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Diet-Mediated Heart Disease Risk via the Gut Microbiome: A Systematic Review of Human Intervention Studies

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Diet-Mediated Heart Disease Risk via the Gut Microbiome: A Systematic Review of Human Intervention Studies

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Table of Contents

ABSTRACT

Background: Recent advances in microbiome research suggest the gut microbiome may play a role in atherosclerosis and the development of coronary artery disease (CAD). Though the American Heart Association (AHA) has dietary guidelines for preventing and managing CAD, the role of the gut microbiome is not considered.

Objective: The aim of this systematic review was to examine existing literature on the role of probiotics, prebiotics, and processed foods on markers of atherosclerosis via the gut microbiome.

Methods: A search was conducted for human intervention studies using PubMed, CINAHL, and Embase. Twenty-two articles met eligibility and were included in this review.

Results: Probiotic ingestion tended to decrease low-density lipoprotein and increase fecal short chain fatty acids (SCFAs), especially butyrate. Prebiotic ingestion had little effect on inflammatory markers, and effects on SCFAs and blood lipids were variable. The best outcomes were seen with almonds, walnuts, whole apples, resistant starch, and multi-fiber breads. Processed foods tended to exhibit pro-inflammatory, pro-cholesterol, and anti-SCFAs effects, especially when fiber was artificially removed. While none of the included studies were powered for microbiome significance, trends between bacterial species and metabolic markers were seen throughout.

Conclusion: Findings suggest that probiotic, prebiotic, and processed foods may play an important role in atherosclerosis and the development of CAD via the gut microbiome. Additional research is needed to further investigate the gut microbiome and to inform dietary guidelines. Nurses can educate patients on the risks of processed and artificial foods and the benefits of unprocessed and probiotic foods.

Keywords: gut microbiome, coronary artery disease, short chain fatty acids, processed

SECTION 1: HONORS THESIS

Introduction

A surge in the prevalence of the Standard American Diet has correlated with increasing heart disease and metabolic disorders across nations. Not only is heart disease the leading cause of mortality in the United Staes (U.S.) – globally it accounts for about one third of all deaths. The gut microbiome, though not fully understood, has been shown to affect multiple body systems and produce metabolites that cause systemic inflammation and atherosclerosis. With better understanding of how certain foods affect the microbiota, we can better target, educate, and prevent the development of heart disease in the U.S. We aim to systematically review the literature concerning probiotics, prebiotics, and processed foods on markers of atherosclerosis via the gut microbiome.

Dissemination of Scholarly Work

- September 2023, Summer Community of Scholars Poster Presentation
    - First Place Dean's and Alabama Space Grant Award
- Manuscript is slated for a December 2024 submission to *Journal of Cardiovascular Nursing*

SECTION II: MANUSCRIPT

Professional Journal Selection

The *Journal of Cardiovascular Nursing* was chosen for first submission but has not yet been accepted for publication.

Scope of Journal

Official journal of the Preventive Cardiovascular Nurses Association, *Journal of Cardiovascular Nursing* is one of the leading journals for advanced practice nurses in cardiovascular care, providing thorough coverage of timely topics and information that is extremely practical for daily, on-the-job use. Each issue addresses the physiologic, psychologic, and social needs of cardiovascular patients and their families in a variety of environments. Regular columns include By the Bedside, Progress in Prevention, Pharmacology, Dysrhythmias, and Outcomes Research.

Aims of Journal

The mission of the Preventive Cardiovascular Nurses Association (PCNA) is to develop and promote nurses as leaders in the prevention of cardiovascular disease.

INTRODUCTION

A surge in global heart disease has paralleled the expansion of the Standard American diet and lifestyle, giving rise to an epidemic (Gaziano et al., 2010; Ikem & Sumpio, 2011). While largely preventable, atherosclerotic heart disease - especially coronary artery disease (CAD) – is persistently prevalent in the United States (U.S.), necessitating a change in education or public narrative. In 2000, just one percent of the American population was estimated to have ideal cardiovascular health, as measured by the American Heart Association's (AHA) "Life's Simple 7" (Ford et al., 2012). In a recent meta-analysis, those who met at least five of these seven heart-health criteria had an 82% risk reduction in myocardial infarction, compared to those meeting two or fewer criteria, and a 62% risk reduction, compared to those meeting three or four (Radovanovic et al., 2023). Out of all seven metrics used in this tool, the three that were significantly predictive of mortality were closely related to food (i.e., blood pressure, dietary score, and hemoglobin A1C) (Ford et al., 2012).

With research on the human gut microbiome rapidly expanding, the relationship between host immunity and nutrition is becoming more apparent. As a "supraorganism," (Glendinning & Free, 2014) humans are host to a rich microbial environment whose metabolites affect many functions, seen and unseen. With the development of technologies that sterilize and process foods economically, the contemporary American's exposure to microbes is necessarily lower than their predecessors (Pfefferle et al., 2021). Though this has allowed an unprecedented increase in standard of living and general abundance, there is evidence that this new era of cleanliness is detrimental to health and contributes to the development of autoimmune diseases (e.g., type 1 diabetes) (Chapman et al., 2012; Okada et al., 2010). Antibiotic usage is increasing and has paralleled the development of diseases like type 2 diabetes, irritable bowel disease, and

celiac (Fenneman et al., 2023). Though diet modification can improve the gut microbiome in many cases, the efficacy of its use as a primary prevention tool has yet to be examined (Arnett et al., 2019).

In the present review, we look at three categories of food (probiotics, prebiotics, and processed foods) to explore how diet may affect heart disease risk factors through the gut microbiome. First, probiotics refer to any form of live microorganism that lives in the gastrointestinal tract in a beneficial, symbiotic relationship with its host (Oniszczuk et al., 2021). Their metabolites affect cardiac health via immune and metabolic processes. Metabolites of gut microbiota help mediate the inflammatory cascade necessary for both the formation of atherosclerotic plaques and their rupture (Gui et al., 2012; Hansson, 2005). Second, prebiotics (typically high-fiber foods) are considered any ingredient fermentable by host microbiota that results in short chain fatty acid by-products and generally improves health (Davani-Davari et al., 2019; Schoeler & Caesar, 2019). The ingestion of these are necessary for overall health and immunity and have been implicated in atherosclerosis via inflammatory axes (Nicholson et al., 2012). Third, processed foods use many ingredients including food additives that improve palatability, processed raw materials (hydrogenated fats, modified starches, etc.) and ingredients that are rarely used in home cooking. Processed foods are mainly of industrial origin and can be stored for a long time (Monteiro et al., 2018). Processed foods tend to be pro-inflammatory and may contribute to negative cardiovascular outcomes (Kong et al., 2014; Z. Zhang et al., 2021)..

The American Heart Association (AHA) dietary guidelines recommend maximizing consumption of fruits, vegetables, oils, and whole grains, while minimizing fats, processed food, added sugar, and salt (Lichtenstein et al., 2021). While these recommendations are evidence-based, there is little consideration of the gut microbiome. In this systematic review, we examined

the existing literature on dietary interventions to examine the effects of probiotic, prebiotic, and processed foods on biomarkers of atherosclerosis and associated gut microbiome shifts.

METHODS

Literature Search

We constructed tailored searches in PubMed, CINAHL, and Embase in June 2023, using key terms associated with three main food groups (probiotics, prebiotics, and processed foods) for the purpose of AHA comparison. We chose terms relating to food, microbiome, and cardiovascular outcomes or risk factors. Table 1 details the search terms that were used. To ensure representative host microbiomes and most up-to-date sequencing methods were used, studies were limited to human interventional research published in the past ten years (2013-2023).

Study Selection / Eligibility Criteria

Screening was conducted in three stages – title, abstract, and full-text examination. Articles progressed past the first two stages if they met all of the following criteria: 1) randomized controlled or clinical trial, 2) healthy adult subjects aged 18-65 years free of clinical disease (e.g., CVD, obesity, diabetes mellitus, non-alcoholic fatty liver disease, food intolerances, autoimmune disorders, irritable bowel disease, cancer), 3) participants' diets were altered via one food or food group, 3) fecal samples were collected and analyzed, and 4) at least one cardiovascular risk factor was measured (e.g., short chain fatty acids [SCFAs], bile acids [BAs], inflammatory markers [CRP, cytokines, IL-6], cholesterol levels, and lipopolysaccharides). Cardiovascular risk factors were chosen using the AHA Risk-Enhancing Factors (Arnett et al., 2019) and current understanding of the microbiome (Tang et al., 2017; Trøseid et al., 2020).

Exclusion criteria consisted of: 1) Multiple foods or food groups included in diet alteration (e.g. Mediterranean diet), in vitro studies (e.g. the dynamic colon model), 2) full data collected on fewer than 15 subjects, 3) Mean participant age >60 years, 4) lack of washout period in crossover studies, 5) intervention period less than one week, 6) pregnant population, 7) antibiotic use, 8) non-systemic oxidative effects measured, 9) concomitant exercise or weight loss interventions, 10) insomnia-driven or chemotherapy-induced dysbiosis, 11) gastric-bypass surgical history, 12) fecal water examination, 13) supplements or ingredients with no synonymous foods in the Western diet (e.g. omega-3 polyunsaturated fatty acids, probiotic pills, extracts without their fibrous origins), 14) foods outside the Western diet (e.g. crickets, konjaka flour, olive pomace, probiotic-infused meat), and 15) non-Western diet populations (e.g. Asia).

Data Extraction

Articles were selected based on quality, relevance, and eligibility criteria. Eligible articles were compiled using a citation manager, and data were extracted/recorded by the authors. An evidence table was developed (Table 2).

RESULTS

Study Characteristics

An overview of article selection is represented via PRISMA flow chart (Figure 1). The initial search yielded a total of 605 articles from all databases. After 42 duplicate articles were removed, 563 titles and abstracts were examined. 314 articles were excluded at the title level and 161 at the abstract level based on exclusion criteria. 88 articles made it to the full-text review where 66 were excluded due to the possible confounding nature of comorbidities (e.g. obesity, diabetes mellitus, postmenopausal women), lack of application to a Standard American diet (e.g.

chitin-glucan supplements), insufficient subjects completing interventions, and inclusion of non-Western populations. Twenty-two articles were included in the final review (Table 2).

Sample sizes ranged from 15 to 81 and consisted of crossover and parallel randomized controlled trials. All studies analyzed fecal samples for microbiome sequencing and/or microbiota metabolite analysis. When SCFAs or BAs were measured, gas or liquid chromatography-mass spectrometry was performed. Microbiomes were sequenced via 16S ribosomal ribonucleic acid (rRNA), shotgun whole genome metagenomic mapping, or a combination of the two. Though 16S rRNA is used in the majority of studies due to cost, shotgun sequencing is considered the gold-standard, as it analyzes host and microbial deoxyribonucleic acid (DNA) to the species level (Wensel et al., n.d.). Though both methods have weaknesses, 16S rRNA is generally less sensitive and reliable since it only samples DNA coding for the 16S ribosomal subunit. Of the included studies, only four included shotgun sequencing (Table 2).

Probiotics

AHA guidelines recognize that microbiome research on probiotics is limited and do not make recommendations based on the current literature (Lichtenstein et al., 2021).

Cardiometabolic Markers

In crossover studies, effects of SCFAs depended on food or microbe ingested. *Bifidobacterium lactis* BB-12 supplementation yielded no significant change from baseline in fecal SCFAs (Lee, Yujin et al., 2017), while cultured cheese exhibited increases in butyrate and propionate compared to nonfermented dairy counterparts (Zheng et al., 2015). Isolated probiotics, like *Bifidobacterium lactis* BB-12, are increasingly being added to foods such as yogurts and supplements. Though no form of the probiotic significantly altered blood lipids or glucose response, acetate was significantly increased from baseline in the control ($p=0.007$) and

probiotic smoothie where bacteria was added pre-fermentation (p=0.009). The groups consuming a capsule form of probiotic or a smoothie with bacteria added post-fermentation did not share these SCFAs effects. This occurred despite consistent dietary intakes across treatment periods. This difference may have been due to manufacturing and/or fermentation process (Lee, Yujin et al., 2017).

When consuming dairy products, there is some evidence that suggests fermented dairy consumption has more positive effects than pasteurized control (Zheng et al., 2015). Though both pasteurized milk and cheese with live cultures increased fecal lipid excretion due to calcium, the cheese intervention had higher levels of SCFAs butyrate, propionate, and malonate while exhibiting lower acetate and glycerol levels. Butyrate, especially, was increased compared to milk (p=0.06) and baseline (p=0.06) (Zheng et al., 2015). Butyrate, propionate, and fecal lipid levels negatively correlated with total and LDL cholesterol.

Evidence that probiotic *Lactobacillus reuteri* affects BAs was inconclusive. Though this microbe hydrolyzes bile salts to form secondary BAs, no significant change was seen to fecal BAs, blood lipids, fasting glucose, or inflammatory markers after the intervention period (Pushpass et al., 2023). In the short term, though, supplementing a low-fiber cornflakes breakfast (1.2 g fiber) with *L. reuteri* resulted in significantly greater levels of unconjugated ($p<0.002$) and secondary BAs concentrations (p=0.016) within the six hours measured post-prandial compared to control.

Synergism

Comparing synbiotics – a combination of probiotic and prebiotic – to the same probiotic given with maltodextrin placebo, a synergistic relationship is not always seen. Though additive effects were apparent when ingesting *Bifidobacterium animalis* subsp. *Lactis* Bi-07 with xylo-

oligosaccharide (8 g/d), the synbiotic did not result in significantly higher counts of *B. lactis* in feces (Childs et al., 2014). The synbiotic did, however, significantly alter the effect on SCFAs and immune mediators compared to placebos ($p<0.05$). When the prebiotic or probiotic was given individually, acetic and butyric acid decreased from baseline which was not the case with synbiotic treatment (Childs et al., 2014). Participants also reported a significantly lower use of analgesics during synbiotic supplementation versus maltodextrin placebo, though self-reports indicated no symptom differences across groups (Childs et al., 2014).

Prebiotics

AHA dietary guidelines suggest that whole grains benefit gastrointestinal symptoms, but do not recommend prebiotic consumption based on microbiome effects (Lichtenstein et al., 2021).

Inflammatory Markers

Prebiotic intervention compared to controls often resulted in insignificant changes to inflammatory markers, including C-reactive protein (CRP) and Interleukin 6 (IL-6) (Holscher et al., 2018; Pushpass et al., 2023; Ranaivo et al., 2022). Comparing whole grain intervention to refined grain, no significant change was seen in lipopolysaccharide binding protein (LBP), stool cytokines, lymphocytes, cytokines, or index of inflammation, though percentage of total terminal effector memory T cells ($p=0.03$) and LPS-stimulated tumor necrosis factor alpha (TNF-a) ($p=0.04$) were significantly higher with whole grain ingestion and correlated to plasma alkylresorcinols (Vanegas et al., 2017). Yet, these effects were due mostly to the decreases from baseline in refined grain groups (Vanegas et al., 2017).

Yet, some studies found prebiotcs to decrease inflammatory markers. After adjusting for decreased body fat during intervention, one study found that whole grain intake significantly

reduced CRP (p=0.004) and IL-6 (p=0.047) compared to baseline and decreased CRP compared to refined grain (p=0.003) (Roager et al., 2019). Another study found whole grains tended to decrease CRP, though similar effects were seen in the low whole grain, high red meat group (Foerster et al., 2014). Apolipoprotein B was significantly reduced from low-fiber cereal control with ingestion of apples for eight weeks (p=0.037).

Prebiotic effects on glycemic response varied by food. Within six hours post prandial, apples versus control resulted in a 19% reduction in maximum concentration of post prandial glucose, and oats and apples reduced insulin concentrations (Pushpass et al., 2023). After eight weeks of intervention, though, fasting glucose and insulin levels were insignificant (Pushpass et al., 2023). Conversely, multifiber bread was shown to significantly decrease insulin levels after eight weeks of ingestion (p=0.049) while having no significant effect on post prandial glycemic response to test meal (Ranaivo et al., 2022).

Fecal Markers and Blood Lipids

Increasing prebiotic intake did not significantly alter total SCFAs levels in the majority of studies (Ampatzoglou et al., 2015; Childs et al., 2014; Creedon et al., 2022; Foerster et al., 2014; Granado-Serrano et al., 2022; Healey et al., 2018; Hess et al., 2018; Hughes et al., 2021; Martínez et al., 2013). In a parallel trial, the whole grain group exhibited increased total SCFAs compared to refined grain (p=0.05), especially acetate (p=0.02), but these effects were in part due to decreases from baseline in the refined grain group (Vanegas et al., 2017). In a similar crossover study, soluble corn fiber had a significant increase in total number of SCFAs compared to resistant starch (p=0.005) and control (p=0.007) (Klosterbuer et al., 2013).

When considering SCFAs, individually, though, different prebiotic types had targeted effects. Common mushrooms, like *Agaricus bisporus*, are a source of low-digestible and non-

digestible carbohydrates and resistant starch, beta-glucans, and mannitol. Only isovalerate was significantly increased with *Agaricus bisporus* mushroom treatment compared to protein-matched red meat group ($p=0.02$) (Hess et al., 2018). Butyrate was heavily affected by resistant starch type 3 (cooked and cooled starchy foods), with and without pullulan, whereas resistant starch with pullulan exhibited the most significant increase in percent butyrate ($p<0.05$) compared to fiber-matched groups of soluble maize fiber (with or without pullulan) and control (Klosterbuer et al., 2013). Almond intervention also increased butyrate production compared to low-fiber control muffins ($p=0.046$) (Creedon et al., 2022).

BAs were not studied in the majority of articles, and results were variable. In walnut intervention, primary BAs were not affected, but secondary BAs deoxycholic acid and lithocholic acid decreased by 25% and 45%, respectively, versus isocaloric control ($p<0.01$). In a study with a healthy population, though, no changes were seen in fasting plasma or fecal BAs versus control, nor did they correlate with inflammatory markers (Pushpass et al., 2023).

Compared to low-fiber controls, prebiotic intervention often resulted in no significant change to any blood lipids (Foerster et al., 2014; Granado-Serrano et al., 2022; Pushpass et al., 2023; Vanegas et al., 2017). Even with elevated body mass index (BMI), lipid effects often did not reach significance (Ampatzoglou et al., 2015; Martínez et al., 2013). In healthy subjects given up to 13.7 g/d of supplemental fiber from whole grain, low-density lipoprotein (LDL) was decreased compared to control refined grain (up to 4.2 g/d supplemented) but did not reach significance when controlling for amount of test foods consumed (Cooper et al., 2017). Though apple intervention showed no significant effects on lipids when compared to cornflake control, whole apple supplementation tended to decrease total cholesterol compared to control diet ($p=0.066$) and cloudy apple juice ($p=0.064$) (Ravn-Haren et al., 2013).

Some prebiotic studies show clearer effects on lipids. In walnut intervention versus isocaloric control, both total cholesterol ($p=0.03$) and LDL ($p<0.01$) significantly decreased (Holscher et al., 2018). Interventions with baked goods found similar results. Multifiber bread reduced total and LDL cholesterol versus sourdough control ($p<0.05$) (Ranaivo et al., 2022), while insoluble fiber cookies tended to exhibit higher LDL and TGs compared to soluble and antioxidant fiber cookies (Granado-Serrano et al., 2022).

Population Effects

Studies concerned with fiber exhibited large interindividual variability and population effects in gut microbiome response. The most significant results came from studies with populations at high risk of developing cardiovascular disease. At baseline and after interventions, body fat was found to significantly affect IL-6 ($p<0.01$), (LBP) ($p<0.0001$), and high-sensitivity C-reactive protein (hs-CRP) ($p<0.0001$) (Martínez et al., 2013).

No changes were seen to inflammatory markers or lipid profiles after any treatment in the normal weight group, while significant decreases were seen in IL-6 and glycemic levels in the overweight population ($p<0.05$) (Martínez et al., 2013). Similarly, subjects with habitually low dietary fiber intake were more resistant to changes in their microbiome with the introduction of soluble fiber when compared to subjects with habitually high intake (Healey et al., 2018). No differences were seen, though, in SCFAs when comparing low-habitual and high-habitual fiber consumers (Healey et al., 2018).

Bacterial Shifts

Though no study had a population size large enough to claim significance, there were distinct trends in the literature. The most predictive species of SCFAs were *Victivallaceae*,

Butyricicoccus, and *Roseburia* where *Victivallaceae* and *Butyricicoccus* exhibited negative correlations to butyrate and *Roseburia* exhibited positive (Hughes et al., 2021).

After three barley and rice interventions, the genus *Blautia* was increased from baseline, while *Eubacterium rectale, Roseburia faecis, Roseburia intestinalis* increased only after whole grain barley intervention (Martínez et al., 2013). Whole grain also tended to decrease the *Enterobacteriaceae* family and increase the *Lachnospira* genus, while *Lachnospira* and *Roseburia* positively correlated with both acetate and butyrate (Vanegas et al., 2017). Multifiber bread compared to baseline bread resulted in decreased *Bacteroides vulgatus* and increased *Parabacteroides distasonis* and *Fusicatenibacter saccharivorans*, and on average bacterial evenness increased without effect to richness (Ranaivo et al., 2022). Resistant starch type II (high-amylose maize starch, raw potato, raw banana starch) enriched wheat in normal to overweight subjects tended to increase *Ruminococcus* and *Gemmiger* genera compared to wild type wheat control, and both the resistant starch and wild type wheat increased *Bifidobacterium, Faecalibacterium,* and *Roseburia* from baseline (Hughes et al., 2021). High whole grain, low red meat diet resulted in an increase in the abundance of *Collinsella aerofaciens* (Foerster et al., 2014).

Whole foods saw similar results. Walnuts increased the abundance of *Firmicutes* and decreased *Actinobacteria* (Holscher et al., 2018). Genera that were increased from control were *Faecalibacterium, Clostridium, Roseburia,*and *Dialister,* while those that were decreased were *Ruminococcus, Dorea, Oscillospira,* and *Bifidobacterium*. Both almonds and walnuts decreased *Bifidobacterium* compared to their low-fiber counterparts (Creedon et al., 2022; Holscher et al., 2018). Genera *Bacteroides, Parabacteroides, Coprococcus, Sutterella*, and *Anaerostipes* were higher in the mushroom diet than meat (Hess et al., 2018).

Bacterial shifts were dependent on the population. Overweight subjects exhibited lower abundances of *Ruminococcaceae* and *Faecalibacterium* which negatively correlated with hs-CRP. *Bacteroidetes, Bacteroidaceae,* and *Bacteroides* in normoweight and overweight groups positively correlated with plasma high-density lipoprotein (HDL), and in both groups *Bacteroidetes* decreased while *Firmicutes* increased (Martínez et al., 2013). After inulin intake, the population of low habitual consumers of fiber saw an increase in proportion of *Bifidobacterium*, while high habitual consumers saw an increase in *Bifidobacterium* and *Faecalibacterium* and a decrease in *Coprococcus*, *Dorea*, and *Ruminococcus* (Healey et al., 2018).

In one study, hypercholesterolemic participants were categorized as responders (n=24) or non-responders (n=23) to fiber based on total change in cholesterol at a threshold of 2.3 mg/dL (Granado-Serrano et al., 2022). Responders had a reduction in *Flavonifractor* while the non-responders had reductions in *Anaerostipes*, *Clostridium XVIa*, *Ruminococcus*, *Butyricoccus*, *Parabacteroides*, and *Odoribacter*. Positive correlations were seen between *Flavonifractor* and total cholesterol and cholesterol in the LDL and very low density lipoprotein particles. Negative correlations were found between *Ruminococcus* and cholesterol levels in HDL particles. Negative correlations were found between *Clostridium* XIVa and the levels of triglycerides (TG) in LDL particles and the size of LDL particles. *Parabacteroides* correlated with the observed difference in responders and non-responders to dietary fiber and the change in SCFAs. Non-responders had a decrease in the *Firmicutes*/*Bacteroidetes* ratio after two months, while responders' ratio remained stable. Both saw an increase in the proportion of *Bifidobacterium* genus (Granado-Serrano et al., 2022).

Processed Foods

AHA guidelines recognize that microbiome research on processed foods is limited and do not make recommendations based on the current literature (Lichtenstein et al., 2021).

Cardiometabolic Markers

Food additives appear to affect microbial metabolites differently. Non-nutritive sweeteners are NOVA Group IV ultra-processed ingredients (Monteiro et al., 2018). After two weeks of aspartame and sucralose ingestion at dosages approximating three cans of diet soda per day, no significant change in SCFAs was observed (Ahmad et al., 2020). Yet, when carboxymethylcellulose, an ultra-processed ingredient and emulsifier, was ingested, fecal analysis revealed depleted SCFAs beginning at day three and remaining throughout the treatment period (Chassaing et al., 2022).

One form of processing involves removing inherent fibers or nutritional content from whole foods. Consumption of clear apple juice – a processed ingredient – increased LDL($p=0.0006$ and $p=0.0074$) and total cholesterol (0.005 and 0.004) compared to whole apples and cloudy juice but not control ($p=0.113$ and $p=0.227$) (Ravn-Haren et al., 2013). Ferric reducing ability of plasma was increased compared to control with whole apples ($p=0.020$) and cloudy juice ($p=0.076$), while pomace ($p=0.228$) and clear juice ($p=0.115$) decreased this antioxidant level. Trolox equivalent antioxidant capacity showed no significant changes between groups, but clear and cloudy juice resulted in higher oxygen radical absorbance capacity than whole apples ($p=0.011$ and $p=0.004$, respectively) (Ravn-Haren et al., 2013). Glutathione peroxidase type 1, another cellular antioxidant (Lubos et al., 2011), decreased in clear ($p=0.023$) and cloudy ($p=0.046$) juice groups compared to whole apple.

Refined grains are a Group III processed food commonly studied in humans. Compared to isocaloric doses of whole-grain products, refined-grain product intervention in healthy

subjects results in significantly increased CRP (p=0.003), IL-6 (p=0.009), and Interleukin-1 beta (IL-1β) (p=0.008) even when adjusted for weight loss in the whole grain groups (p=0.004, p=0.047 and p=0.009, respectively) (Roager et al., 2019). One study reported that refined grains significantly decreases TNF-a compared to whole grain which they attribute to addition of B-vitamins and fortification of the refined products (-2404 vs. -273 pg/mL, p<0.04) (Vanegas et al., 2017).

Bacterial Shifts

Shifts in microbiome depended on the type of processed food. After two weeks of aspartame and sucralose ingestion, few differences were seen in bacterial proportions (Ahmad et al., 2020). Carboxymethylcellulose tended to decrease the evenness of bacteria in both 16S and shotgun sequencing without affecting overall stool weight or bacterial density. Sequence variants especially decreased were *Faecalibacterium prausnitzii* and *Ruminococcus* sp., while those especially increased were *Roseburia* sp. and *Lachnospiraceae* (Chassaing et al., 2022). In a refined grain study, a majority of subjects consuming half or more of whole grain products had increased abundances of *Akkermansia* and *Lactobacillus*, while high consumers of the refined grain products exhibited opposite shifts with a unique increase in *Erysipelotrichales* (Cooper et al., 2017).

Interindividual Variation

Many studies had a high level of variation between subject microbiomes (Ravn-Haren et al., 2013). A treatment-gender interaction was observed for women in the case of heightened LDL in response to low-fiber juices (n=14, p=0.026), while men showed less significant but similar tendencies (Ravn-Haren et al., 2013). Comparing responses between healthy and pre-diabetic subjects, introducing a processed prebiotic, fructo-oligosaccharide, to a high polyphenol

smoothie resulted in significantly higher LDL levels compared to the control smoothie in prediabetic subjects (2.77 mmol/L vs. 2.97 mmol/L, p=0.0027), while healthy subjects exhibited an insignificant and opposite shift (2.59 mmol/L vs. 2.56mmol/L, p=0.8766) (X. Zhang et al., 2022). Differences in microbiome shifts between these subpopulations were also evident in this study.

Ingestion of an emulsifier resulted in disproportionate microbiome response in two individuals. This observation did not reach significance (p=0.175) likely due to sample size, but the bacterial changes relative to the other subjects did (p=0.0002) (Chassaing et al., 2022). Both were male and older than the average with no difference in anthropomorphic measures. These subjects exhibited significantly reduced bacterial-epithelial distance as measured by comparison of distal colonic biopsies before and after treatment. Their bacterial response increased in the same direction as the other participants but with a heightened intensity (p=0.004) (Chassaing et al., 2022). Though their inflammatory markers were roughly the same as other participants, their fecal lipopolysaccharide levels were significantly increased (p=0.005).

DISCUSSION

Twenty-two randomized controlled trials using human diet interventions were systematically reviewed for dietary effects on atherosclerotic risk factors and changes to the gut microbiome. Largely positive effects were observed on cardiometabolic markers with diets low in processed foods and high in probiotics and prebiotics in healthy or subclinical subjects. These relationships are thought to be mediated at least in part by the gut microbiome, but due to the nature of human interventional studies, sample sizes were not powered to detect significant results at the metagenomic level. Therefore, we can only accept the microbiome results as correlative and are not likely to be indicative of variations at the population level. Here we used

SCFAs as an important indicator of microbiome shift, as low abundances of SCFAs have been linked to atherosclerotic heart disease via *Ruminococcaceae, Roseburia, Faecalibacterium* spp., and other SCFAs-producers (Verhaar et al., 2020).

Fiber Versus Processing in the Western Diet: Who's to Blame?

The greatest anti-inflammatory effects were seen with foods that increased SCFAs, suggesting these sources may be important mediators in the gut microbiome and systemic inflammation. Butyrate is preferred by colonocytes and provides the majority of energy required to maintain homeostasis (Zeng et al., 2019). Similar to the effects seen with almonds, cultured cheese, and resistant starch type II intervention, previous research has established protective effects of these molecules against systemic inflammation and cancer in humans (Koh et al., 2016) and cardiovascular events in hypertensive mice (Marques et al., 2017).

These findings are also in keeping with the AHA's statement that the highest fermentable starches may have the greatest metabolic benefit (like resistant starch type II included in this review) (Lichtenstein et al., 2021). Foods that were highly processed, low in nonfermentable fiber, or both tended to increase atherosclerosis risk factors, though fibrous foods failed to reliably affect SCFAs. Effects of dietary fibers were especially apparent with crossover comparison to refined grains. Significant differences between groups were often attributed to worsening markers in the refined grain treatment group, suggesting processed foods may have a greater negative effect than whole grains do positive. Yet, refined grains have shown no significant effects on cardiovascular risk in prospective cohort studies (Gaesser, 2022).

Processed foods tended to exhibit pro-inflammatory, pro-cholesterol, and anti-SCFAs effects, which were especially apparent when fiber was artificially removed, like in refined grains or fruit juices. Even low amounts of sugar-sweetened beverages result in increased

morbidity and mortality related to cardiovascular and chronic inflammatory diseases (Ma et al., 2022; Sun et al., 2023). High fructose corn syrup, for example, is both devoid of fiber, ultra-processed, and plentifully found in typical American food products. Since positive effects of prebiotic interventions were often due to the presence of refined and processed ingredients, it appears that the processed nature of foods may have a greater effect than the strict prebiotic content.

Cardiovascular health decreases significantly with ultra-processed food consumption, and the effects of ingredients on SCFAs suggest a microbiome link (Z. Zhang et al., 2021). Therefore, it may be beneficial to target education toward modification of quality of food products. Though public health campaigns like *MyPlate* (https://www.myplate.gov/) suggest proportions of food groups, the dangers of consuming nutrient-devoid, industrially-produced ingredients are not often communicated to the public (U.S. Department of Agriculture, 2020).

Interindividual Variability

Interindividual variability in microbiome response to food appears to be partially dependent on subject comorbidities. This is in keeping with previous literature and suggests diet modification has the capacity to improve cardiovascular risk factors in at-risk populations. Vascular health and inflammatory markers have been shown to improve significantly in patients with cardiac-related dysbiosis (Garrett et al., 2010; Jin et al., 2019; Marques et al., 2017). Immune pathways are heavily implicated in these effects, as BMI correlates closely to inflammatory intestinal macrophages in the gastrointestinal tract (Rohm et al., 2021). This will also lead to increased gut permeability and endotoxemia which predisposes arterial plaque. Pathogenic bacteria have been identified as a common denominator in cultures of atherosclerotic plaque, and microbiota are heavily implicated in this discovery (Ziganshina et al., 2016).

The ability of the microbiome to adapt and shift is unique to the individual, though it is suggested that sterility of the gut and antibiotic usage puts individuals at high risk of low adaptability. Antibiotic use, for instance, is increasing throughout the world and has paralleled the development of diseases like type 2 diabetes, irritable bowel disease, and celiac (Fenneman et al., 2023).

Potential Targets for Probiotic Prevention

Bifidobacterium has been linked to many metabolic and bowel diseases when low in abundance and has a major protective effect due to its role in antioxidant and bacteriocins production and tended to increase with improvements in cardiovascular risk factors (Rivière et al., 2016). As was previously demonstrated, dietary fiber was reported in a majority of studies to increase *Bifidobacterium* abundance (So et al., 2018).

Though not many studies on probiotics met our eligibility criteria, the results largely support the potential benefits of fermented food on the gut microbiome and cardiovascular health. Similar to the effects seen with fermented dairy, fermented red ginseng intervention in a female Korean population was shown to decrease fasting glucose, LDL, and total cholesterol and shift the microbiome in major metabolic pathways (Lee et al., 2022). Comparable foods often have antioxidant and anti-inflammatory effects associated with decreased pathogenic bacteria, as in fermented brown rice drinks, fermented plant extract, and fermented sobya (Akamine et al., 2022; Chiu et al., 2017; Gouda et al., 2016).

Limitations

Since no studies were powered for metagenomic significance, further research is needed to examine the relationship between microbiota and atherogenesis in human subjects. This is due in large part to the limited number of studies utilizing whole-genome shotgun sequencing.

Clinical Implications

Though our findings are mostly in keeping with the AHA heart-healthy dietary guidelines, it is evident that a shift is needed to account for the rapidly expanding microbiome research. Cardiovascular nurses are in a unique position to initiate this change, as cardiac patients are especially receptive to health education after a major cardiac event or surgery (Faggiano et al., 2019). Diet education may be improved with an emphasis on the dangers of processed, artificial ingredients and the benefits of consuming raw, unprocessed fiber and probiotics. Change occurs on the margin, and improving just one person's health-span through educational empowerment contributes to the betterment of our communities. Diet modification has the potential to do so through the amelioration of heart disease risk factors via the gut microbiome.

CONCLUSION

Though not understood in its entirety, the gut-heart axis has been linked to atherogenesis and CAD via inflammatory pathways associated with dysbiosis. Twenty-two randomized control human intervention studies were included in this review to investigate the role of probiotics, prebiotics, and processed foods on markers of atherosclerosis via the gut microbiome. Our findings suggest that current AHA dietary guidelines may benefit from the inclusion of a microbiome context. Nursing dietary education may emphasize the potential dangers of ultra-processed foods and benefits of probiotic and prebiotic foods.

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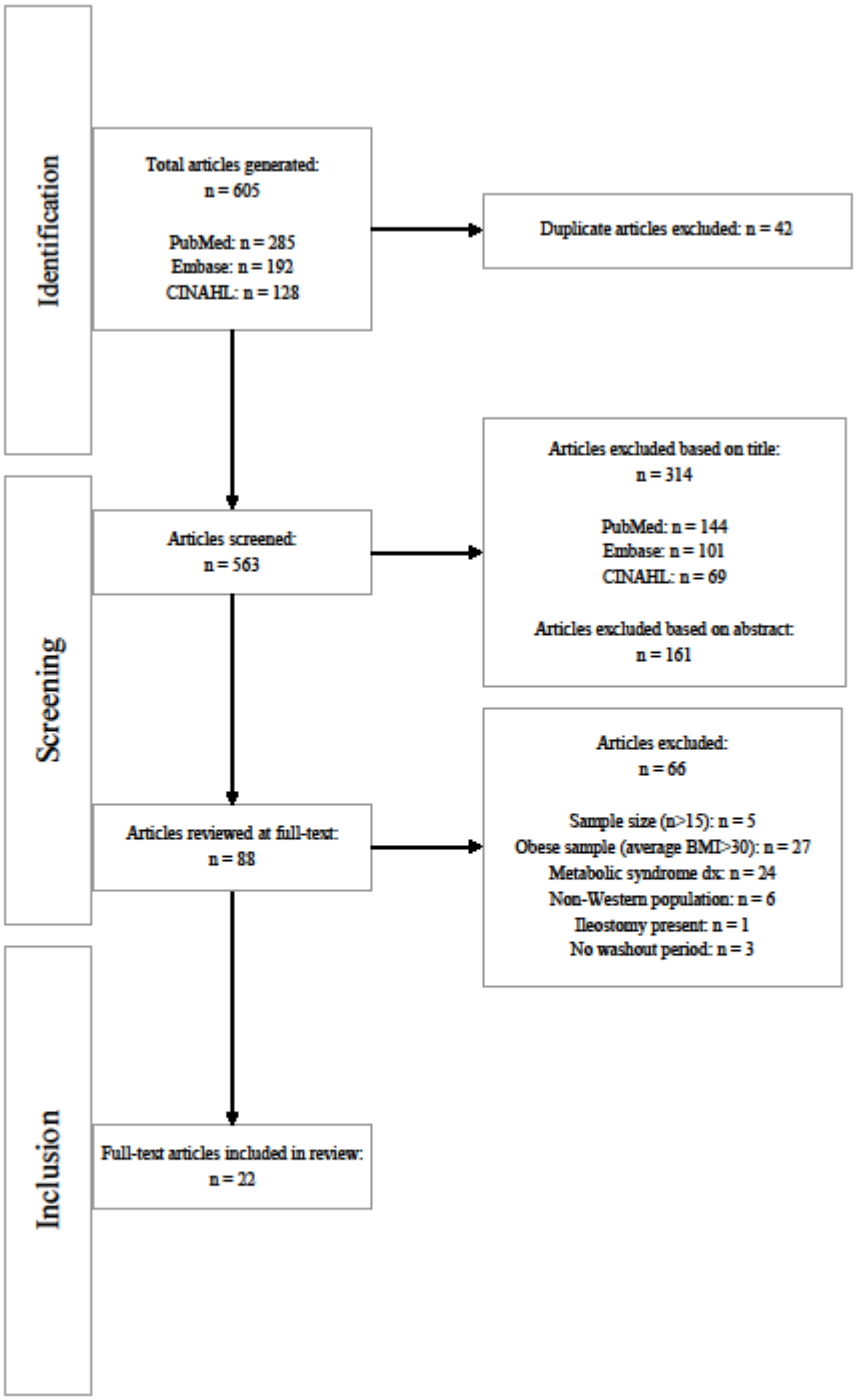


FIGURE 1. Preferred Reporting Items for Systematic Reviews and Meta-Analysis flowchart.

TABLE 1. Database search summary

	Search Terms				
CINAHL	sweet*	AND	"microbio*"	AND	"coronary artery disease"
	fried		"intestinal permeab*"		"coronary heart disease"
	additiv*		"dysbio*"		"cardiovascular disease*"
	processed		"metagenom*"		"atherosclero*"
	fiber*		"gut-microbio*"		CAD
	dietary fiber*		WGS		ischemia
	vegetable		WES		SCFA
	nut		whole genome sequenc*		"short chain fatty acid*"
	yogurt		fecal transplant*		oxidat*
	ferment*				"oxidative stress"
	artificial*				lipid*
	monounsaturated fat*				cholesterol
	polyunsaturated fat*				butyrate
	refined grain*				cytokin*
	refined wheat				
	whole grain*				
	whole wheat				
Embase	fermented*	AND	microbiot*	AND	"coronary artery disease"
	yogurt		"intestinal permeab*"		"coronary heart disease"
	fiber*		dysbio*		"cardiovascular disease*"
	"dietary fiber*"		microbiome		"atherosclero*"
	vegetable				CAD
	nut				ischemia
	fried				SCFA
	"processed meat*"				"short chain fatty acid*"
	additiv*				oxidat*
	"artificial sweet*"				lipid*
	"monounsaturated fat*"				endothel*
	"polyunsaturated fat*"				vascular*
	"refined grain*"				endotoxin
	"refined wheat"				"systemic inflammation"
	"whole grain*"				"chronic inflammat*"
	"whole wheat"				platelet*
	SSB				
	processed				
PubMed	fermented*	AND	microbiot*	AND	dysbio*
	yogurt		"intestinal permeab*"		"coronary heart disease"
	fiber*		dysbio*		"cardiovascular disease*"
	"dietary fiber*"		microbiome		"atherosclero*"
	vegetable				CAD
	nut				ischemia
	"processed food*"				SCFA
	"processed meat*"				"short chain fatty acid*"
	"food additive*"				oxidat*
	"artificial sweet*"				lipid*
	"monounsaturated fat*"				endothel*
	"polyunsaturated fat*"				vascular*
	"refined grain*"				endotoxin
	"refined wheat"				"systemic inflammation"
	"whole grain*"				"chronic inflammat*"
	"whole wheat"				platelet*
	SSB				

Table 2. Dietary intervention studies investigating the effects of probiotics, prebiotics, and processed foods on biomarkers of atherosclerosis through the gut microbiome

	Reference	Study Design	Country	Participant Characteristics	Intervention Food*	Control Food	16S rRNA Microbiome Sequencing	Relevant Metabolic Outcomes	Duration (weeks)	Results
Probiotics	Childs *et al.* (2014)	Crossover, Double-blind, Factorial	U.K.	n=41, Age 43+/-12 years, BMI 25 +/- 3 kg/m^2	Prebiotic xylo-oligosaccharide with or without probiotic *Bifidobacterium animalis* subsp. *Lactis*	Maltodextrin	Yes	Blood lipids, fecal SCFAs, T cells, interleukins, probiotic fecal count	3	Supplementation of fiber with the probiotic produced additive but not synergistic anti-inflammatory effects and decreased circulating B and T cells, suggesting a potential to decrease autoimmunity.
	Lee *et al.* (2017)	Crossover, Partially-blinded	U.S.	n=30, Age 18-40 years, BMI 20-35 kg/m2	Yogurt smoothies with probiotic Bifidobacterium animalis subsp. lactis added pre- or post-fermentation	Yogurt smoothie without probiotics	No	Blood lipids, fecal SCFAs, HOMA-IR, CRP	4	Capsule probiotics and post-fermentation probiotic smoothie exhibited no change in SCFAs, while control smoothie and pre-fermentation probiotic smoothie exhibited an increase in acetate, suggesting a difference in efficacy depending on manufacturing and/or fermentation process.
	Pushpass *et al.* (2022)	Parallel, Partially blinded	U.K.	n=61, Age 52+/-12 years, BMI 24.8+/-3.4 kg/m2	Cornflakes with probiotic lactobacillus reuteri capsules, oats, and apples	Cornflakes with placebo capsules	Yes	Blood lipids, fecal BAs, plasma BAs, interleukins, CRP	8	Cornflakes with probiotic capsules increased postprandial circulating BAs compared to control, though no significant differences were found in fecal BAs after full intervention period. Apple and oat ingestion decreased serum glycemia and insulin levels, though no group exhibited changes to fasting lipids, glucose, insulin, or inflammatory markers.
	Zheng *et al.* (2015)	Crossover	Denmark	n=15, Age 18-50 years, BMI 20-28 kg/m2	Semiskimmed milk, isocaloric semihard cow's cheese	Butter without dairy in diet	No	Blood lipids, fecal SCFAs	2	Cheese increased fecal SCFAs butyrate and propionate and decreased fecal SCFA acetate compared to milk. Butyrate, propionate, and fecal lipids negatively correlated with total and LDL cholesterol, and LDL increased in all groups from baseline but decreased in intervention groups compared to control.
Prebiotics	Cooper *et al.* (2017)	Parallel, Partially blinded	U.S.	n=46, Age 25.8+/-0.9 years, BMI 23.4+/-0.6 kg/m2	Various whole grain products via market basket	Refined grain market basket	Yes	Blood lipids, fasting blood glucose, body composition, and GI symptoms log	6	Supplemental fiber from whole grain appeared to decrease LDL compared to control refined grain group but did not reach significance when controlling for amount of test food consumed. Whole grain tended to decrease blood glucose but did not reach significance. *Akkermansia* and *Lactobacillus* associated with whole grain and *Erysipelotrichales* associated with refined.
	Creedon *et al.* (2022)	Parallel, Researcher blinded	U.K.	n=74, Age 27.5+/-6.2 years, BMI 22.9+/-2.8 kg/m2	Whole and ground almonds	Isocaloric snack muffin	Yes	Fecal SCFAs, volatile organic compounds, gut symptoms, and gut transit time	4	Almonds increased fecal butyrate and associated with decreased *Bifidobacteria* and increased *Lachnospiraceae*.
	Foerster *et al.* (2014)	Crossover	Germany	n=20, Age 40.1+/-11.6 years, BMI 24.4+/-2.9 kg/m2	High whole grain, low red meat diet	Isocaloric high red meat, low dietary fiber diet	Yes	Blood lipids, CRP, uric acid, creatinine, and fecal SCFAs	3	Whole grain led to decrease in BMI and fat mass and increase in microbial diversity.
	Granado-Serrano *et al.* (2022)	Parallel, Researcher blinded	Spain	n=63, Age 53.9+/-7.0 years, BMI 27.1+/-3.3 kg/m2	Insoluble fiber (wheat bran) cookies, Antioxidant fiber (onion-based) cookies	Isocaloric soluble fiber (psyllium plantago) cookies	Yes	Blood lipids, fecal SCFAs	9	Interindividual variability identified in blood lipid response and associated with SCFA and microbial differences. *Bifidobacterium* increased and blood pressure decreased from baseline in all groups.
	Healey *et al.* (2018)	Crossover, Double-blinded	New Zealand	n=33, Age 19-65 years, BMI 18.5-30 kg/m2	Prebiotic inulin-type fructan drink mix	Digestible maltodextrin placebo drink mix	Yes	Fecal SCFAs	3	Large variability in microbiota response to prebiotic appeared to be mediated by habitual fiber intake. Low habitual intake associated with resistance to microbiome shifts.
	Hess *et al.* (2018)	Crossover, Open-label	U.S.	n=32, Age 18-65 years, BMI 18.5-30 kg/m2	Lean ground beef	Protein-matched raw mushrooms	Yes	GI tolerance, and fecal SCFAs	1.5	Mushrooms and meat associated with distinct microbiome changes without evidence of fecal SCFA shifts.
	Holscher *et al.* (2018)	Crossover, Controlled-feeding	U.S.	n=18, Age 53.1+/-2.2 years, BMI 28.8 +/-0.9 kg/m2	Walnuts with base diet	Base diet	Yes	Blood lipids and fecal BAs	3	Walnuts led to decreased fecal BAs and LDL with distinct microbiome shifts including decreased fungal taxonomy.
	Hughes *et al.* (2021)	Crossover, Double-blinded	U.S.	n=30, Age 53.9+/-6.6 years, BMI 26.5+/-3.8 kg/m2	Resistant starch type 2 enriched wheat rolls	Conventional wheat roll placebo	Yes	Blood glucose and fecal SCFAs	1	Resistant starch decreased postprandial glucose and insulin and exhibited distinct microbial shifts without change to fecal SCFAs.
	Klosterbuer *et al.* (2013)	Crossover, Double-blinded	U.S.	n=20, Age 29+/-8 years, BMI 23+/-2 kg/m2	Resistant starch type 3 cereal bars, soluble corn fiber cereal bars, and pullulan drink mix	Low-fiber bars and digestible maltodextrin drink mix	Yes	GI tolerance and fecal SCFAs	1	Soluble corn fiber increased total SCFAs compared to control and resistant starch, though resistant starch with/without pullulan exhibited the highest increase in SCFA butyrate, specifically. Distinct microbial shifts seen.
	Martinez *et al.* (2013)	Crossover	U.S.	n=28, Age 25.9+/-5.5 years, BMI 22.7+/-3.0 kg/m2	Whole-grain barley and whole-grain barley with brown rice	Brown rice	Yes	Blood glucose, blood insulin, hs-CRP, interleukin 6, lipopolysaccharide-binding protein, and fecal SCFAs	4	Whole grain barley with/without brown rice associated with improved circulating interleukin-6, peak glucose, and CRP with high interindividual variation and disproportionate effects to overweight subpopulation.
	Ranaivo *et al.* (2022)	Crossover, Double-blinded	France	n=23, Age 18-69 years, BMI 29.0+/-2.8 kg/m2	Multifiber bread	Sourdough bread	No**	Blood lipids, blood glucose, and CRP	8	Multifiber bread decreased LDL, insulin, and HOMA-IR without change to inflammatory markers.
Processed	Ahmad *et al.* (2020)	Crossover, Double-blinded	Canada	n=17, Age 24+/-6.8 years, BMI 22.9+/-2.5 kg/m2	Aspartame	Sucralose	Yes	Blood glucose and fecal SCFAs	2	Sweeteners did not significantly alter glucose regulation, SCFAs, or bacterial proportions from baseline.
	Ampatzoglou *et al.* (2014)	Crossover	U.K.	n=33, Age 48.8+/-1.1 years, BMI 27.9+/-0.7 kg/m2	High whole grain diet	Low whole grain diet	Yes	Blood lipids, blood glucose, plasma alkylresorcinols, and fecal SCFAs	6	Fiber intake correlated with body fat percentage. No change to SCFAs or blood lipids.
	Chassaing *et al.* (2022)	Crossover, Double-blinded, Controlled-feeding	U.S.	n=16, Age 18-60 years, BMI Control: 24.5+/-3.5 kg/m2, BMI CMC: 25.3+/-4.0 kg/m2	Carboxymethylcellulose added to dessert	Emulsifier-free dessert	Yes**	GI tolerance, fecal SCFAs, fecal amino acids, fecal lipopolysaccharide, fecal CMC, and bacterial-epithelial distance	1.5	Carboxymethylcellulose decreased SCFAs by day 3 and depleted richness of microbiome. Bacterial-epithelial distance was decreased in some respondents but exhibited high interindividual variability.
	Ravn-Haren *et al.* (2013)	Crossover, Single-blinded	Denmark	n=23, Age 36.2+/-17.9 years, BMI 22.3+/-2.59 kg/m2	Whole apples, apple pomace, cloudy apple juice, and clear apple juice	No fruit	Yes	Blood lipids, plasma lipid resistance to oxidation, serum antioxidants, serum insulin, CRP, and fecal BAs	4	Clear apple juice increased and whole, pomace, and cloudy groups decreased LDL and lithocholic acid. Whole apples and pomace increased antioxidant enzyme GXP1.
	Rouger *et al.* (2017)	Crossover	Denmark	n=50, Age 20-65 years, BMI 25-35 kg/m2	High whole grain diet	High refined grain diet	Yes**	GI tolerance, blood lipids, serum insulin, blood glucose, AST, ALT, CRP, interleukins, plasma alkylresorcinols, and intestinal transit time	8	Whole grain decreased CRP and IL-6 while decreasing the number of calories consumed per sitting. No significant differences in fecal microbiota.
	Vanegas *et al.* (2017)	Parallel	U.S.	n=81, Age RG: 54+/-0.79 years, Age WG: 55+/-0.94 years, BMI 26+/-0.47 kg/m2	High whole grain diet	High refined grain diet	Yes	Blood lipids, lymphocytes, cytokines, T cells, tumor necrosis factor, and fecal SCFAs	6	Whole grain ingestion increased SCFAs compared to refined grain ingestion and tended toward higher memory T cells and tumor necrosis factor. Whole grain was associated with anti-inflammatory and SCFA-producing bacterial species.
	Zhang *et al.* (2022)	Crossover	U.S.	n=21, Age Reference 31+/-3 years, Age PreDM 35+/-2 years, BMI Reference 22.5+/-1.2 kg/m2, BMI PreDM 28.7+/-1.2 kg/m2	Red raspberries with prebiotic fructo-oligosaccharide	Red raspberries	No**	Blood lipids, blood glucose, serum insulin, and hepatic insulin resistance	4	In pre-diabetic population, red raspberries decreased hepatic insulin resistance and LDL. Total cholesterol decreased and insulin secretion rate increased in all groups compared to baseline but most significantly in pre-diabetics. Distinct microbiota shifts most significant in pre-diabetic group and apparent with and without prebiotic.

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; BA, bile acid; BMI, body mass index; CRP, C-reactive protein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; PCR, polymerase chain reaction; PreDM, pre-diabetic; RG, refined grain; rRNA, ribosomal ribonucleic acid; SCFA, short chain fatty acid; WG, whole grain;

*Intervention food in crossover studies defined as primary food/s of interest

**Shotgun Metagenomic Sequencing was performed

Appendix A
Summer Community of Scholars Poster

THE UNIVERSITY OF ALABAMA IN HUNTSVILLE | COLLEGE OF NURSING

Honors Capstone Research (HCR)
Summer Program 2023

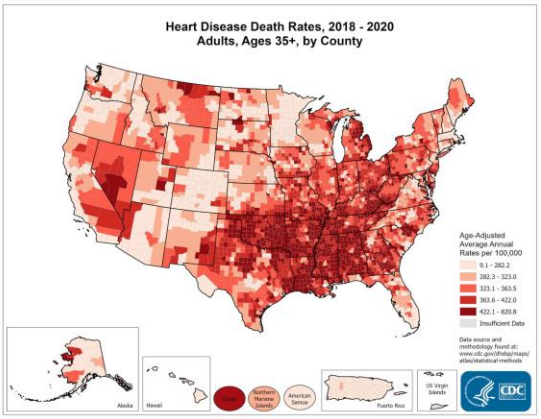
Diet-Mediated Heart Disease Risk via the Gut Microbiome

Emily Bolton, mentor Dr. Jennifer Bail
College of Nursing

Overview

A surge in global metabolic and heart disease has paralleled the expansion of the Western diet and lifestyle, giving rise to an epidemic. Though both noncommunicable and largely preventable, atherosclerotic heart disease - especially coronary artery disease (CAD) - continues to rise, necessitating a change in education or public narrative.

In this **systematic review**, we examined twenty-two randomized controlled trials using human diet intervention to elucidate the effects of specific foods and food groups on biomarkers of atherosclerosis and associated gut microbiome shifts. To identify current gaps and potential targets of diet education in the microbiome context, we used the heart-healthy guidelines from the American Heart Association (AHA) for reference.



Methods

- Search conducted until June 20, 2023, via PubMed, CINAHL, and Embase
- **Eligibility criteria**:
  - Randomized controlled or clinical trials
  - Human subjects aged 18-65 years old, healthy or exhibiting subclinical disease manifestations
  - Diet intervention consisting of one food or food group
  - Fecal samples were collected and analyzed
  - Cardiometabolic markers were measured (short chain fatty acids, inflammatory / immune markers, and cholesterol)
- Twenty-two articles included from 605 initial results on the basis of inclusion and exclusion criteria
  - Exclusion criteria: n<15, whole-diet alterations (e.g. Mediterranean diet), lack of washout period, pregnant populations, history of disease that could cause dysbiosis

Results

- **Probiotics:**
  - Fermented dairy decreased low-density lipoprotein (LDL) and total cholesterol while increasing short chain fatty acid (SCFA) production, especially butyrate
- **Prebiotics:**
  - Dietary fiber and whole grains tended to reduce inflammatory markers, C-reactive protein (CRP) and interleukin-6 (Il-6), especially when compared to refined grain
  - SCFA and blood lipid changes were variable
- **Processed foods:**
  - NOVA grades 3 and 4 tended to exhibit pro-inflammatory, pro-cholesterol, and anti-SCFA effects, especially when fiber was artificially removed (commercial fruit juices)
- Interindividual variability of microbiota shifts prevalent
- No trial was powered to detect significance at the metagenomic level, but trends were seen throughout
  - Cardiac health correlated with *Bifidobacterium* genus
  - Positive effects: *Parabacteroides*, *Lachnospira*, *Roseburia*, *Ruminococcaceae* and *Faecalibacterium*
  - Negative effects: *Victivallaceae, Butyricicoccus, Erysipelatoclostridium ramosum*, *Flavonifractor* and *Ruminococcus*

Discussion

NOVA group 3 **processed foods** in the form of refined grain products had greater negative effect on cardiometabolic health than whole grains did positive. In crossover studies with refined grains used as control, improvements in whole grain treatments were attributed to worsening markers in the control group from baseline. Yet refined grains have shown no significant effects on cardiovascular risk in prospective cohort studies[1]. This may suggest that the gut microbiome is an important mediator in our studies since those that altered **SCFAs** had the greatest results. Previous research has established a protective effect of these molecules against systemic inflammation and cancer in humans[2], while others revealed protective cardiovascular effects in hypertensive mice[3].

Interindividual variability in microbiome shifts appear to be partially dependent on subject comorbidities. This is in keeping with previous studies that indicate a large potential for use of diet modification in patients with cardiac-related dysbiosis to improve vascular health[3-5]. Increasing gut permeability decreases endotoxemia which predisposes arterial plaque, and some studies have found that **pathogenic bacteria** are a common denominator in cultures of atherosclerotic plaque[6].

Though our findings are mostly in keeping with the AHA **heart-healthy guidelines**, it is evident that a shift is needed to account for the burgeoning microbiome research. The rapid expansion in gut microbiome research serves as a reminder of the intricate connectedness of organ systems which requires a holistic approach to improve **health-span**.

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