4-1-2020

Atherosclerotic Cardiovascular Disease –The Gut Microbiome as a Risk Factor

Kristin J. Gedda
University of the Pacific, k_gedda@hotmail.com

Atherosclerotic Cardiovascular Disease –The Gut Microbiome as a Risk Factor

By

Kristin J. Gedda

April 2020

Capstone Project

Submitted to the Faculty of the

Department of Physician Assistant Education

of University of the Pacific

in partial fulfilment of the requirements

for the degree of

MASTER OF PHYSICIAN ASSISTANT STUDIES

Introduction

As it remains, cardiovascular disease (CVD) has been the leading cause of mortality across the globe accounting for as many as 17.9 million deaths per year and projected to increase to more than 23.6 million deaths by 2030.[1] Of the CVD related deaths, the majority are due to acute myocardial infarctions (AMI) and cerebral vascular accidents (CVA) and are associated with modifiable risk factors such as obesity, dyslipidemia, hypertension, and diabetes.[2] Lifestyle modifications for decreasing these risk factors have been actively emphasized in healthcare, but do not eliminate the risks entirely. In addition to diet, exercise, and smoking cessation, other modifiable risk reduction should be explored. One area of particular interest pertains to the gut microbiome. As the understanding of gut health expands, the connections between microbiome disruptions and disease processes, such as cardiovascular disease, have suggested microbiota may affect health risks. Advances in the ability to analyze the plethora microbiota in the human gut have made it possible to both identify and quantify specific innate bacteria. This ability has provided a better understanding of the roles, functions, and potential alterations of the gut microbiome that are seen in various disease processes.

Analyzing alterations of the gut microbiome, if any, in persons with CVD or its risk factors is a key first step. Then, identifying any associations between an altered gut microbiome and dietary ASCVD risk factors, specifically obesity and dyslipidemia, may enhance not only the understanding of the underlying pathophysiology, but also the prospects of finding means to reduce these risks.

2

Background

The gut microbiome is comprised of trillions of bacteria, grouped by six major phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, and *Verrucomicrobia* phylae, which are under the constant influence of external factors, such as dietary intake, antibiotic use, and infection.[3] The gut microbiome is responsible for a number of biological functions through various mechanisms including saccharolytic metabolism, activation of immune responses through Toll-like receptors (TLRs), and trimethylamine *N*-oxide (TMAO) production. By way of saccharolytic metabolism, comes essential sources of energy from the byproducts of short-chain fatty acids (SCFAs) including acetate, butyrate, and propionate. [4]

The gut microbiome serves to mitigate immune responses through TLR ligands by sensing bacterial components which in turn generate proinflammatory cytokine cascades. A study by Miura et al found that chronic low-inflammatory states such as obesity are mediated by increased activation of TLR signaling. In obese mice, alterations in gut microbiota, are evidenced by an increased number of bacteria from the *Firmicutes* phylum in the gut microbiota and a decreased number of bacteria from the *Bacteroidetes* phylum, in addition to elevations in specific TLRs .[5] While immune response activation is essential for the host to ward off offending pathogenic microbes, constant activation can promote a perpetual state of inflammation leading to organ and tissue destruction.

The gut microbiome might increase CVD risk through its generation of TMAO, an atherogenic metabolite. Numerous animal and human studies have shown that the gut microbiota affect metabolism of substrates from phosphatidylcholine which is found in animal proteins. The breakdown of phosphatidylcholine is mediated by gut microbes and produces

TMAO. High levels of plasma TMAO were seen in one study in mice that were administered radiolabeled phosphatidylcholine and were followed by greater arterial plaque development. Hence, TMAO levels may be an indicator of ASCVD risk.

Extrapolating this hypothesis from animal studies, human subjects were examined for a possible relationship between TMAO plasma levels and ASCVD. In two separate studies the levels of TMAO were measured in large cohorts of patients who were undergoing cardiac evaluations. Those with the most significant risk of having a major cardiac event had the highest levels of TMAO. Thus, in humans, high TMAO are associated with increased ASCVD risk [6].

Discussion

Many of the risk factors for ASCVD, like obesity and lipid dysfunction, have overlapping pathophysiologies in which metabolic dysregulation has a major role. This overlap makes identifying the effects of isolated microbes and their mechanisms for dysfunction difficult to separate from other possible effects associated with other CVD risk factors, such as obesity and dyslipidemia. Furthermore, much of the current evidence evaluating the gut microbiome, metabolic dysregulation, and influence on ASCVD and risk factor disease states are through case-control and cross-sectional analysis in human subjects. Randomized, controlled trials with manipulation of the gut microbiome to assess its effects on ASCVD outcomes are not feasible at this time, although evaluating the effects of gut microbiota on surrogate endpoints of ASCVD (ASCVD modifiable risk factors, TMAO levels, and other markers) is certainly achievable. Thus, research specifically examining TMAO levels, obesity, and dyslipidemia, other risk markers in relation to the composition of the gut microbiome may provide new knowledge of the

pathophysiology of ASCVD as well as suggest means of decreasing ASCVD risks by altering the gut microbiome.

Dietary Influences and TMAO Levels

Dietary trends are constantly changing; albeit they are not always supported by scientific evidence. Moreover, such diets are recommended by media outlets, personal fitness coaches, or family, who may not have expertise in nutrition. Although weight loss fads and diets move in and out of popularity, omnivorous and vegetarian diets remain constant. The ongoing CARNIVAL non-blinded, randomized, interventional clinical trial is currently comparing these two diets by examining the effect of L-Carnitine, a compound isolated from meat. In this study, 100 healthy omnivores and vegan/vegetarians were administered oral L-carnitine to comparatively assess plasma and fecal levels of L-carnitine before and after oral antibiotics. These levels were examined within several hours of ingestion and again after a two-month period of L-carnitine supplementation. After oral L-carnitine, the omnivore group produced greater than 20 times more TMAO compared with vegan/vegetarians (a statically significant difference). The presumed route of TMAO formation by gut microbiota was by way of microbial transformation of TMA to its newly confirmed intermediary γ-butyrobetaine (γBB). Although various gut microbiota are involved in the transformation of TMA from L-carnitine, the differences between the groups' microbiota were not specially detailed, and thus warrant further investigation. The CARNIVAL trial is tentatively scheduled to be complete June 2020. These preliminary results are limited by small sample size which restricts generalizability. Further evaluation with larger sample is necessary to improve external validity. Furthermore,

investigations identifying the specific gut microbes involved in TMAO production and other possible microbial mechanisms leading to atherogenesis are needed.[7] If elevated TMAO levels are a risk factor for ASCVD, as suggested in the foregoing evidence, then L-carnitine may promote ASCVD risk because of its conversion to TMAO by gut microbiota [see illustration below]. Since this amino acid is highly prevalent in meat and in some food supplements, diets high in meat or L-carnitine supplements may therefore increase ASCVD risk.
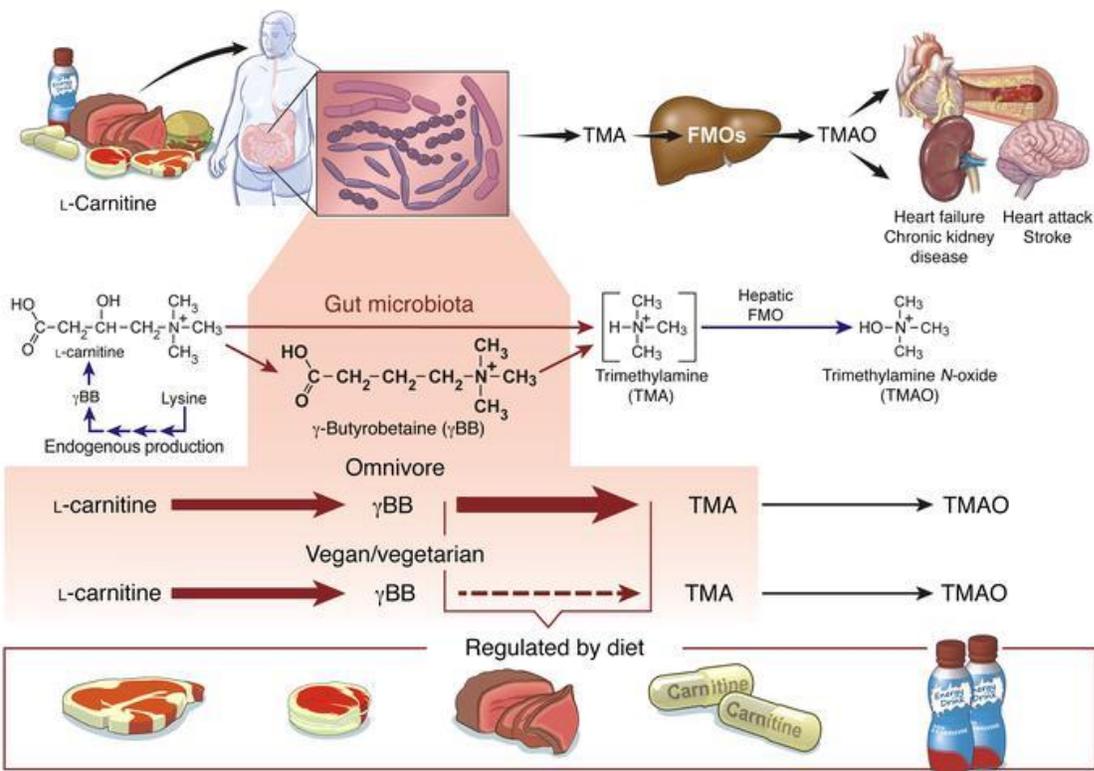
[7] Koeth RA, Lam-Galvez BR, Kirsop J, et al. l-Carnitine in omnivorous diets induces an atherogenic gut microbial pathway in humans. *Journal of Clinical Investigation*. 2018;129(1):373-387. doi:10.1172/jci94601.

Dietary Influences and Obesity

An observational cross-sectional-study by Cuesta-Zuluaga et al, examined SCFA levels and their association with the gut dysbiosis seen in subjects with obesity and other

cardiometabolic risk factors. A relatively large sample of persons was selected throughout

populations in Colombia and other South American countries. Multiple variables including age,

gender, diet, physical activity, adiposity (measured by body mass index (BMI), percent body fat,

and waist circumference), blood chemistries, blood pressure, operational taxonomic unit (OTU)

richness of gut microbiomes, and fecal SCFAs. Greater fecal butyrate excretion in subjects

classified as obese was found and was associated with an overall decreased diversity of gut

microbiota as measured by OTU richness. Inversely, rich variation in gut microbiota diversity

was associated with the decreased gut permeability seen in subjects with lower BMIs and less

central abdominal fat. Strengths of this study included its large sample size and its ability to

control for many confounders including limiting the sample to subjects who did not take any

medications. A limitation of the study was its design, cross-sectional in contrast to an RCT. The

measurement of SCFAs in fecal samples only and not serum was another limitation.

Nonetheless, evidence was found that the microbiome of the obese subjects differs from that

of the controls, posing the question of why the altered microbiome increases the risk of ASCVD

in obese persons of mechanistic link. [8]

Lipid Regulation and Dyslipidemia

Modulation of lipids is a multivariate process and is influenced by many factors,

including gut microbiome function and composition. The composition of the gut microbiome in

individuals with ASCVD was successfully characterized by a metagenome-wide association

study. Specific microbial strains, specific functional modules linked to ASCVD, and enriched

virulent factors were identified. Of the 218 subjects with known atherosclerotic cardiovascular

7

disease, the richness of *Enterobacteriae* and *Streptococcus* spp. was greater than the richness

of those species in the 187 healthy control subjects. Of the *Enterobacteriaceae* richness in the

ASCVD group, specific bacterial genes that encode for lipid synthesis or destruction were found

and were in contrast to the lower level of *Enterobacteriaceae* and the otherwise rich

Bacteroides seen with the control group. Components that contribute to atherogenic plaque

formation were identified in the bacterial functional gene modules. A greater synthesis of O-

Antigen (a component of lipopolysaccharides) and reduced synthesis of lipid A (the innermost

component of lipopolysaccharides), was found in high density in the in the Enterobacteriaceae-

associated ASCVD groups. This finding could explain the link between microbiome composition

and ASCVD. However, the various drug therapies among subjects may have constituted a

confounding factor. Further isolation of the disease-associated strains and more in-depth

knowledge of their functional capacities are necessary before interventions affecting these

bacteria, and hence ASCVD, can be evaluated. [9]

In January of this year, Mayo Clinic researchers studied 53 patients with advanced

coronary artery disease (CAD). Through primary fecal analysis, they identified specific bacteria

associated with this disease state and compared these results with those from healthy controls.

An overall decreased gut microbial richness was found in the CAD group compared with the

matched control group. Specifically, a heavier concentration of *Ruminococcus gnavus* and a

decreased concentration of *Lachnospiraceae* NK4B4 and *Ruminococcus gaureauii* groups (all are

of the *Firmicutes* phylum) were seen in persons with CAD. Limiting factors in this study included

use of a low phylogenic power of the method of rDNA microbiome analysis, the fact that not all

CAD patients were identified by angiography, and the small sample size.[10] As with many of the

aforementioned studies, causal evidence between specific microbes and either cardiovascular disease or its risk factors require further substantiation with additional research.

In a cross-sectional study of 561 participants, evaluating the interplay between the gut microbiome and individual metabolism, 283 subjects with insulin resistance were assessed by body composition, lipid profiles, and glucose tolerance in relation to dietary intake. Data was gathered by using the semi-quantitative food frequency questionnaire (SQFFQ) to inventory particular dietary habits. Stool analysis was performed to identify gut microbiome diversity by their OTU and RDP Classifier algorithm. A richness of *Bacteroidetes* was seen in those subjects with lower LDL-C levels and larger amounts of *Tenericutes* were seen in those with higher total cholesterol levels. An abundance of *Actinobacteria* was found in persons with greater BMIs and larger waist and hip circumference. These findings suggested that these specific phyla may have played a role in the metabolism of lipids and therefore, alteration in these microbes might have contributed to dyslipidemia and metabolic dysfunction. This study was limited by its observational design. The use of the subjective SQFFQ tool could have created bias. Further investigation is needed to ascertain the exact mechanism of the gut microbiome's influence on lipid regulation and its effect on metabolism that contributes to obesity and lipid dysregulation.[11]

Conclusion

Evidence does indeed indicate that the composition of the gut microbiota in patients with ASCVD and known risk factors of obesity and lipid dysregulation compared with those without are different. While variation is seen among those persons with ASCVD and known risk

factors, the exact cause is still poorly understood. The gut microbiota is influenced by metabolic pathways that both downregulate and upregulate synthesis of beneficial and pathogenic microbes, respectively. Mechanisms behind these metabolic pathways, including TMAO mediated synthesis, warrant further investigation to better understand the involvement in atherogenesis. More large-scale metagenome-wide association studies across more diverse patient populations could further categorize microbe trends in those persons with ASCVD and would provide more insight into what phyla may play a role in the pathogenesis of ASCVD. This evidence does not currently provide a basis for pharmacological interventions, but does support lifestyle modification for those patients with ASCVD and its risk factors.

References

1. Benjamin EJ, Muntner P, Alonso A, et al. Heart Disease and Stroke Statistics—2019 Update: A Report from the American Heart Association. *Circulation.* March 2019. https://www.ahajournals.org/doi/10.1161/CIR.0000000000000659#. Accessed November 23, 2019.

2. Cardiovascular Diseases. World Health Organization. https://www.who.int/health-topics/cardiovascular-diseases#tab=tab_2. Accessed December 24, 2019.

3. Jin M, Qian Z, Yin J, Xu W, Zhou X. The role of intestinal microbiota in cardiovascular disease. *J Cell Mol Med*. 2019;23:2343-2350. doi:10.1111/jcmm.14195

4. Korakas E, Dimitriadis G, Raptis A, Lambadiari V. Dietary Composition and Cardiovascular Risk: A Mediator or a Bystander? *Nutrients*. 2018;10(12). doi:10.3390/nu10121912.

5. Miura K, Ishioka M, Iijima K. The Roles of the Gut Microbiota and Toll-like Receptors in Obesity and Nonalcoholic Fatty Liver Disease. *Journal of Obesity & Metabolic Syndrome*. 2017;26(2):86-96. doi:10.7570/jomes.2017.26.2.86.

6. Hills RD, Pontefract BA, Mishcon HR, Black CA, Sutton SC, Theberge CR. Gut Microbiome: Profound Implications for Diet and Disease. *Nutrients*. 2019;11(7):1613. doi:10.3390/nu11071613.

7. Koeth RA, Lam-Galvez BR, Kirsop J, et al. l-Carnitine in omnivorous diets induces an atherogenic gut microbial pathway in humans. *Journal of Clinical Investigation*. 2018;129(1):373-387. doi:10.1172/jci94601.

8. Cuesta-Zuluaga JDL, Mueller N, Álvarez-Quintero R, et al. Higher Fecal Short-Chain Fatty Acid Levels Are Associated with Gut Microbiome Dysbiosis, Obesity, Hypertension and Cardiometabolic Disease Risk Factors. *Nutrients*. 2018;11(1). doi:10.3390/nu11010051.

9. Jie, Z., Xia, H., Zhong, S. *et al.* The gut microbiome in atherosclerotic cardiovascular disease. *Nat Commun* 8, 845 (2017). doi:10.1038/s41467-017-00900-1

10. Toya T, Corban MT, Marrietta E, et al. Coronary artery disease is associated with an altered gut microbiome composition. *Plos One*. 2020;15(1). doi:10.1371/journal.pone.0227147.

11. Nuli R, Cai J, Kadeer A, Zhang Y, Mohemaiti P. Integrative Analysis Toward Different Glucose Tolerance-Related Gut Microbiota and Diet. *Frontiers in Endocrinology*. 2019;10. doi:10.3389/fendo.2019.00295.