Effects of a high-fiber diet on gut microbiota and the risk of cardiovascular disease: a systematic review

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SUMMARY

Within the past decade, the essential processes and molecular changes linked to the gut microbiome have become important factors that influence human physiology. An imbalance in the composition of gut microbiota, a process referred to as gut dysbiosis, has shown to be correlated with the development and progression of different cardiovascular diseases such as hypertension and atherosclerosis. Dysbiosis is linked with the production of particular metabolites within the intestinal mucosa that may further facilitate the advancement of cardiovascular diseases. The physiological benefits of a high-fiber diet have been well documented in association with patients suffering from different cardiovascular diseases. A diet that is high in fiber has been found to play a protective role in the occurrence of cardiovascular disease. The beneficial effects can be attributed to the production and distribution of a crucial metabolite of the gut microbiome, short-chain fatty acid acetate. The goal of this review is to highlight the existing data regarding the role of the gut microbiome in the onset of cardiovascular disease. Specifically, emphasis will be placed on the cellular and molecular components resulting from the microbiome that by gut dysbiosis, may increase the risk for developing cardiovascular disease. The underlying physiological changes induced by changes to acetate levels in the body, both directly and indirectly associated with cardiac health improvement, will also be examined. Collectively, the data illustrates that correcting imbalances in the composition of gut microbiota, along with a high-fiber diet are components that in the near future, may serve as new therapeutic targets for the prevention and treatment of cardiovascular diseases.

Received: 03/03/2020 **Accepted:** 04/07/2020 **Published:** 11/21/2020

Keywords: Gut Microbiota, High-fiber Diet, Cardiovascular Disease, SCFA, Atherosclerosis, TMAO, Hypertension, Gut Dysbiosis

INTRODUCTION

Cardiovascular disease (CVD) has been documented by many studies to be the leading cause of death worldwide, including in developed countries (Ahmadmehrabi and Tang, 2017; Ascher and Reinhardt, 2018; Al-Rubaye, Perfetti and Kaski, 2019) and accounts for over a third of all deaths worldwide (Battson, et al., 2018). In 2016, 31% of all deaths in Canada were a result of cardiovascular disease, and in 2017, it costed 66, 922 lives in Canada (Khalili, et al., 2020). The healthcare costs associated with CVD was $12 billion (CAD) in 2008, and has been the leading expense in Canadian healthcare (Boisclair, et al., 2018). It is therefore crucial to understand the underlying mechanisms in the body that contribute to the development of CVD in order to minimize the burden on healthcare and deaths

worldwide. The gut microbiome, associated with the gastrointestinal (GI) tract, has been recently studied in its ability to affect the risk of developing CVD (Battson, et al., 2018). The GI tract contains trillions of microbes including over a thousand different bacterial species (Battson, et al., 2018). The gut microbiome accounts for approximately 1-3% of total body mass by weight (Brial, et al., 2018).

Recently, many studies have investigated the potential link between gut dysbiosis and the development of CVD (Johansson-Persson, et al., 2014; Jie, et al., 2017; Haghikia, et al., 2018). It is primarily due to the effect of diet on the gut microbiome which can modify the activity of specific fatty acids called short-chain fatty acids (SCFAs), that have a variety of functions in the body (Battson, et al., 2018). SCFAs are fatty acids composed of less than six carbons primarily produced by the gut microbiome (Ahmadmehrabi and Tang, 2017). The gut microbiome converts polysaccharides from an individual's diet into SCFAs as a by-product of fermentation, specifically from dietary fiber (Ascher and Reinhardt, 2018; Battson, et al., 2018). SCFAs are responsible for providing energy for the body, around 5-10% of a person's total energy (Li and Tang, 2018). There are three major types of SCFAs: acetate, butyrate and propionate (Brial, et al., 2018). SCFAs have two major roles, the first being a source of energy for gut mucosal cells, and the second being sent into circulation to behave more as a signaling molecule (Brial, et al., 2018). A large majority of SCFAs are metabolized in the large intestine, however, a small portion of them can be absorbed into the systemic circulation where they have been shown to have their most profound effects on the cardiovascular system (Battson, et al., 2018).

Fiber is considered a non-digestible carbohydrate fermented by the microbiome in the colon and is also referred to as a prebiotic (Battson, et al., 2018). It acts as a prebiotic by increasing acetate-producing microbiota (SCFAs) (Battson, et al., 2018). It is also important to note that prebiotics are more efficient than probiotics at decreasing blood pressure, an integral relationship that will be discussed in detail later on (Ma and Li, 2018). The Western diet—, unlike the heavily studied Mediterranean diet (which is high in fiber)— is low in fiber and many studies have shown that it drastically diminishes the diversity and richness of the gut microbiome, including beneficial species such as *Eubacterium* (Battson, et al., 2018). The Mediterranean diet consists of foods that are high in fiber such as whole grains, vegetables, fruits, nuts and fish (Widmer, et al., 2015). The Mediterranean diet has been investigated in many studies and is beneficial in reducing the risk of CVD and diabetes (Widmer, et al., 2015). The effect of dietary changes on the gut microbiome are rapid, and can be observed within 24 hours (Battson, et al., 2018). The gut microbiome can be a contributor to many conditions other than cardiovascular disease, such as obesity and diabetes (Jin, et al., 2019).

There are a variety of conditions that have been shown to behave as precursors to developing CVD, such as atherosclerosis (Chen, et al., 2018; Ma and Li, 2018; Ding, et al., 2019), hypertension (Al-Rubaye, Perfetti and Kaski, 2019; Schiattarella, et al., 2019), and chronic kidney disease (Jovanovich, Isakova and Stubbs, 2018). Atherosclerosis is a condition characterized by the inflammation and thickening of artery walls (Brown and Hazen, 2018). Hypertension is a condition caused by environmental or genetic factors that causes improper blood pressure regulation (Schiattarella, et al., 2019). Hypertension affects over one billion people worldwide (Battson, et al., 2018), and is the most common precursor to CVD that can be modified in terms of its occurrence (Al-Rubaye, Perfetti and Kaski, 2019). Approximately 10-15% of people suffering from hypertension are resistant to current chemical treatments, so natural treatments such as dietary interventions are necessary (Yang, et al., 2015).

Recent studies have also investigated the impact of gut microbiota-produced trimethylamine oxide (TMAO) levels in blood plasma to see the potential link between it and the risk of developing CVD (Ahmadmehrabi and Tang, 2017; Heianza, et al., 2017; Li, et al., 2017; Chambers, et al., 2018; Haghikia, et al., 2018; Al-Rubaye, Perfetti and Kaski, 2019). TMAO levels have been shown to be a biomarker for the risk of CVD (Brown and Hazen, 2018). Flavin monooxygenases (FMOs) in the liver oxidize trimethylamine (TMA) gas which is produced by the gut microbiome (Schiattarella,

et al., 2019) that is ultimately transformed into TMAO by flavin-containing monooxygenase 3 (FMO3) (Li and Tang, 2018). The metabolite TMAO is produced by phosphatidylcholine and choline which can be obtained through diet, such as through red meat (Kanitsoraphan, et al., 2018). Studies have also proposed that bacteria that enter systemic circulation are capable of entering host tissues, ultimately resulting in the introduction of diseases (Brown and Hazen, 2018). Recent studies focusing on animals have shown that a high-fiber diet can decrease TMAO and TMA (the precursor to TMAO) metabolism by 62.6% and 40.6%, respectively (Chambers, et al., 2018). In this systematic review, the potential for a high-fiber diet to influence the risk of CVD will be investigated, specifically to understand how a high-fiber diet can make changes to the gut microbiome and how it can affect the risk of CVD.

MATERIALS AND METHODS

The protocol employed was the standard procedure for conducting systematic reviews by analyzing observational findings within peer-reviewed studies and reporting the results. The online software Covidence was utilized for information synthesis and appropriate screening. Google Scholar, Science Open and PubMed databases were searched to identify research surrounding the consumption of high-fiber diets in association with varying cardiovascular disease as a result of changing concentrations of gut microbiota populations. The references of highly relevant articles were also examined to identify other applicable articles. Keywords pertaining to gut microbiota and its effects on cardiovascular disease in correlation with a high-fiber diet were included. Specifically, the following terms were used: Gut microbiome AND Cardiovascular disease OR Atherosclerosis OR gut microbiome OR inflammation OR high-fiber diet OR TMAO.

Independent evaluation was conducted of the shortlisted studies based on keywords and relevance using the following inclusion criteria: (i) direct and indirect association between high-fiber diet and gut microbiota populations on the prevalence of cardiovascular disease; (ii) peer-reviewed studies after the year of 2000 for improved accuracy and relevance; (iii) research and literature reviews involving clinical trials conducted on mice and/or humans (iv) full-text PDF availability in only the English language. A low-fiber diet, obesity, or any other condition unrelated to cardiovascular disease in the study population was omitted and was the only exclusion criteria.

Beginning with 534 total studies, 51 duplicates were removed. Initial screening for article titles and abstracts was performed by two reviewers. This helped to remove the articles that were irrelevant, some which included editorials and case study observations. Since the link between gut microbiota and CVD was the main connection being examined, studies with participants having a history of exercise were excluded. Upon title and abstract screening, 250 studies were excluded based on the exclusion criteria. The exclusion criteria included diseases such as obesity and diabetes simply because this review solely focuses on examining relationships between the effects of the gut microbiome and a high-fiber diet on CVD only. Moreover, since diabetes and obesity are conditions that are already well studied in scientific literature, it would only lead to unnecessary confusion. Next, the full length of articles meeting the titles, abstract and overall inclusion criteria were obtained. Upon full-text screening, another 201 studies were excluded while referring to the exclusion criteria, along with studies that did not have relevant information for this review. A final total of 32 studies were analyzed for this systematic review. A simple schematic showing the process of the selection of studies for this systematic review is depicted in Figure 1.

RESULTS

Gut Microbiota and Trimethylamine Oxide (TMAO)

Increased evidence suggests that microbiota-related dysbiosis and activation interferes with the proper functioning of the cardiovascular system (Ahmadmehrabi and Tang, 2017). TMAO, an important marker for cardiovascular disease, was analyzed to view the related molecular pathways leading to major adverse cardiovascular events

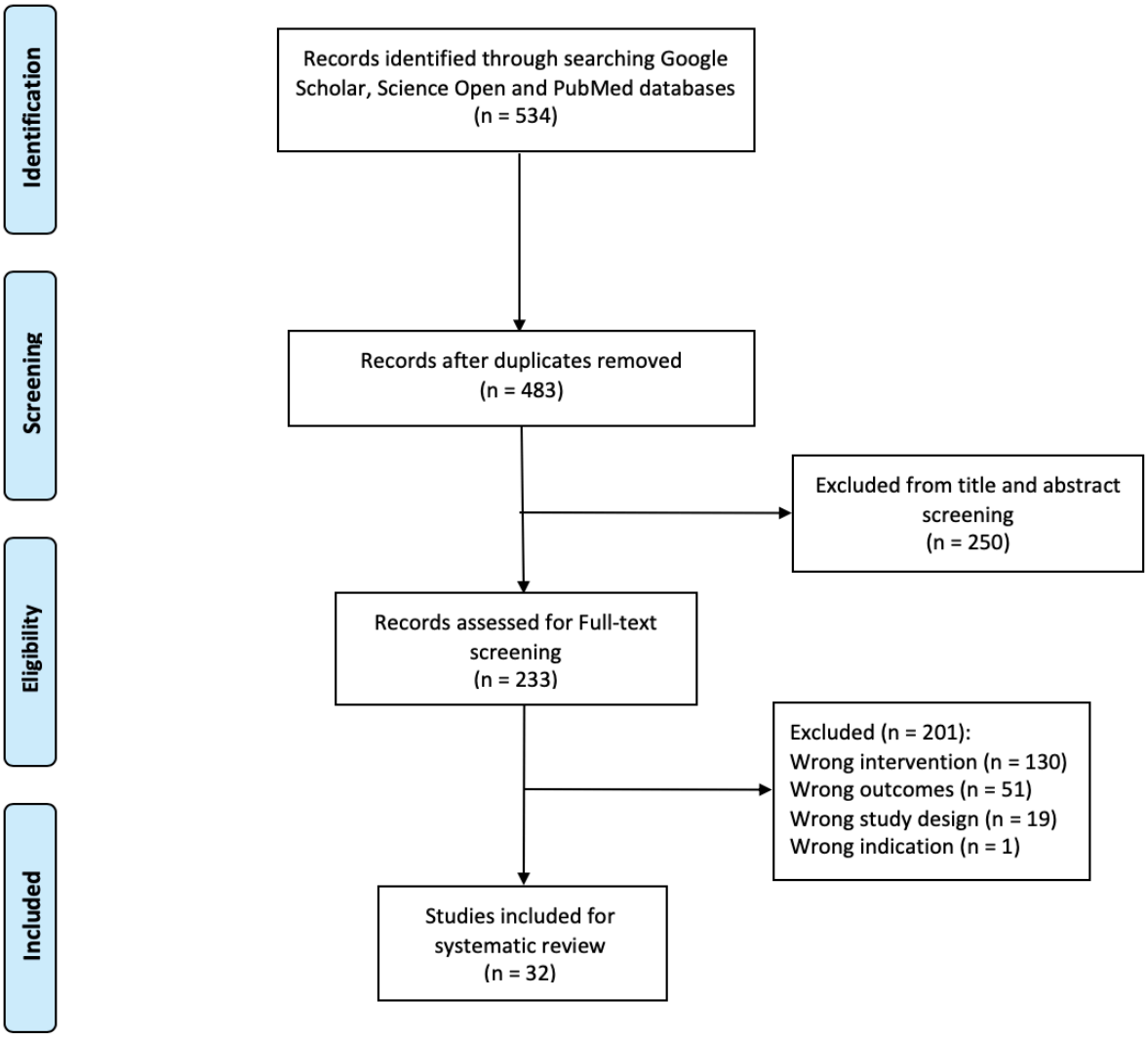

Figure 1: The process of identifying and screening studies to be included in this systematic review.

(MACE) (Ascher and Reinhardt, 2018). The changing composition of the gut microbiome has been shown to be directly linked with high levels of plasma TMAO (Ascher and Reinhardt, 2018). The gut microbiome is able to produce the TMA gas through the metabolization of phosphatidylcholine- and trimethylamine-containing compounds usually found in the human gut pathways (Ahmadmehrabi and Tang, 2017). The TMA gas is then metabolized by the mucosal enzymes to generate TMAO, a small molecule that is highly correlated with the development of MACE, such as coronary vascular disease and atherosclerosis (Albenberg and Wu, 2014). Through the production of TMA and the subsequent generation of TMAO as shown in Figure 2, some physiological effects include the processes through which cholesterol transport is interrupted causing MACE and atherosclerosis. Several studies have outlined increased levels of plasma TMAO being a principal factor in greater risks of MACE (Al-Rubaye, Perfetti and Kaski, 2019). A clinical cohort study demonstrated that 4007 patients that had belonged to the highest bracket for TMAO saw a 2.5-fold increase in the development of MACE (Ahmadmehrabi and Tang, 2017). A meta-analysis conducted by Heianza, et al., (2017) showed there was a major increase in the risk of MACE (by 62%) or death (by 63%) for individuals with higher plasma TMAO levels. Furthermore, increased levels of TMAO resulted in a 1.7-fold increased risk in developing MACE (Heianza, et al., 2017). However, a limitation of this meta-analysis was

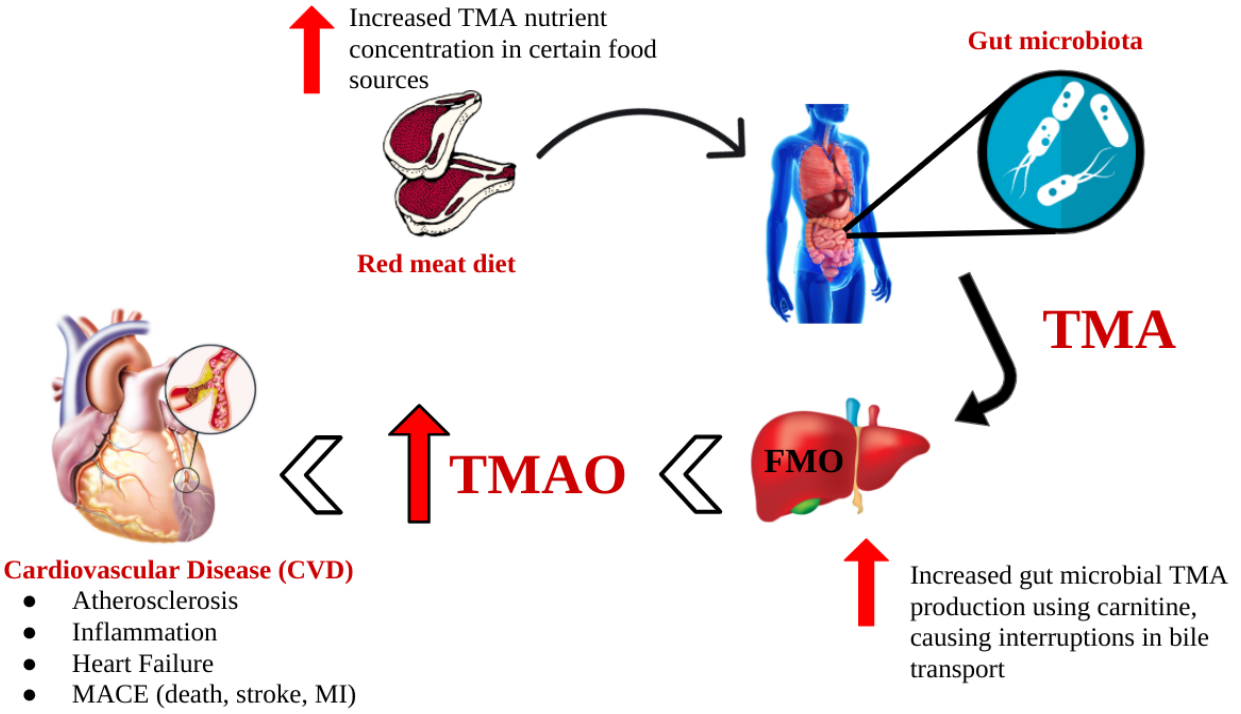

Figure 2: The pathophysiology of TMAO production from red meat which can result in severe negative health impacts, including an increased risk of death.

that the studies reviewed were just prospective cohort studies so they could only provide information based on reports and not account for confounding variables such as level of exercise (Heianza, et al., 2017).

Another meta-analysis demonstrated a major increase in the risk of MACE and eventual mortality as a result (Al-Rubaye, Perfetti and Kaski, 2019). In a prospective cohort study, the researchers concluded that individuals who were experiencing cardiovascular diseases such as myocardial infarction did have increased levels of plasma TMAO after a one-year follow-up ($P<0.01$) (Haghikia, et al., 2018). Individuals who were in the highest bracket of plasma TMAO levels indicated the progression of diseases pertaining to cardiovascular activity (Haghikia, et al., 2018). This was supported after accounting for several confounding variables such as age, gender, or precursors to cardiovascular disease such as hypertension ($P=0.04$) (Haghikia, et al., 2018). Other clinical studies have outlined the reduction of atherogenic diets containing mainly red meat and egg yolks, which lead to the metabolization of TMAO (Brown and Hazen, 2018). The data from these studies suggest a positive correlation between high blood plasma TMAO and the development of CVD, independent of other conditions such as obesity, renal disease and diabetes (Kim, et al., 2013). Within these studies, the levels of TMAO were represented as a significant predictor of death after 7 years ($P<0.05$) and was a significant predictor of MACE within 30 days or 6 months ($P<0.01$) (Li, et al., 2017; Li and Tang, 2018). The study also found that TMAO may interfere with cholesterol, lipid and sterol metabolism eventually leading to the development of atherosclerosis (Li, et al., 2017; Li and Tang, 2018). It was also demonstrated that reverse cholesterol transport was inhibited through gut-microbiota mechanisms as a result of TMAO (Li, et al., 2017; Li and Tang, 2018).

Short-Chain Fatty Acids and the *Firmicutes: Bacteroidetes* Ratio

The anaerobic fermentation of fiber by the gut microbiome is what generates short-chain fatty acids (SCFAs) (Jin, et al., 2019). SCFAs can modify blood pressure levels and control systemic inflammation (Li and Tang, 2018). SCFAs have been shown to minimize inflammation by decreasing the proinflammatory cytokine IL-12 and increasing the anti-inflammatory cytokine IL-10 (Li and Tang, 2018). The binding of SCFAs to

four key receptors can induce effects on the body such as delaying the progression of cardiovascular disease (Jin, et al., 2019). SCFAs work by stimulating G protein-coupled receptor (GPCR) pathways to target secretion of renin which in turn works to modulate blood pressure (Ma and Li, 2018). These receptors include vascular olfactory receptor 78 (Olfr78), G protein-coupled receptor 109A (GPR109A), G protein-coupled receptor 43 (GPR43), and G protein-coupled receptor 41 (GPR41) (Jin, et al., 2019). Specifically, the receptors Olfr78 and GPR41 can control renin secretion in the kidneys as a means of modulating blood pressure (Schiattarella, et al., 2019). Olfr78 has been shown to increase blood pressure, and GPR41 has been described to decrease blood pressure (Jin, et al., 2019). These two receptors work against each other, and studies have shown Olfr78 to promote conditions such as hypertension by increasing blood pressure, while GPR41 antagonizes its effects (Jin, et al., 2019). The transition from the fermentation of dietary fiber to forming SCFAs, ultimately leading to the changes mentioned earlier are displayed in Figure

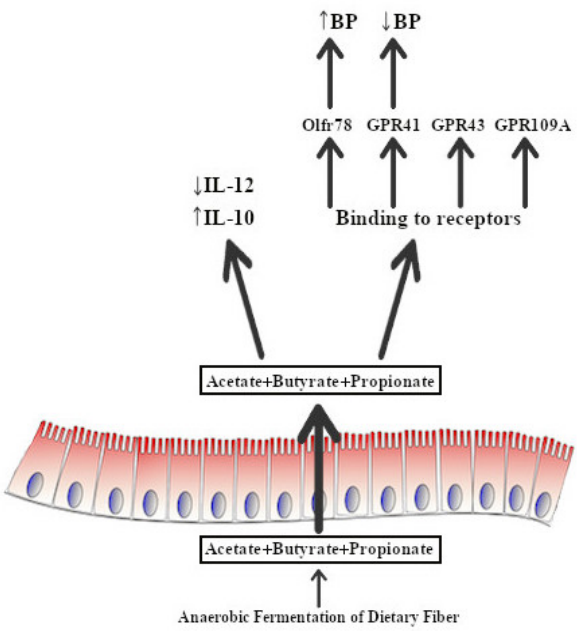

Figure 3: A simple illustration of the pathway of short-chain fatty acids (SCFAs) as they are produced through anaerobic fermentation, travelling past enterocytes to ultimately impact physiological processes of the body.

3.

There are four key phyla in the gut microbiome: Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria (Jin, et al., 2019). Particular interest surrounds the Firmicutes:Bacteroidetes ratio because it has been discovered to be a biomarker for the determination of gut dysbiosis (Jin, et al., 2019). Microbiota dysbiosis is linked with high blood pressure, and patients with hypertension tend to have less microbiota diversity (Razavi, et al., 2019). A high-fiber diet acts as a prebiotic by increasing acetate-producing microbiota (SCFAs), and prebiotics are more efficient than probiotics at mitigating negative impacts on the cardiovascular system such as decreasing blood pressure (Razavi, et al., 2019). Gut dysbiosis results in inflammation and a higher Firmicutes:Bacteroidetes ratio (Razavi, et al., 2019). Another study investigated other bacterial species that potentially had an effect in individuals affected by a form of CVD (Jie, et al., 2017). This study involved 218 participants affected by atherosclerotic cardiovascular disease, along with 187 controls (Jie, et al., 2017). This study concluded that two bacterial species, Streptococcus spp. of the phylum Firmicutes was shown to be increased in richness in individuals affected by CVD, and a decreased richness in Bacteroides spp. of the Bacteroidetes phyla was also observed (Jie, et al., 2017). This indicates a high Firmicutes:Bacteroidetes ratio, which further supports the claim for this ratio being involved in the risk of developing CVD.

Correction of Gut Dysbiosis Through A High-Fiber Diet

Various studies have also noted the impact of short-term changes in diet which have been shown to significantly impact the constitution of gut microbiota (Albenberg and Wu, 2014). One study in particular examined mice and saw significant drops in systolic and diastolic blood pressure when administering a high-fiber diet alone, or acetate alone (Marques, et al., 2017). This finding has caused many researchers to manipulate gut microbiota populations with a high-fiber diet (Ding, et al., 2019).

The Western diet, consisting of red meat, eggs, fish and poultry, is low in fiber and has been shown to diminish the diversity and richness of the gut microbiome, including beneficial bacterial species such as Eubacterium (Battson, et al., 2018). Diets

high in fats or sugars similar to the Western diet, have been shown to increase levels of TMAO in circulation, which in turn leads to the disruption of cardiac function (Battson, et al., 2018). Upon the implementation of a high-fiber diet as part of a 5-week study, scientists found a statistically significant drop in elevated fibrinogen levels when high-fiber diets were administered (P=0.044) (Johansson-Persson, et al., 2014). This is especially important considering increased fibrinogen levels are a principal risk factor of myocardial ischaemia and a predictor of accelerated atherosclerosis (Benisi-Kohansal, et al., 2016). The study also saw a significant drop in levels of C-reactive protein (CRP), a biomarker for inflammation leading to heart trauma, when the participants were provided a high-fiber diet (P=0.017) (Johansson-Persson, et al., 2014). This demonstrates how increased dietary fiber minimizes inflammatory conditions of the heart, such as vasculitis, by modifying gut microbiota (Ahmadmehrabi and Tang, 2017). Heavily vegetarian diets have also been shown to mimic the beneficial effect of high-fiber diets, though to a lesser degree (Kim, et al., 2013). This suggests vegetarian diets are an alternative but an inferior option (Kim, et al., 2013). Fiber from heavily vegetarian diets is capable of decreasing the Firmicutes:Bacteroidetes ratio (Kim, et al., 2013). One particular study described how the source of fiber also plays a role in the development of CVD (Mirmiran, et al., 2016). This study showed fruits and vegetables to have the highest inverse relationship with the risk of CVD, but nuts and grains did not have as much of a profound effect (Mirmiran, et al., 2016). However, a major limitation of this study was that only 57 of the subjects developed CVD, while 2202 did not develop it (Mirmiran, et al., 2016).

Determining the potential of a high-fiber diet to reduce the risk of CVD was studied in a cohesive manner, with a large meta-analysis of 757,966 individuals from 11 different studies (Benisi-Kohansal, et al., 2016). In this meta-analysis, 25,595 individuals of the 757,966 were reported dead from all 11 studies combined (Benisi-Kohansal, et al., 2016). They determined that an increased consumption of whole-grain foods led to a 16% reduced risk of cardiovascular-related death (Benisi-Kohansal, et al., 2016). Due to the nature of statistical analysis when comparing multiple different studies and the enormous sample size, these results were not statistically significant (P=0.11) (Benisi-Kohansal, et al., 2016).

Moreover, the high-fiber diet was shown to be beneficial as it helped to prevent and reduce the number of MACE occurring in individuals (Ahmadmehrabi and Tang, 2017). Researchers from another prospective cohort study administered the high-fiber diet in relation with subjects who experienced myocardial infarction (Li, et al., 2014). This study involved 1840 men and 2258 women who did not have cardiovascular disease and had suffered a myocardial infarction (Li, et al., 2014). Follow-up for men was an average of 9.0 years and 8.7 years for women upon occurrence of myocardial infarction (Li, et al., 2014). Out of a total 682 deaths for women, 336 deaths were linked with the cardiovascular system, and 222 deaths of a total 451 deaths for men of the same reason (Li, et al., 2014). It was concluded that a high-fiber diet upon occurrence of myocardial infarction resulted in a significantly lower incidence of death (Li, et al., 2014). Increasing the level of dietary fiber had significantly lowered the chance of cardiovascular death in women (P=0.003), but not for men (P=0.07) when adjusting for time since the myocardial infarction occurrence and age of subjects (Li, et al., 2014).

A meta-analysis of prospective cohort studies found that whole grains alone led to a 21% reduction in mortality and events caused by cardiovascular disease (Widmer, et al., 2015). Another systematic review on prospective studies found that individuals with diets that had a 10-gram dietary fiber addition per day demonstrated a significant decrease of the risk of heart attacks (P=0.001) (Liu, Wang and Liu, 2015). A similar study found the addition of fiber and acetate minimized gut dysbiosis by increasing the richness of Bacteroides acidifaciens (Marques, et al., 2017). These bacteria, which are SCFAs, displayed the ability to assist in the prevention of heart failure and hypertension, and positively modulate blood pressure (Marques et al., 2017). Other studies examining other MACE, defined as a composite of heart failure, ischemic cardiovascular events and cardiovascular death, concluded that the high-fiber diet is linked with decreased cardiac hypertrophy, fibrosis, and decreased blood pressure (Tang, Kitai and Hazen, 2017). The

American Heart Association (AHA) have provided baseline dietary amounts for individuals with some sort of cardiac disease history (Schiattarella, et al., 2019; Brial, et al., 2018). They have recommended 25-30 g of fiber daily, specifically from whole grain foods as an important approach to minimize the risk of CVD, especially in later years of one's life (Schiattarella, et al., 2019; Brial, et al., 2018).

CONCLUSIONS AND FUTURE DIRECTIONS

The existing literature has made it clear that the potential for a high-fiber diet to be used as an intervention to minimize the risk of developing cardiovascular disease is feasible given the large variety of studies conducted in the recent years. The effect of a high-fiber diet to influence the gut microbiome has also been highlighted and has shown to have profound effects such as increasing levels of acetate-producing microbiota. This increased consumption of high-fiber foods has been shown to be a useful means of treating hypertension, a common precursor to cardiovascular disease, as the intervention involved decreasing blood pressure. Treatment of gut dysbiosis through a high-fiber diet has been shown to decrease the Firmicutes:Bacteroidetes ratio, a biomarker for gut dysbiosis, and decreases inflammation associated with the development of cardiovascular diseases. The use of a vegetarian diet, which is also relatively high in fiber, has shown promise in the prevention of cardiovascular disease, but not to the extent of a solely high-fiber diet. This is because the source of fiber is a key determinant in the process of treating gut dysbiosis, with whole grains being a leading source of fiber in the prevention of cardiovascular disease.

Some possible limitations of this systematic review include that studies with low-fiber diets were excluded, and studies that focused on the relationship between non-cardiovascular disease-related conditions to the gut microbiome were also excluded. This includes studies that focused on diabetes and obesity. By reviewing studies that focused on low-fiber diets, a better overall understanding could be achieved through analyzing both high-fiber and low-fiber interventions in tandem. Although the level of fiber was the primary parameter analyzed in this systematic review, the potential benefits of other ingredients in different diets, such as the Mediterranean diet or a vegetarian diet, need to be further studied. This may potentially identify new key molecules that can have more profound impacts than fiber on the treatment of gut dysbiosis, as there are many different ingredients that have yet to be studied to the same degree as same as high-fiber diets. Furthermore, investigating the relationship of other bacterial genus' Enterobacteriaceae and Streptococcus spp. is another area of study that could use more focus, since a study in this review showed high levels of these species in individuals who were affected by atherosclerotic cardiovascular disease. The potential for these bacterial species to be involved in the pathophysiology of other precursors to cardiovascular disease such as hypertension should also be studied in the future.

AUTHOR CONTRIBUTIONS

M.A.M. and A.S. collected studies to be incorporated into Covidence. M.A.M and A.S. split up title and abstract screening and full text screening. M.A.M was responsible for data extraction. A.S. wrote the abstract, M.A.M wrote the introduction, and M.A.M and A.S. collaborated on the results. M.A.M wrote the conclusion and future directions, A.S. found figures, and M.A.M wrote the references list.

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