	Other metabolites
Seethaler et al. (158)	Feces (SCFAs), blood/plasma (LBP)	↑ propionate ( $q < 0.001$ , +19%), ↑ butyrate ( $q < 0.001$ , +44%) after MedDiet vs. baseline; ↑ propionate ( $p = 0.09$ ) after MedDiet vs. control	—	—	↓ LBP ( $q < 0.001$ , -6%) after MedDiet vs. baseline	↓ zonulin ( $q < 0.001$ , -30%) after MedDiet vs. baseline
Seethaler et al. (161)	Feces (SCFAs), blood/plasma (LBP)	NR	—	—	↓ LBP ( $p < 0.001$ ) after MedDiet vs. baseline; ↓ LBP ( $p = 0.017$ ) after MedDiet vs. control	↓ zonulin ( $p < 0.01$ ) after MedDiet vs. baseline
Shankar et al. (162)	Feces	↑ propionate ( $p < 0.05$ ) in Egyptian vs. American	↓ BAs ( $p < 0.05$ ) in Egyptian vs. American	↓ choline ( $p < 0.01$ ) in Egyptian vs. American	—	↑ nucleotides [hypoxanthine ( $p < 0.01$ ) and uracil ( $p < 0.05$ )] in Egyptian; ↓ amino acids [aspartate, isoleucine, leucine, lysine, tyrosine, valine (all $p < 0.01$ )] in Egyptian
Shoer et al. (163)	Blood (serum)	↑ butyrate-related compounds after PPT diet	—	—	—	↑ 10 uncharacterized biochemicals, 7 lipids, 6 AA, 1 xenobiotic (3-bromo-5-chloro-2,6-dihydroxybenzoic acid), 1 peptide (HWESASXX), 1 nucleotide (dihydroorotate) and bilirubin after MedDiet
Strauss et al. (63)	Feces	↑ valerate ( $p = 0.05$ ), ↔ acetate, propionate, and butyrate after MedDiet vs. habitual diet	↑ GCDCA ( $p = 0.02$ ), ↔ CA, CDCA, and DCA after MedDiet vs. habitual diet	—	—	↓ FCP associated with ↑ MedDiet score ( $p = 0.004$ )
Tanaka et al. (165)	Blood (plasma)	—	DCA (MDS, $p = 0.04$ ; MIND, $p = 0.004$ ); GUDCA (MDS, $p = 0.04$ ; MIND, $p = 0.001$ ); GCDCA (MIND, $p = 0.05$ ); GDCA (MIND, $p = 0.04$ ) with MedDiet indices Other NS bile acids reported in Table S4 within the original article.	Not correlated with MDS	—	↑ or ↓ TG with ↑ MedDiet adherence
Vitale et al. (167)	Blood (serum)	↑ butyric acid IAUC in MedDiet group ( $p = 0.019$ )	—	—	—	↓ LDL-cholesterol in MedDiet group ( $p = 0.04$ )
Zhu et al. (168)	Blood (plasma)	—	No significant changes	No significant changes	—	kynurenine to tryptophan ratio ↓ after FF diet and ↑ after MedDiet ( $p = 0.005$ ); ↑ indole-3-lactic acid ( $p = 0.003$ ) and indole-3-propionic acid after MedDiet

↑, increase; ↓, decrease; ↔, no change; SCFA, Short Chain Fatty Acid; BA, Bile Acid; BCFA, Branch Chain Fatty Acid; TMAO, Trimethylamine N-oxide; LPS, lipopolysaccharide; FA, Fatty Acid; MedDiet, Mediterranean Diet; CanDiet, Canadian Diet; EC, Endocannabinoid; DHEA, N-docosahexaenoyl-ethanolamine; EPEA, N-eicosapentaenoyl-ethanolamine; EPG, 1/2-eicosapentaenoylglycerol; OEA, N-oleoyl-ethanolamine; DHG, 1/2-docosahexaenoyl-glycerol; OG, 1/2-oleoyl-glycerol; AEA, anandamide; CA, cholic acid; GCDCA, glycochenodeoxycholic acid; CDCA, chenodeoxycholic acid; LPB, Lipopolysaccharide Binding Protein; EVOO, Extra Virgin Olive Oil; LDL, Low-Density Lipoprotein; PPT, Personalized Postprandial Glucose-Targeting; MCP-1, Monocyte Chemoattractant Protein-1; PCSK9, Proprotein Convertase Subtilisin/Kexin type 9; FCP, Fecal Calprotectin; DCA, Deoxycholic Acid; MDS, Mediterranean Diet Score; MIND, Mediterranean-DASH Intervention for Neurodegenerative Delay; GUDCA, Glycoursodeoxycholic Acid; GDCA, Glycodeoxycholic Acid; TLCA, Taurolithocholic acid; TCA, Taurocholic acid; TG, Triglyceride; IAUC, Incremental Area Under the Curve; FF, Fast Food; MMDS, Modified Mediterranean Diet Score; rMED, relative Mediterranean Diet Score; MACE, major adverse cardiovascular event; TxB2, urinary 11-dehydro-thromboxane B2; AA, amino acids; MMKD, Mediterranean Ketogenic diet; OTU, operational taxonomic unit.



TABLE 3 Results summary of microbiome and other changes in recent clinical studies investigating associations between gut-derived metabolites and the MedDiet.

Reference	Microbiome composition	Other health-related changes	Main results
André et al. (121)	—	—	Greater adherence to Mediterranean and prudent diets associated with lower circulating 3-OH FAs.
Baratta et al. (41)	—	↑ sNox2-dp in NAFLD (MASLD); ↑ sNox2-dp = ↑ GGT, AST, ALT (all $p < 0.001$ );	In NAFLD (MASLD) patients, highest sNox2-dp tertile associated with highest LPS tertile and low adherence to MedDiet (esp. wine and fish).
Barber et al. (87)	↑ <i>Agathobaculum</i> spp., <i>Anaerostipes</i> spp., <i>Anaerostipes hadrus</i> , <i>Agathobaculum butyriciproducens</i> with MedDiet	↑ flatulence ( $p = 0.048$ ), borborigmi ( $p = 0.016$ ), stool consistency ( $p = 0.014$ ), stool weight ( $p < 0.001$ ), colonic content ( $p < 0.001$ ) after MedDiet	MedDiet, associated with higher gas and larger colonic content, changed microbial metabolism, but less dramatically in volunteers with higher beta-diversity.
Barrea et al. (123)	—	TMAO positively correlated to BMI, WC, total cholesterol, LDL cholesterol, TG (each $p < 0.001$ )	Women, who consumed more plant protein and $\omega$ -3 PUFA, had higher adherence to MedDiet and lower TMAO levels than men.
Barrea et al. (125)	—	↑ TMAO with ↑ BMI and ↓ physical activity (each $p < 0.001$ ); ↑ TMAO with evening chronotype ( $p < 0.001$ )	Morning chronotype had significantly lower BMI, WC, TMAO levels, and highest adherence to MedDiet.
Barrea et al. (127)	—	↑ TMAO in HS ( $p < 0.001$ ) and ↓ MedDiet score in HS ( $p = 0.002$ )	HS patients, esp. with highest disease severity, had increased inflammation, TMAO levels, and lower adherence to MedDiet compared to healthy controls.
Bourdeau-Julien et al. (65)	↑ <i>Bacteroides</i> spp., <i>Butyricoccus</i> spp., <i>Coprococcus.1</i> spp., <i>Lachnospiraceae</i> spp., <i>Lachnospiraceae UCG 001</i> spp., <i>Parasutterella</i> spp., and <i>Lachnospira</i> spp. with MedDiet	—	Lead-in MedDiet and CanDiet both showed immediate and reversible metabolite (SCFA, BCFA, EC) changes, which correlated with changes in gut microbiota composition. BCFAs more strongly reduced after second MedDiet. Higher initial gut microbiota diversity resulted in more stable microbiota response.
De Filippis et al. (128)	↑ <i>Prevotellaceae</i> with plant-based diets; ↑ Bacteroidetes in vegans and vegetarians compared with omnivores ( $p < 0.05$ ); ↑ F/B ratio in omnivores	—	Consumption of plant-based diets, associated with high MedDiet adherence, increased levels of SCFA and altered gut microbiota composition.
Forteza et al. (66)	↑ <i>Oscillospiraceae</i> ( $p = 0.039$ ) and <i>Prevotellaceae</i> ( $p = 0.047$ ) after MedDiet vs. CanDiet	—	Consumption of short-term MedDiet vs. CanDiet leads to differential response in EC and SCFA metabolites before or immediately following acute maximal aerobic exercise.
Galie et al. (130)	Cluster of <i>Lachnospiraceae</i> spp., <i>Ruminococcaceae UCG002</i> spp., <i>Lachnospiraceae</i> spp., and <i>Prevotellaceae</i> positively associated with changes in metabolites C16-OH, C12:0, C12-OH, PC35:1, PC40:6, TGs 56:6, 46:7, 56:5, and ChoE 20:5, while negatively associated with changes in phosphoethanolamine and taurine	↓ glucose ( $p = 0.02$ ), insulin ( $p = 0.01$ ), and HOMA-IR ( $p = 0.01$ ) after MedDiet	MedDiet, rather than consumption of nuts in context with a non-MedDiet, was associated with a plasma metabolic profile related to metabolic disease improvements.
Garcia-Mantrana et al. (132)	↑ <i>Catenibacterium</i> spp. with high MedDiet adherence; ↑ <i>Butyricimonas</i> spp., <i>Desulfovibrio</i> spp., and <i>Oscillospira</i> spp. with BMI < 25; ↓ trend F/B ratio ( $p = 0.057$ ) with higher MedDiet score	—	Dietary habits, adherence to MedDiet pattern, and BMI affect gut microbiome and metabolite changes in healthy adults. MedDiet adherence associated with increased SCFA, <i>Catenibacterium</i> spp., and higher intake of vegetable proteins and polysaccharides.
Ghosh et al. (42)	↑ <i>Faecalibacterium prausnitzii</i> , <i>Roseburia</i> spp. ( <i>R. hominis</i> and some unclassified), <i>Eubacterium</i> spp. ( <i>E. rectale</i> , <i>E. eligens</i> , <i>E. xylanophilum</i> ), <i>Bacteroides thetaiotaomicron</i> , <i>Prevotella copri</i> and <i>Anaerostipes hadrus</i> with high MedDiet adherence	↓ Frailty with MedDiet ( $p < 0.06$ ); ↓ frailty with ↑ DietPositive taxa ( $p < 0.05$ ); ↓ hsCRP and IL-17 with DietPositive taxa	Adherence to MedDiet resulted in a changed gut microbiota and metabolites, reduced frailty, improved cognitive function, and negatively correlated with markers of inflammation.

(Continued)

TABLE 3 (Continued)

Reference	Microbiome composition	Other health-related changes	Main results
Griffin et al. (134)	↑ <i>Akkermansia mucinophilia</i> in colon biopsies with ↓ TMAO, choline, and betaine	—	No significant changes in TMAO or TMAO precursor ratios in MedDiet or Healthy Eating diet groups. Relative abundance of <i>Akkermansia mucinophilia</i> in colon biopsies negatively correlated with TMAO and some precursors (betaine, choline, carnitine).
Guasch-Ferre et al. (136)	—	↑ choline ( $p < 0.001$ ) in cases vs. controls; baseline B/C ratio inversely associated with CVD; baseline choline metabolite score associated with a 2.21-fold higher risk of CVD across extreme quartiles ( $p < 0.001$ for trend) and a 2.27-fold higher risk of stroke ( $p < 0.001$ for trend)	Baseline B/C ratio negatively associated with CVD while baseline choline associated with increased risk of CVD and stroke. MedDiet associated with lower risk of CVD compared to control diet. No significant correlations between metabolites and CVD found after 1-year MedDiet intervention.
Gutierrez-Diaz et al. (137)	↑ Bacteroidetes ( $p = 0.001$ ), <i>Prevotellaceae</i> ( $p = 0.002$ ), and <i>Prevotella</i> spp. ( $p = 0.003$ ); ↓ Firmicutes ( $p = 0.003$ ) and <i>Lachnospiraceae</i> ( $p = 0.045$ ) with MDS $\geq 4$ vs. MDS $< 4$	—	High MedDiet score associated with higher abundance of Bacteroidetes and <i>Prevotellaceae</i> , and increased fecal SCFAs, propionate and butyrate.
Haskey et al. (55)	↑ <i>Alistipes finegoldii</i> , <i>Flavonifractor plautii</i> , <i>Ruminococcus bromii</i> after MedDiet	—	MedDiet lowered FCP and increased SCFAs compared to CanDiet. MedDiet associated with gut microbiota species known to be protective against colitis ( <i>Alistipes finegoldii</i> and <i>Flavonifractor plautii</i> ) and promote the production of SCFAs ( <i>Ruminococcus bromii</i> ).
Krishnan et al. (141)	—	TMAO positively associated with HOMA-IR, a surrogate for insulin resistance ( $p = 0.036$ )	TMAO levels reduced when lower amounts of red meat (200 vs. 500 g) consumed with MedDiet.
Maldonado-Contreras et al. (142)	↑ trend <i>Prevotella copri</i> in individuals with higher 18:3 alpha linolenic fatty acid intake ( $p = 0.09$ ); ↑ Enterobacteriales in T2D ( $p = 0.01$ )	butyrate ( $p = 0.03$ ), propionate ( $p = 0.02$ ), acetate ( $p = 0.04$ ) correlated with % calories from fat	Caribbean Latino adults showed poor adherence to MDS or HEI-2015. Microbiome samples clustered into two groups depending on <i>Prevotella copri</i> abundance, which was related to higher alpha linolenic fatty acid intake. Individuals with T2D had higher Enterobacteriales and trend lower SCFAs.
Meslier et al. (91)	↑ <i>Faecalibacterium prausnitzii</i> , <i>Roseburia</i> spp., and <i>Lachnospiraceae</i> after MedDiet	↓ total cholesterol 4 weeks after MedDiet ( $p < 0.05$ )	MedDiet increased fiber and reduced animal protein intake, reduced levels of carnitine, cholesterol, and BAs. Shotgun metagenomics showed MedDiet increased abundance of fiber-degrading <i>Faecalibacterium prausnitzii</i> and genes linked to butyrate metabolism.
Mitsou et al. (144)	↓ <i>Escherichia coli</i> ( $p = 0.022$ ), ↑ bifidobacteria: <i>E. coli</i> ratio ( $p = 0.025$ ), and ↑ <i>Candida albicans</i> ( $p = 0.039$ ) with high MedDiet adherence	↑ total number of evacuations ( $p = 0.028$ ), GI pain ( $p = 0.029$ ), and bloating ( $p = 0.028$ ) with high MedDiet adherence	High MedDiet adherence associated with lower <i>Escherichia coli</i> counts, an increased bifidobacteria: <i>E. coli</i> ratio, increased levels of <i>Candida albicans</i> , higher molar ratio of acetate, and more pronounced GI symptoms.
Nagpal et al. (146)	↓ <i>Bifidobacteriaceae</i> and <i>Bifidobacterium</i> spp. after MMKD; ↑ <i>Akkermansia</i> spp., <i>Verrucomicrobia</i> , and <i>Verrucomicrobiaceae</i> after MMKD	In adults with mild cognitive impairment eating the MMKD, ↑ Tenericutes and <i>Enterobacteriaceae</i> = ↓ CSF Aβ42, ↑ <i>Lachnospiraceae</i> , <i>Rikenellaeae</i> , and <i>Parabacteroides</i> = ↑ CSF Aβ42, and ↑ <i>Sutterella</i> and <i>Mollicutes</i> = ↑ and ↓ tau-p181, respectively	MMKD can modulate the gut microbiome and serum metabolites in those at risk for Alzheimer's disease. These changes are associated with improved Alzheimer's disease biomarkers in cerebrospinal fluid.
Pagliai et al. (90)	↑ <i>Enterorhabdus</i> spp. ( $p = 0.002$ ), <i>Lachnoclostridium</i> spp. ( $p = 0.039$ ), and ↓ <i>Parabacteroides</i> spp. ( $p = 0.037$ ) pre- vs. post-MedDiet; ↑ <i>Clostridium sensu stricto</i> ( $p = 0.005$ ), <i>Enterorhabdus</i> spp. ( $p = 0.003$ ), <i>Veillonella</i> spp. ( $p = 0.029$ ), and ↓ <i>Anaerostipes</i> spp. ( $p = 0.048$ ) after MedDiet vs. vegetarian diet	<i>Anaerostipes</i> positively correlated with LDL cholesterol and total cholesterol; HDL-cholesterol and IFN- $\gamma^*$ negatively correlated with <i>Enterorhabdus</i> spp.; <i>Parabacteroides</i> spp. positively correlated with MCP-1; <i>Lachnoclostridium</i> spp. related to negative variations of IL-6, AST*, ALT and vitamin B12 ( $p < 0.05$ or * $p < 0.01$ )	MedDiet and vegetarian diet changed some gut microbiota composition and SCFA propionate differentially. After MedDiet, variations of SCFAs negatively associated with some inflammatory cytokines (VEGF, MCP-1, IL-17, IP-10, and IL-12).

(Continued)

TABLE 3 (Continued)

Reference	Microbiome composition	Other health-related changes	Main results
Park et al. (148)	N/A	↑ TMAO with high fat diet vs. low fat diet ( $p = 0.01$ )	Baseline diet and 4-week low-fat diet reduced TMAO and BCAA levels compared to high-fat. Few changes in moderate fat Med-like diet.
Pastori et al. (149)	—	↑ LPS with ↑ MACE ( $p = 0.021$ ), ↓ survival free of MACE ( $p = 0.001$ , 3rd vs. 1st LPS tertile); Log-LPS is a predictor of MACE ( $p = 0.009$ )	Log-LPS, age, and previous CV or cardiac events were predictors of MACE. MedDiet score (esp. higher intake of fruits and legumes) significantly affects circulating log-LPS.
Pastori et al. (150)	—	↑ of LPS ( $p < 0.001$ ) and ↑ sNox2-dp ( $p < 0.001$ ) with PCSK9 above the median, and these were directly correlated	LPS and PCSK9 levels significantly correlated. LPS, sNox2-dp, and high adherence to MedDiet associated with PCSK9 above the median range. Olive oil and wine intake negatively correlated with PCSK9. Patients with high levels of LPS and PCSK9 had increased incidence of CV events.
Pignanelli et al. (151)	—	↑ TMAO associated with ↓ eGFR ( $p = 0.02$ ) or ↑ renal impairment	Impaired renal function associated with higher plasma metabolites and higher carotid plaque burden. No correlations detected between plasma metabolites and MedDiet score.
Quercia et al. (153)	Not significant	—	Gut microbiome profiles did not differ between 3-day vegan (Ma-Pi 2) and MedDiet group. SCFA levels increased only with vegan diet.
Ruiz-Saavedra et al. (154)	↑ <i>Faecalibacterium prausnitzii</i> levels positively associated with DII ( $p = 0.030$ ), HEI ( $p = 0.035$ ), DQI-I ( $p = 0.047$ ), and MMDS ( $p = 0.044$ ), while <i>Lactobacillus</i> spp. levels negatively correlated with AHEI ( $p = 0.027$ ) and MMDS ( $p = 0.012$ )	—	DII, HEI, DQI-I, and MMDS were positive predictors of <i>Faecalibacterium prausnitzii</i> . AHEI and MMDS were negatively associated with <i>Lactobacillus</i> spp. HEI, AHEI, and MMDS positively associated with SCFA. Lower IL-8 detected with higher MedDiet scores.
Seethaler et al. (158)	—	↓ WC ( $p = 0.005$ ), ↓ WHR ( $p = 0.07$ ) after MedDiet vs. control	High MedDiet adherence led to decreased LPB and zonulin levels and increased SCFAs. Propionate and butyrate identified as mechanistic links between diet and intestinal barrier integrity.
Seethaler et al. (161)	—	↑ n-3 PUFA, n-3 DHA ( $p < 0.001$ ) with MedDiet adherence	MedDiet adherence associated with increased n-3 DHA levels and decreased LBP and zonulin levels, however the effect of n-3 DHA on intestinal barrier integrity was mild compared to SCFAs reported previously.
Shankar et al. (162)	↑ <i>Prevotella</i> spp. ( $p < 0.01$ ), <i>Gammaproteobacteria</i> , <i>Methanobacteria</i> , <i>Megasphaera</i> spp. ( $p < 0.05$ ), <i>Eubacterium</i> spp. ( $p < 0.01$ ), <i>Mitsuokella</i> spp. ( $p < 0.01$ ), <i>Catenibacterium</i> spp. ( $p < 0.01$ ), and <i>Succinivibrio</i> spp. ( $p = 0.028$ ) in Egyptian; Egyptian = <i>Prevotella</i> spp. enterotype; American = <i>Bacteroides</i> spp. enterotype	—	The Egyptian (MedDiet pattern) gut had higher levels of SCFAs, increased prevalence/proportions of microbial polysaccharide degradation-encoding genes/genera and belonged to <i>Prevotella</i> spp. enterotype compared to American gut.
Shoer et al. (163)	↑ microbiome diversity after MedDiet ( $p < 0.05$ ) and PPT diet ( $p < 0.01$ ); ↑ <i>Ruminococcaceae</i> , <i>Clostridiaceae</i> , <i>Clostridium</i> spp. CAG 122 (SGB_4659, $p = 0.01$ ), <i>Clostridium</i> spp. (SGB_4714, $p = 0.01$ ), <i>Faecalibacterium prausnitzii</i> (SGB_15332, $p = 0.03$ ; SGB_15333, $p = 0.008$ ), and ↓ <i>Eubacterium ventriosum</i> after MedDiet	↑ cytokines [Axin 1 (AXIN1) and Sirtuin 2 (SIRT2)] after MedDiet	PPT diet had larger impact on microbiome and metabolites (several linked to butyrate metabolism) compared to MedDiet. Oral microbiome found to be genetically more dynamic than the gut.
Strauss et al. (63)	↑ <i>Roseburia</i> spp., <i>Lachnospiraceae</i> spp. and <i>Bifidobacterium</i> spp. with ↓ FCP; ↑ <i>Bacteroides fragilis</i> , <i>Ruminococcus</i> spp., and <i>Eikenella corrodens</i> with ↑ FCP levels; ↑ <i>Faecalibacterium prausnitzii</i> ( $p = 0.02$ ), <i>Dorea longicatena</i> (NS), and <i>Roseburia inulinivorans</i> ( $p = 0.002$ ) mediators between ↑ MedDiet and ↓ FCP	↑ benzyl alcohol, 3-hydroxyphenylacetate, 3-4-hydroxyphenylacetate and phenylacetate as mediators between ↑ MedDiet and ↓ FCP	MedDiet intervention significantly increased some SCFA and BA compared to habitual diet. Identified three taxa and four metabolites as strong mediators between MedDiet and fecal calprotectin.

(Continued)

TABLE 3 (Continued)

Reference	Microbiome composition	Other health-related changes	Main results
Tanaka et al. (165)	—	—	All dietary scores (MDS, MIND, and AHEI-2010) inversely correlated with frailty index. Of 466 metabolites measured, 236, 218, and 278 associated with MDS, MIND, and AHEI-2010, respectively; 176 metabolites overlapped between the three diet scores. Some signatures of MIND and AHEI-2010 identified as potential mediators of diet and frailty index.
Vitale et al. (167)	↑ <i>Intestinimonas butyriciproducens</i> and <i>Akkermansia muciniphila</i> ; ↓ <i>Ruminococcus torques</i> , <i>Coprococcus comes</i> , <i>Streptococcus galloyticus</i> and <i>Flavonifractor plautii</i> (all $p < 0.05$ ) after MedDiet	—	MedDiet reduced glucose and insulin response after a meal, increased postprandial butyric acid (which negatively correlated with insulin sensitivity) and increased relative abundance of <i>Intestinimonas butyriciproducens</i> and <i>Akkermansia muciniphila</i> .
Zhu et al. (168)	↑ <i>Butyricoccus</i> spp. ( $p = 0.0001$ ), <i>Lachnospiraceae_UCG-004</i> spp. ( $p = 0.01$ ) after MedDiet; <i>Collinsella</i> spp. ( $p = 0.004$ ), <i>Parabacteroides</i> spp. ( $p = 0.004$ ), <i>Escherichia</i> spp./ <i>Shigella</i> spp. ( $p = 0.03$ ), <i>Bifidobila</i> spp. ( $p = 0.03$ ) after FF diet	—	Four-day MedDiet increased fiber-fermenting bacteria, while fast-food diet increased bile-tolerant species. Indole derivatives significantly higher after MedDiet. Interindividual variability may be due to differences in habitual diet.

↑, increase; ↓, decrease; SCFA, Short Chain Fatty Acid; BA, Bile Acid; BCFA, Branch Chain Fatty Acid; FA, Fatty Acid; TMAO, Trimethylamine N-oxide; LPS, lipopolysaccharide; BMI, Body Mass Index; NAFLD, Nonalcoholic Fatty Liver Disease; MASLD, Metabolic Dysfunction-Associated Steatotic Liver Disease; MedDiet, Mediterranean Diet; CanDiet, Canadian Diet; GGT, gamma glutamyltransferase; AST, aspartate transaminase; PUFA, Polyunsaturated Fatty Acid; HS, Hydradenitis Suppurativa; EC, Endocannabinoid; F/B, Firmicutes/Bacteroidetes; LPB, Lipopolysaccharide Binding Protein; CVD, Cardiovascular Disease; HOMA-IR, Homeostasis Model Assessment-Estimated Insulin Resistance; GI, Gastrointestinal; VEGF, Vascular Endothelial Growth Factor; LDL, Low-Density Lipoprotein; HDL, High-Density Lipoprotein; PPT, Personalized Postprandial Glucose-Targeting; MCP-1, Monocyte Chemoattractant Protein-1; PCSK9, Proprotein Convertase Subtilisin/Kexin type 9; WHR, Waist-to-Hip ratio; WC, Waist Circumference; DHA, Docosahexaenoic Acid; FCP, Fecal Calprotectin; MDS, Mediterranean Diet Score; MIND, Mediterranean Diet Score; AHEI, Alternative Healthy Eating Index; TG, Triglyceride; HEI, Healthy Eating Index; T2D, Type 2 Diabetes; BCAA, Branched-Chain Amino Acid; MMDS, Modified Mediterranean Diet Score; DII, Dietary Inflammatory Index; DQI-I, Diet Quality Index-International; B/C, betaine/choline ratio; 1st, primary; 2nd, secondary; MACE, major adverse cardiovascular event; eGFR, estimated glomerular filtration rate; Spp., species; MMKD, Mediterranean Ketogenic diet; FF, fast food; IFN, interferon; ChoE, cholesterol ester; PC, phosphatidylcholine; CSF, cerebrospinal fluid; AB, amyloid beta. \*Indicates  $p < 0.01$ .

bloodstream (12). While dietary fiber has been shown to promote weight loss and improve glycemic control, the complete biological role of SCFAs in this process remains unclear (83). Some SCFAs have been shown to beneficially affect host metabolism through secretion of gut hormones, such as glucagon-like peptide-1 (GLP1) and peptide YY, that affect appetite, reduce inflammation, and increase fat oxidation, as reviewed by Hernandez et al. (169). Studies in mice, which allow experimental designs that cannot be performed in humans, have added greatly to our understanding of diet contributions to gut microbiota-derived metabolic changes. Recently, Bachem et al. showed the impact of gut microbiota on the fate of CD8<sup>+</sup> T-cells through the production of SCFAs in mice consuming a high-fiber diet (Figure 1) (171). SCFA supplementation also restored the number of enteric neurons that were depleted following antibiotic treatment in mice (177). In mice, reduction of SCFAs by a fiber-deficient diet led to alterations of the gut microbiota, increased intestinal permeability, inflammation, and cognitive impairment. Furthermore, SCFA supplementation improved these deficits (178). These recent studies support a role for SCFAs in modulation of immunity, inflammation, and potentially obesity.

Dietary fiber that affects production of SCFAs can come from many sources, but mostly fruits, vegetables, and whole grains, which are staples of a MedDiet. Several recent clinical studies have shown that there is increased production of some SCFAs, based on blood and/or feces measurements (see Table 2), following MedDiet intervention in healthy volunteers (66) or individuals with disorders such as ulcerative colitis (63), intestinal barrier impairment (158), overweight, or obesity (90, 167). Observational studies have also shown that better adherence to the MedDiet has been associated with increased levels of SCFAs (128, 132, 144). Another study of individuals who followed a hypocaloric MedDiet ( $n = 21$ ), a very-low-calorie ketogenic diet ( $n = 18$ ) and volunteers who underwent sleeve gastrectomy bariatric surgery ( $n = 22$ ) showed MedDiet was enriched in several pathways related to SCFA fermentation (179). Following MedDiet intervention in volunteers with overweight, SCFAs were negatively correlated with changes in some inflammatory cytokines, including VEGF, MCP-1, IL-17, IP-10, and IL-12 (90). Meanwhile, a few studies have shown no significant changes (91) or decreases (65) in SCFAs following a MedDiet. Tracking the changes in specific SCFAs may be useful to our understanding as future studies continue.

Due to resource constraints, many intervention studies are limited to a very short time interval with the longest dietary intervention investigating changes in SCFAs described above spanning 3 months. This short time frame sets a significant limitation on the conclusions that can be drawn from the studies in terms of generalizing to a stable effect over years. In a small study of 21 healthy-weight individuals who consumed a MedDiet for 3 days, then a Canadian diet [reflecting the average Canadian dietary intake, which would be considered a Western diet (180)] for 13 days, followed by a MedDiet for an additional 3 days, circulating SCFAs and branched-chain fatty acids were not significantly altered by the first MedDiet intervention, but propionate, valerate, isobutyrate, and isovalerate were increased by the Canadian diet, then decreased after the second MedDiet (65). While the data show that circulating SCFA concentrations can be altered following MedDiet, and changes can occur over a short period of time, one



must be careful about extrapolation of results from a few days to an effect that might occur after years of following a specific dietary pattern. Additional interventional long-term studies are needed to resolve discrepancies between study results and to assess whether these changes remain stable over time.

A systemic meta-analysis of 34 animal studies showed that diets rich in anthocyanin-rich fruits and vegetables significantly reduced the Firmicutes/Bacteroides ratio and increased SCFA production in rodents. They found that higher production of acetic acid, butanoic acid, and propionic acid was observed with longer periods of dietary intervention ( $\geq 4$  weeks) and higher doses of anthocyanins. Anthocyanin supplementation had the greatest effect on acetic acid concentration in high fat/cholesterol diet models, while the greatest effect on butanoic acid and propionic acid were in HFD-induced obesity models (181). These studies provide an initial understanding of the role of specific components of the MedDiet in modulating the gut microbiota and gut-derived SCFAs.

Because humans with obesity have been reported to display excessive levels of fecal SCFAs (potentially due to lack of ability to metabolize and absorb these metabolites), as reviewed in (11, 112, 182), a strategy was proposed to combat obesity by altering the gut microbiota with a goal to modulate the number of SCFA-metabolizing or SCFA-producing bacteria through a dietary change. In a small study of 20 elderly women, obesity was associated with an increase in pro-inflammatory *Collinsella* spp. and *Streptococcus* spp. There was also a decrease in SCFA-producers, including *Lachnospiraceae* and *Ruminococcaceae*. Relative abundance of *Collinsella* spp. was reduced following both a hypocaloric MedDiet for 15 days and a hypocaloric MedDiet enriched with a probiotic mixture for 15 days (and both included an individual-based exercise regimen) (88). These studies make associations between MedDiet, SCFA, inflammation, and obesity.

In a sub-study of the PREDIMED trial in volunteers with overweight/obesity, an energy-restricted MedDiet resulted in weight loss and changes in the gut microbiota after a 1-year intervention. While SCFAs were not measured directly, there was an increase in some SCFA-producing microbes, including *Lachnospira* spp. and *Lachnospiraceae* NK4A136 (57). When selecting subjects with the highest fecal butyrate increase at 4 weeks after MedDiet initiation, higher relative abundances of *Faecalibacterium prausnitzii* and *Lachnospiraceae* family were also observed (91). Likewise, the Obekit study found SCFA-producing bacteria, including *Bifidobacterium animalis*, *Oscillibacter valericigenes*, *Oscillospira (Flavonifractor) plautii*, *Ruminococcus bromii*, *Roseburia faecis*, and *Paraprevotella clara*, in a northern-Spanish population with overweight/obesity and high MedDiet adherence (89). While our discussion is focused on the MedDiet, many studies have combined caloric restriction with the MedDiet, so interpretation of results cannot distinguish the contribution of each of these two variables in many cases. However, it is evident that diets rich in fiber, flavonoids, and polyphenols, such as the MedDiet, are shown to impact the gut microbiota composition, and importantly, increase the number of bacteria with the ability to produce and metabolize SCFAs. The studies summarized in Table 2 report circulating and/or fecal SCFA levels. One cautionary note derives from a study by Farhat et al. that demonstrated poor correlation between serum and fecal SCFAs, concluding that one is not a good

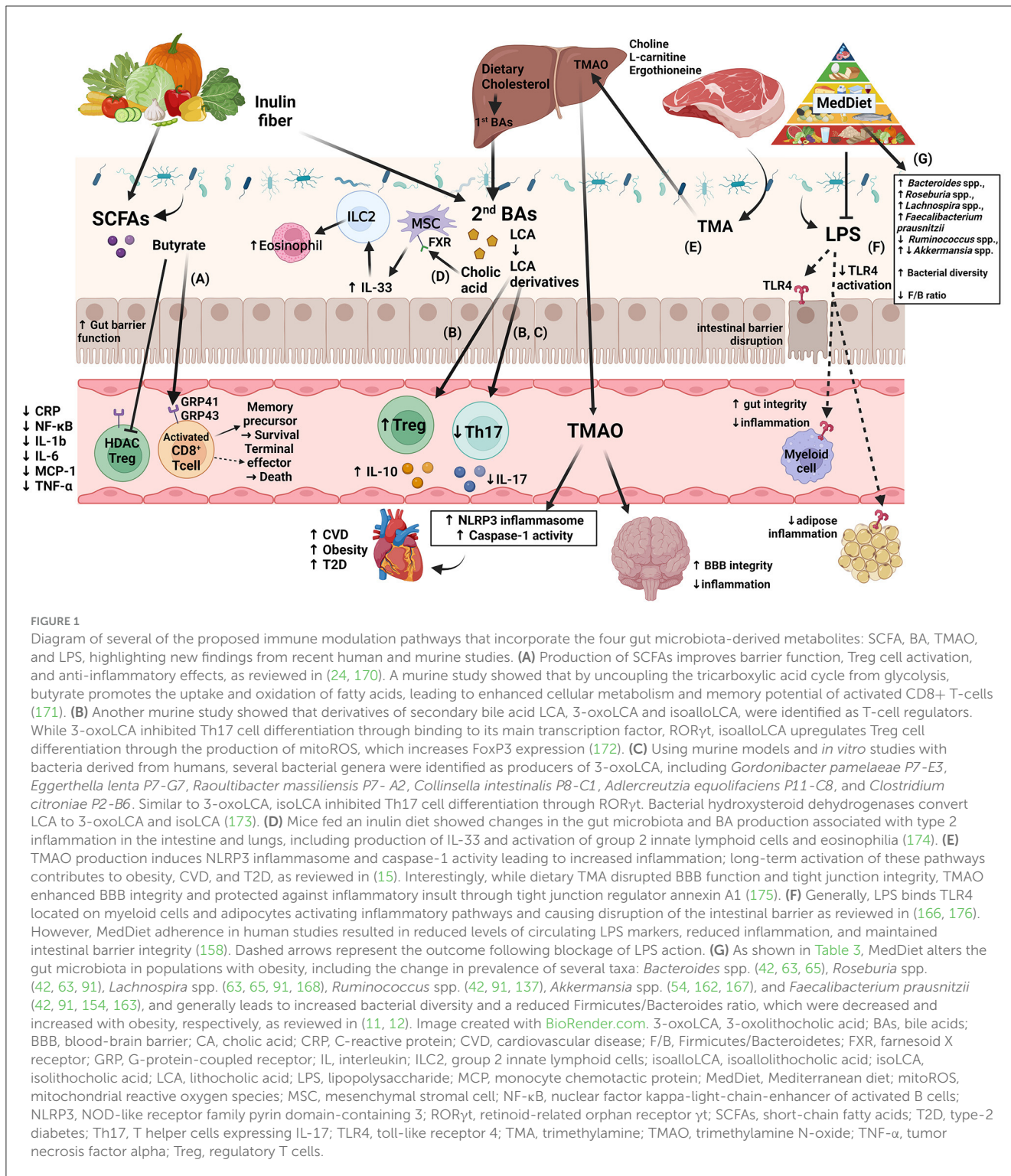
proxy for the other (183). In summary, many studies associate a MedDiet with an increase in SCFAs, and mechanisms are proposed by which SCFAs modulate immunity/inflammation; however, results are not entirely consistent across studies, so work is ongoing.

## 5.2 Bile acids

BAs, which are secreted into the intestine in the presence of fats as part of the digestive process, are generated from dietary lipids, cholesterol, and fat-soluble vitamins in hepatocytes via two main synthetic pathways. Primary BAs are stored in the gallbladder and secreted in the gut after conjugation. Secondary BAs are generated via further interaction of primary BAs with the gut microbiome. Similar to SCFAs, BAs are critically important in gut physiology, and secondary BAs alter the gene expression of enterocytes and of gut bacteria (184). Elevated secondary BAs in serum and feces have been associated with increased inflammation. Secondary BAs act as ligands for G-protein-coupled bile acid receptor 1 and farnesoid X receptor (FXR), the activation of which mediates immunity and promotes anti-inflammatory effects. Under normal conditions, there is a balance between primary and secondary BAs; however, this balance can be disrupted by gut microbiota dysbiosis (185).

While some mechanisms of BA metabolism are known and can be reviewed here (186), others have yet to be explored. Hang et al. showed that derivatives of the secondary BA lithocholic acid (LCA), mediate host immune response by mediating T helper cells expressing IL-17A ( $T_H17$ ) and regulatory T (Treg) cell differentiation. The metabolites 3-oxoLCA and isoLCA were shown to inhibit  $T_H17$  cell differentiation by binding to transcription factor retinoid-related orphan receptor ( $\gamma$ )t (172, 173), while production of mitochondrial reactive oxygen species by isoalloLCA increased expression of FoxP3 and Treg cell differentiation (172) (Figure 1). A diet of inulin fiber altered the composition of mouse microbiota and lead to increased production of BAs, which is presumed to have aided in the production of IL-33 and activation of innate lymphoid cells and eosinophils to promote type 2 inflammation (Figure 1). These affects were BA-dependent because (1) depletion of the BA receptor FXR and (2) genetic deletion of a BA-metabolizing enzyme abrogated these affects (174). HFD-fed mice with obesity had significantly increased taurine-conjugated BAs, but these affects were nearly abrogated in NLRP3-deficiency (81). During the last several years, there have been significant discoveries regarding the role of BAs in immunity and inflammation; it is proposed that manipulation of the gut microbiota, and thus of BA production, may be a useful approach to treatment for obesity.

There is an interdependent relationship between the host biological pathways and bacterial metabolism. The gut microbiome has been shown to impact the chemistry of all organs, including amino acid conjugations of host BAs (186). Conversely, BAs have considerable effects on the structure of the gut microbial community; they can stimulate the growth of microbes that utilize BAs as an energy source and repress the growth of microbes that are intolerant of its effects (184). A recent study suggested that human gut bacteria from many families within



the Actinobacteria and Firmicutes phyla produce 3-oxoLCA, including *Gordonibacter pamelaee* P7-E3, *Eggerthella lenta* P7-G7, *Raoultibacter massiliensis* P7-A2, *Collinsella intestinalis* P8-C1, *Adlercreutzia equolifaciens* P11-C8, and *Clostridium citroniae* P2-B6 and these may work together to affect the immune system (173).

In a fecal microbiota transplant pilot clinical trial in volunteers with obesity, BA profiles were modified to match that of

the lean donor after 12 weeks, including sustained reduction in taurocholic acid, without any change in BMI (104). This trial did not document any change in BMI, however the 12-week time period may not have been long enough to capture significant weight change and future studies with a longer trial period are needed. A secondary analysis from fecal samples collected from these volunteers identified *Bacteroides ovatus* and

*Phocaicola dorei*, which positively correlated with unconjugated BAs, and *Bifidobacterium adolescentis*, *Collinsella aerofaciens*, and *Faecalibacterium prausnitzii*, which positively correlated with secondary BAs, as the bacterial species candidates that affected gut BA metabolism (187). In addition to dietary components, the caloric level of a diet must be considered, as calorie restriction has been shown to decrease production of BAs (188, 189). Supplementation with non-12 $\alpha$ -hydroxylated BAs in mice increased thermogenesis and slowed weight gain (188). It is well-known that BAs impact the gut microbiota and are influenced by dietary changes, however, there are limited studies that have incorporated the measurement of BAs in relation to the MedDiet pattern.

The MedDiet pattern limits the amount and types of dietary fat intake, and therefore has potential to change the amounts and types of BAs produced by the host. In MedDiet intervention studies, lower production of primary and secondary BAs has been observed (42, 91). Fecal secondary BAs were significantly reduced by 4 weeks and primary BAs were reduced by 8 weeks following initiation of a MedDiet. Volunteers with the greatest reduction of total BAs also had higher baseline levels of *Bilophila wadsworthia*, which significantly decreased after 4 weeks (91). Although circulating BA levels remained unchanged after just 4 days of a MedDiet compared to a fast-food diet, the primary to secondary BA ratio was found to positively correlate with *Bifidobacterium* spp. and negatively correlate with *Roseburia* spp. (168).

Dietary diversity has been shown to inversely correlate with several circulating secondary BAs (190), and a MedDiet tends to have more diverse foods than a Western diet (191). A variety of fruits and vegetables eaten on a MedDiet contain flavonoids, which are shown to have anti-inflammatory properties, in part through pathways involving BAs. In murine studies, administration of the hops-derived prenylated flavonoid xanthohumol, and its semi-synthetic derivative tetrahydroxanthohumol, altered the gut microbiota and BA metabolism, and reduced adipose tissue inflammation (192). The MedDiet also promotes intake of whole grains compared to refined grains, as eaten in the typical Western diet. Two secondary BAs were lowest in a diet of unrefined carbohydrates composed from a high proportion of whole grain foods (193). A single fat source alone may not be enough to alter BA production in humans, as consumption of virgin olive oil with or without thyme did not alter BA production when volunteers were asked to limit their polyphenol-rich food intake (96). Compared to Western diet, MedDiet promotes reduced overall fat intake, with primary intake of healthy fats like olive oil, resulting in lowered production of secondary BAs, favoring reduced inflammation and a decreased risk of obesity.

### 5.3 TMAO and its dietary precursors

TMAO is a product of choline, L-carnitine, betaine, and ergothioneine via metabolism by the gut microbiota. Trimethylamine is generated within the intestinal lumen by enzymatic changes of the aforementioned precursors, absorbed from the intestine, and transferred to the liver where flavin-dependent monooxygenase isoforms 1 and 3 convert it to TMAO.

Dietary choline and L-carnitine are primarily found in animal products while betaine is mostly from plants. Dietary ergothioneine is found in both some animal (mostly liver and kidney organs) and plant (including mushrooms and beans) products (83, 194). Krueger et al. describe the negative effects of elevated TMAO on adipose tissue as it relates to the discussion of obesity (108). Increases in TMAO has been found to correlate with an increase in BMI and visceral adipose and TMAO levels over 8.2  $\mu$ M predict the occurrence metabolic syndrome (77). Obese mice that had a TMAO-producing enzyme (FMO3) conferred protection against obesity (109). TMAO also have an inflammatory effect through activation of the NLRP3 inflammasome (195). However, similar to other metabolites, the physiology of TMAO is complex, as TMAO acts on multiple organs, with evidence of beneficial effects in the brain. Long-term exposure to TMAO in mice protected the brain from inflammatory challenge with LPS and reduced activation of astrocytes and microglia (Figure 1) (175). While some studies might paint TMAO as a negative factor and many proposed healthy diets eliminate or strictly limit red meat, the study by Hoyles et al. suggest that the full picture of TMAO's role in obesity and other metabolic syndromes has yet to be understood.

Elimination of red meats, which are known to be a source of dietary choline, is encouraged on the MedDiet. An observational study comparing healthy adults found circulating levels of TMAO negatively correlated with MedDiet score after adjusting for BMI, physical activity, and total energy intake (123). A similar result was seen in a separate study with a population of volunteers including 30% with obesity, as those with a morning chronotype (a term used to describe a person's circadian preferences) had the highest adherence to a MedDiet and the lowest circulating TMAO concentrations (125, 127). Choline participates in multiple essential functions, including serving as a precursor of essential cellular components, and is oxidized to betaine in the methylation cycle of multiple pathways.

A lower betaine/choline (B/C) ratio is associated with features of metabolic syndrome, so the B/C ratio is a biomarker of metabolic function. A case-cohort study within the PREDIMED trial found that volunteers assigned to the MedDiet intervention with a high B/C ratio had a lower risk of CVD compared to controls with a low B/C ratio (136). In contrast, a separate case-cohort of the PREDIMED study, found that individuals with the highest quartile of baseline TMAO and  $\alpha$ -glycerophosphocholine had a lower risk of T2D (196). One-year follow-up data from the Spanish PREDIMED-Plus trial showed the greatest increase in dietary choline or betaine intake was associated with improved serum glucose and HbA1c levels, as well as reduced body weight and total cholesterol in subjects with overweight/obesity (197). A secondary analysis of a randomized clinical trial in adults with overweight/obesity comparing the effects of consuming different concentrations of unprocessed lean red meat, along with a MedDiet, found that lower consumption of red meat resulted in lower serum TMAO concentrations after 5 weeks (141). In individuals with healthy weight, there was a 1.5-fold increase in urinary TMAO after 2 weeks of a MedDiet compared to a Western-type diet (87). However, another study involving adults with overweight/obesity found urinary carnitine was significantly reduced by 4 weeks following MedDiet intervention and remained reduced at 8 weeks (91).



Few studies have examined associations between TMAO, MedDiet, and gut microbiome composition. *Prevotella copri* was significantly lower in female non-human primates fed a MedDiet compared to a Western Diet. Interestingly, among those fed a Western-diet, those who had highest amounts of *P. copri* also had elevated levels of urinary carnitine-based metabolites (18). Another study found no difference in plasma TMAO levels, or its precursors, before and after a 6-month MedDiet intervention in healthy adults at risk of colon cancer. However, the relative abundance of *Akkermansia muciniphila* in colon biopsies was modestly and inversely associated with TMAO, betaine, choline, and carnitine at baseline, and this association was weaker at 6 months following MedDiet introduction (134).

A study involving children and adolescents with obesity found that non-responders (defined as subjects whose BMI z-score was maintained or increased) to nutritional or exercise regimens had significantly increased choline and a decreased B/C ratio after 6 months. Increased choline was associated with *Romboutsia timonensis*, *Granulicatella adiacens*, and *Aminipila butyrlica*, while decreased choline was associated with *Enterocloster aldensis*. *Anaerotignum faecicola* and *Bacteroides stercoris* were associated with a decreased B/C ratio. Volunteers with both increased choline and a decreased B/C ratio had higher abundance of *Romboutsia timonensis*, *Granulicatella adiacens*, and *Pediococcus stilesii* (198). While it is exciting to see specific bacterial species identified as playing a role in TMAO biology, future RCTs that examine the effects of MedDiet on TMAO are required to draw conclusions on this topic.

## 5.4 Lipopolysaccharide

LPS is also called endotoxin and is derived from Gram-negative bacterial membranes. Previous studies have shown trends toward lower endotoxemia in association with Mediterranean-like diets, while Western-style diets are associated with increased endotoxemia (149, 199, 200). A mechanism by which the MedDiet may contribute to improved metabolic health is through the modulation of the gut microbiota which can lead to a reduction of metabolic endotoxemia (184). We are beginning to discover some of the detailed physiology of LPS action. LPS has also been shown to correlate with the incidence of cardiovascular events, potentially through upregulation of proprotein convertase subtilisin/kexin type 9 involved in a mechanism associated with NADPH oxidase (Nox2)-related oxidative stress (150). Yogurt supplementation, with associated probiotic bacteria, attenuated metabolic endotoxemia and inflammation in mice with obesity likely through reduced activation of the TLR4 signaling pathway (201). In mice, HFD significantly increased levels of LPS binding protein (LBP). However, depletion of the NLRP3 inflammasome using knock-out genotyping abrogated the levels of LBP, implicating the NLRP3 inflammasome as a target to mediate obesity-related inflammation (81).

The MedDiet is rich in polyphenols from various foods such as berries, spices, nuts, cocoa, wine, and olive oil, among others. Polyphenol-rich diets have beneficial effects against obesity-related dysbiosis and circulating LPS levels. For example, isoflavones showed reduced production of nitric oxide species and reduced pro-inflammatory cytokine (TNF- $\alpha$  and IL-6) release in response

to LPS (202). In a cross-sectional study of older adults (60% with overweight or obesity) greater adherence to Mediterranean-like diets (MedDiet and prudent diet) were associated with lower circulating 3-hydroxy fatty acids levels, a proxy of LPS burden (121). As part of the Progression of Liver Disease and Cardiovascular Disorders in Non-alcoholic Fatty Liver disease (PLINIO) study, soluble Nox2-derived peptide, a marker of systemic oxidative stress, and serum LPS, were higher in patients with overweight/obesity and correlated with low adherence to MedDiet (41). In the LIBRE study, women with intestinal barrier impairment were allocated to follow MedDiet ( $n=124$ ) or a control diet ( $n=136$ ) for 3 months. Adherence to MedDiet was associated with decreased LBP and gut permeability (Figure 1) (158). While circulating LPS is typically enhanced in obesity, the MedDiet may help lessen these levels and reduce inflammation.

Olive oil is a critical component of the MedDiet and it has been shown to be protective against inflammation. Virgin olive oil phenolic extract was protective against LPS treatment in murine and human brain cells by reducing activation of TLR4 and the NLRP3 signaling cascade (80). Olive oil consumption was also associated with a less significant increase in blood glucose, a more marked increase in blood insulin and GLP1, and a significant reduction in LPS and gut permeability, in individuals with impaired fasting glucose (203). These recent studies summarize the ability of the MedDiet to reduce the risk of obesity by maintaining intestinal barrier integrity and reducing the amount of circulating LPS, thus reducing the associated inflammation.

## 6 Conclusions and future directions

Diet has a major impact on obesity and the composition of the gut microbiota, and in turn, the types of microbial metabolites in the gut. While some studies suggest that diet may be a key component of an effective treatment for obesity and for the restoration of homeostasis (11), a recent systemic review examining the effects of the MedDiet on the gut microbiota and gut metabolites found inconsistent and few significant changes, which may be attributed to differences in methods, cohort characteristics, and study quality (86). For example, scales to evaluate the MedDiet used in the studies we reviewed range from 8- to 24-point scores, thus emphasizing different dietary components (Table 1). The populations evaluated in each study also varied, from healthy adults (65, 77, 128), to older adults (42, 121, 154), to individuals with health conditions such as ulcerative colitis (55, 63) and MASLD (41). While adherence to the MedDiet improves health and has some effect on the gut microbiome, more work needs to be done to determine the extent to which the MedDiet-associated changes in gut microbiota and their metabolites mediate these health-promoting effects. Two other critical elements important in assessing the risk of obesity are an individual's physical activity level and the total calories consumed per day, so examination of diet patterns without controlling for both exercise and caloric intake, and other potential confounders, may contribute to the conflicting results for various studies.

In a recent review, Gundogdu and Nalbantoglu note the mixed results may be due to lack of standardization, study design limitations, and differences in the defined MedDiet (204). Though widely accepted, amplicon sequencing approaches (i.e.,



16S ribosomal RNA gene sequencing) lack the depth to capture most strain-specific microbes and their functionality (43, 204). Additionally, an individual's specific dietary preferences and responses, whether from a baseline diet or self-selected food during an intervention, also affect gut microbiota composition, as reviewed in Fassarella et al. (205), and further complicate adequately controlled studies. Given the number of potentially confounding variables, rigorous studies that incorporate measuring or restricting as many of these variables as possible, in addition to sequencing of the gut microbiota and measuring metabolites, are needed.

Obesity is a growing public health concern, and a better understanding of the pathophysiology related to diet and lifestyle is needed. Harnessing the ability to systematically change the gut microbiota, and correspondingly change the microbial metabolites, is a therapeutic target for investigators and clinicians. However, larger and more rigorous clinical trials are needed, in addition to animal studies that can decode the mechanisms, to define the pathophysiology of obesity. This will be challenging given the number of interacting parts between the gut microbiota, metabolites, and host immunity. While unsettling to consider the huge task of dissecting the complex biology of dietary nutrients, inflammation, and health consequences, tremendous progress has been made in the past few decades. The heterogeneity between studies is not surprising given the number of potential confounding variables; nevertheless, there is clear evidence to support the benefits of a Mediterranean-like dietary pattern as a means to alter the gut microbiota, gut metabolites, and essential biological pathways within populations with overweight/obesity.

## Author contributions

MF: Writing – original draft. EA: Writing – original draft. KF: Writing – review & editing, Conceptualization. AB: Writing – review & editing, Writing – original draft, Conceptualization.

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## Conflict of interest

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