EXAMINING THE ASSOCIATIONS BETWEEN DIFFERENT TYPES OF CARBOHYDRATES AND VASCULAR HEALTH

by

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ABSTRACT

Arterial stiffness, endothelial dysfunction, and high blood pressure (BP) are risk factors for cardiovascular disease (CVD), all of which can be influenced by diet. Different types of carbohydrates, such as added sugar, have been shown to cause endothelial dysfunction acutely, while high dietary fiber intake has been associated with reduced arterial stiffness and BP in various populations. One of the proposed mechanisms for these findings is postprandial increases in blood glucose concentration. The extent of this increase in blood glucose level is measured by a food's glycemic index (GI). While previous research focused on the acute effects of various types of carbohydrates on vascular health, less is known about habitual consumption and whether these associations begin in early adulthood. This study aimed to examine the relations between added sugar, fiber, and diet GI with measures of vascular health. Fifty-five participants (21M/34W; 26.7±6.1 years; BP 113±12/69±9 mmHg) completed this cross-sectional study. Dietary intake was assessed using 3-day diet records. Measures of vascular health included pulse wave velocity (PWV), a measure of arterial stiffness, augmentation index (AIx), a measure of wave reflection, brachial artery flow-mediated dilation (FMD), a measure of endothelial function, and 24-hour ambulatory blood pressure monitoring (ABPM). Associations between the types of carbohydrate and vascular measures were assessed using Pearson bivariate and partial correlations. Participants consumed an average of 2039±553 kcal with 9.6% (193.6 kcal) of this energy coming from added sugar, which meets the USDA guideline. The average fiber intake was 22.7±8.7 grams/day, which is below the recommendation. Added sugar intake was negatively associated with PWV (r=-0.291, p=0.033) but was no longer significant after controlling for age and

sex (r=-0.228, p=0.119). There were no associations between added sugar intake and AIx, FMD, or BP (all p>0.05). Both fiber and the GI were not associated with PWV, AIx, or FMD (all p>0.05). However, fiber intake was positively associated with systolic BP (SBP; r=0.315, p=0.026) and pulse pressure (PP; r=0.293, p=0.039); however, these no longer remained significant when controlling for age and sex (both p>0.05). GI was not associated with any measure of BP (all p>0.05). In conclusion, while added sugar was negatively associated with arterial stiffness and fiber was positively associated with SBP and PP, both counter to our hypotheses, neither relation remained significant when controlling for sex and age. In our cross-sectional study of young, healthy adults, various types of carbohydrate were not associated with declines in vascular function. Future work should include a wider range of intakes for both added sugars, fiber, and diet GI as well as account for the nutrient density of the sources of added sugar and fiber.

Chapter 1

INTRODUCTION

1.1 Cardiovascular Disease Significance

Cardiovascular disease (CVD) is the leading cause of death in the United States.[1] CVD is a blanket term for complications of the heart and blood vessels. This includes both chronic illness of the cardiovascular system and cardiovascular events. Chronic conditions involve long-term physiological impairments whereas CVD events are the potentially fatal consequences of these impairments. The most common CVD related causes of death are heart attack and stroke, both of which are cardiovascular events.[1] In the United States, 48% of the adult population has some form of CVD.[2] The high incidence of CVD places importance on identifying factors that contribute to the early progression of the disease.

1.2 CVD Indicators

Among CVD risk factors, high blood pressure (BP) is one of the most significant contributors to CVD development.[3] BP measurements include both systolic blood pressure (SBP) and diastolic blood pressure (DBP). SBP refers to the pressure during ventricular contraction while DBP represents the pressure during ventricular relaxation.[4] A larger portion of the cardiac cycle occurs during ventricular relaxation compared to ventricular contraction. Mean arterial pressure (MAP) represents the average pressure through one cardiac cycle and weighs DBP more heavily to account for this. Pulse pressure (PP), the difference between SBP and DBP is also used to assess CVD risk.[5] These BP values have several applications, but the most common use is to diagnose chronically high BP, also known as hypertension.

Recent guidelines define hypertension as a SBP value greater than 130 mmHg and/or a DBP value greater than 80 mmHg.[6] Hypertension is a CVD risk factor and is associated with the highest number of deaths due to CVD related complications.[7] According to NHANES data, 46.7% of adults in the U.S. have hypertension.[8] Hypertension development has been attributed to lifestyle factors, such as diet and physical activity, as well as genetics.[3,9] Overactivity of the sympathetic nervous system has also been linked to hypertension.[9] The sympathetic nervous system is associated with increased vasoconstriction which narrows the blood vessels, as opposed to vasodilation or the widening of blood vessels.[9]

Even at non-hypertensive BP levels, elevations in BP have been associated with increased CVD risk.[10] In a study that followed a healthy cohort, Whelton et al. found that increasing SBP levels below a hypertension diagnosis still resulted in an increased incidence of CVD and CVD risk factors.[10] The CVD risk factors included total cholesterol, low-density lipoprotein (LDL) cholesterol, fasting blood glucose, body mass index (BMI), and prediabetes.[10] A potential consequence of hypertension is atherosclerosis. Atherosclerosis is a local vascular disease in which plaque forms on the wall of the artery, resulting in it narrowing.[5] It develops through the oxidation of LDL in the endothelial cells of the arterial wall.[11] Oxidized LDL triggers an immune response, which leads to the formation and accumulation of foam cells on the arterial wall.[9] The buildup of foam cells hardens into plaque, which decreases blood flow and could eventually lead to CVD.[11] CVD has several different etiologies, but they all involve atherosclerosis.[12]

The hardening of the blood vessels seen in atherosclerosis can be evaluated by assessing arterial stiffness.[3] Arterial stiffness increases with age and has been

associated with CVD events and risk factors.[13] Central arteries are elastic, which is advantageous due to the large amount of blood that travels through them.[13] These vessels are comprised of three layers: the innermost tunica intima, which is made up of endothelial cells, the middle tunica media, which contains the protein elastin, and the outermost tunica externa, which is comprised of collagen. When arteries are damaged, in most cases due to increased BP, the flexible elastin is replaced with the more rigid collagen, resulting in increased stiffness.[13]

Two of the most common assessments of arterial stiffness are carotid to femoral pulse wave velocity (PWV) and pulse wave analysis (PWA), which entails the calculation of an augmentation index (AIx).[14,15] PWV involves measurements of the pulse wave at two different points in the arterial tree, typically at the carotid artery and femoral artery.[15] PWV is calculated by measuring the distance between the two points divided by the time between the upstroke in the proximal waveform and the upstroke in the distal waveform.[15] Blood flows faster in stiffer arteries, which means the delay between the two waveforms will decrease and therefore PWV will increase.[14] AIx represents wave reflection.[15] As blood travels forward through the arteries, an initial wave is created and followed by an additional, reflected wave moving backwards towards the heart.[16] Increased arterial stiffness causes changes in the amplitude and timing of the reflected wave.[16] Due to decreased elasticity of the blood vessels, the reflected wave returns to the heart during systole, augmenting SBP.[16] Augmentation pressure is the contribution to SBP by the reflected wave.[16] AIx is the ratio between augmentation pressure and PP.[16] Higher augmentation pressures represent a larger reflected wave, which indicates stiffer vessels.[16] As a result, a higher AIx is associated with stiffer arteries, similar to PWV.

While impairments in the tunica media and the tunica externa are related to arterial stiffness, impairments in the tunica interna are related to endothelial dysfunction. Endothelial dysfunction involves the impaired production and/or bioavailability of nitric oxide (NO), a vasodilator, by endothelial cells.[17] In addition, vasoconstrictors produced by the endothelium are increased.[17] Vasoconstriction causes increases in platelet deposition, which could lead to atherosclerosis.[17] In healthy endothelial cells, NO is produced by the enzyme eNOS (endothelial nitric oxide synthase) from L-arginine.[18] eNOS synthesis is induced through several different mechanisms, such as the mechanical shear stress caused by blood flow.[18] High BP results in vasoconstriction, which increases shear stress and leads to the damage and dysfunction of endothelial cells.[19] Another cause of damage to endothelial cells is oxidative stress, which can cause uncoupling of eNOS or oxidation of important cofactors necessary for eNOS activity.

A common way to assess endothelial function is flow-mediated dilation (FMD).[20] FMD involves comparing the diameter of an artery during reactive hyperemia to a baseline measurement.[20] To find the diameter of a given artery, an ultrasound is used.[21] The brachial artery is commonly used in FMD.[20] Other arteries (i.e., the femoral artery) have been used in special populations, such as children.[21] The procedure for brachial-artery FMD involves the use of a blood pressure cuff placed distal to the brachial artery.[20] A baseline diameter measurement of the brachial artery is taken before the cuff is inflated. Once inflated, blood flow to the forearm is restricted.[20] Typically after five minutes, the cuff is deflated, resulting in increased blood flow or shear stress in the brachial artery. This method can test signaling pathways involved in NO synthesis.[18,20] FMD is calculated as the change in diameter

of the brachial artery divided by the baseline diameter and is expressed as a percentage.[18] A higher FMD indicates healthier arteries which are capable of producing sufficient NO to react to the increase in blood flow following the release of the cuff.[20] Although, it should be noted that no guidelines exist establishing healthy and diseased values or cut-offs.

1.3 Nutrition and CVD

Several nutrients are associated with CVD and CVD risk factors, including sodium, potassium, and certain types of fat. A high sodium diet has been shown to have adverse effects on vascular health, mainly due to its association with BP.[22] However, work over the last 10 years has shown that sodium has BP-independent effects on the vasculature.[23,24] Sodium is an extracellular electrolyte; a high sodium intake leads to increased sodium concentration in the blood, resulting in an increase in blood volume which would then exert more pressure, leading to elevations in BP which could over time lead to hypertension.[25] The American Heart Association currently recommends a sodium intake of less than 2,300 mg/day for general cardiovascular health.[26] Despite this, the average American currently has an intake of 3,400 mg/day.[26] Similar to sodium, potassium has been linked to CVD through its relation to BP. However, there is less evidence to establish specific guidelines for CVD prevention like there is with sodium. The current adequate intake (AI) value for potassium is 3,400 mg/day for adult men and 2,600 mg/day for adult women.[27] Both of these values were previously set at 4,700 mg/day.[27] Due to this change, a higher percentage of the population is now meeting their potassium requirements, making it less of a concern, especially when compared to sodium. Although, there is evidence to

suggest that consumption of a high potassium diet can be beneficial for the vasculature even in the presence of a high sodium diet.[28]

Another nutrient that has been studied due to its connection to CVD is fat. As previously mentioned, LDL cholesterol contributes to the development of atherosclerosis.[29] LDL levels have been associated with intake of trans fatty acids (TFAs) and saturated fat.[30] TFAs are used to increase the shelf life of food, but they also lead to increased LDL levels.[30] Despite their use in food manufacturing, TFAs impose too great of health risk to be continued to be used.[31] As a result, the FDA banned the use of TFAs in 2018.[32] Similar to TFAs, it had been commonly accepted that increased total fat intake is associated with CVD risk and mortality through increases in LDL levels, but a meta-analysis by Zhu et al. in 2019 goes against this notion.[33] This study found that across 43 studies, total fat intake was not associated with CVD risk.[33] TFA intake, as expected, was associated with increased CVD risk, but it was the only dietary fat variable to have this relation.[33] In fact, polyunsaturated fatty acids (PUFAs) were found to decrease CVD risk in studies lasting at least 10 years.[33] This was explained by PUFAs decreasing oxidative stress and LDL levels.[33] Although the study by Zhu et al. found no association between saturated fatty acid (SFA) intake and CVD risk, several of the individual studies included in the analysis found increased CVD risk with high SFA intakes.[33] A different meta-analysis found that reduced SFA intake resulted in lower risk of CVD events.[34] In general, the relations between fat intake and CVD demonstrates that most fats have a deleterious effect on CVD risk.

In light of the relation between nutrition and CVD, dietary patterns to both treat CVD and reduce the risk of developing CVD have been developed.[35] Two well

recognized dietary patterns include the Mediterranean diet and the Dietary Approaches to Stop Hypertension (DASH) diet. The Mediterranean Diet prioritizes fruit and vegetable intake while replacing saturated fat with unsaturated fat sources, such as olive oil.[36] The Dietary Approaches to Stop Hypertension (DASH) diet aims to reduce blood pressure through decreasing sodium intake and increasing potassium intake.[37] Both of these dietary patterns are shown to be effective in treating CVD, but adherence when transitioning from a typical Western diet has proven difficult.[35-37] The reason for using these approaches is that the current Western diet increases CVD risk.[38] The more that these dietary recommendations are ingrained into average eating habits, the easier it will be to not only fully switch to CVD-treating diet but also prevent CVD from developing in the first place.

1.4 Carbohydrates and CVD

Inspired by the evidence that nutrition plays a role in CVD development, recent studies have looked into the role of nutrients that have received less attention to this point. Nutrients are classified based on function.[39] The three macronutrients (carbohydrates, proteins, and fats) can be used to create energy in the cell.[39] Of these three, carbohydrates are the preferred energy source by many cell types in the human body.[39] A study by Kelly et al. found that total carbohydrate intake was not associated with CVD risk and events.[40] However, total carbohydrate intake may be a poor measure of diet quality as different types of carbohydrates have been associated with varying CVD outcomes, similar to different types of fats.[40] Due to this, specific carbohydrates have been looked at independently to evaluate their relations to CVD without being obscured by other carbohydrates.

Different types of carbohydrates serve different functions and are classified based on their structure.[41] Structurally, carbohydrates vary by size and digestibility.[41] Complex carbohydrates are long, sometimes branched, chains of monosaccharides which need to be broken down before they are metabolized.[41] For example, starch has to be broken down into individual sugar molecules to be absorbed.[41] Fiber is a type of complex carbohydrate that is unable to be broken down by the human digestive system.[41] Despite this, dietary fiber has several biological roles.[41] This includes involvement in the gut microbiota, blood glucose regulation, and defecation.[41] Fiber has an established relation with CVD as it decreases cholesterol absorption.[42] This subsequently decreases the LDL concentration, a risk factor for CVD.[43] Dietary sources of fiber include fruits, vegetables, and whole grains.[44] However, eighty-five percent of American adults do not meet the recommendations for these food groups.[44] Similarly, 90% of women and 97% of men do not meet their respective daily fiber recommended dietary allowances of 25 grams and 38 grams.[44,45]

Simple carbohydrates are comprised of only one or two sugar molecules, meaning they can be absorbed faster than complex carbohydrates.[41] Glucose is the most common simple carbohydrate and its concentration in the bloodstream is tightly regulated by several hormones, most importantly insulin.[41] Simple carbohydrates incorporated into a substance during food processing are called added sugars.[46] This differs from natural sugar found in foods like fruits. Generally, foods high in added sugar are less nutrient-dense than foods low in added sugar.

Both fiber and added sugar affect blood glucose concentration, but in opposing directions.[47] Fiber slows absorption, delaying the postprandial increases in blood glucose.[41] On the other hand, added sugar causes blood glucose levels to rise

quickly as little to no bonds between sugar molecules have to be broken before absorption.[41] Since substances contain different types of carbohydrates in variable amounts, the number of carbohydrates alone is insufficient to predict the glycemic response.[48] The glycemic index (GI) of a food refers to its effect on blood glucose concentrations relative to a reference, which is usually either glucose or white bread.[49] A food's GI depends on composition of the carbohydrates in the food rather than the quantity of carbohydrates.[50] High fiber foods generally have a low GI while high added sugar foods generally have a high GI.[47] Although a food's GI does not account for the amount consumed, glycemic load (GL) takes into account carbohydrate quantity in addition to carbohydrate quality.[51]

1.4.1 Added Sugar

A systematic review by Song et al. found the relation between added sugar and CVD mortality to be inconsistent across studies.[52] That being said, they did note a general trend across the 17 eligible cohort studies that CVD mortality was higher in adults whose added sugar intake exceeded 20% of total energy.[52] For reference, the USDA 2020-2025 Dietary Guidelines for Americans recommends that less than 10% of energy should come from added sugar.[44] While this study establishes a relation between added sugar intake and CVD mortality, the methods are only observational.

A review of 21 randomized controlled trials (RCTs) using differing amounts of added sugar in adults was conducted.[53] The primary outcomes were all-cause mortality and cardiovascular events.[53] However, no studies met their eligibility criteria of non-diabetic adults with no history of CVD.[53] As a result, they focused on secondary outcomes, which included BP, blood lipid levels, and blood glucose levels.[53] The authors divided the participants into a high added sugar intake group and a low added

sugar intake group, although the definitions for these interventions varied across the individual studies.[53] Those who consumed more added sugar had a higher SBP and DBP as well as total cholesterol and triglyceride levels.[53] There were no differences in LDL cholesterol, HDL cholesterol, or fasting blood glucose.[53] The authors noted that these were minor differences that have no clinical implications.[53]

Markey et al. conducted a crossover RCT which included a diet containing regular sugar and a diet containing sugar-reduced foods and beverages.[54] The study was designed to look at body weight outcomes with an added sugar intervention, but they also evaluated biomarkers of CVD and arterial stiffness using AIx.[54] Each diet was followed for 8-weeks with a 4-week washout period in between.[54] There were no differences in body weight between the two diets, which was attributed to participants compensating for decreased amounts of sugar with additional fat and protein.[54] While there was a decrease in added sugar intake, there was no difference in arterial stiffness or CVD-related variables, such as BP and blood lipid levels.[54] Although this study does compare differing added sugar intakes with arterial stiffness, it was not designed to evaluate arterial stiffness as a primary outcome and as a result does not include additional measurements such as PWV.[55] A study by The et al. did compare both AIx and PWV to added sugar intake, but the population studied was type I diabetic adolescents. This study found a relation between added sugar intake and PWV only when controlling for BMI.[55] There was no association between added sugar intake and AIx, even when controlling for BMI.[55]

A crossover study by Loader et. al found that consumption of a sugar-sweetened beverage (SSB) resulted in acute decrements in endothelial function when compared to consumption of water in healthy, non-smoking, sedentary adult males.[56]

The intent behind using a SSB was to isolate the effects of added sugar. In this study, water was used as a control.[56] Participants had an average brachial artery FMD of 6.53% 40 minutes after consuming an SSB compared to 8.56% 40 minutes after consuming water.[56] The proposed mechanism for this significant difference is increased oxidative stress due to hyperglycemia which can decrease NO bioavailability, resulting in endothelial dysfunction.[56] Even though this study showed that added sugar has a negative effect on endothelial function in the short-term, it cannot make any conclusions about the long-term vascular consequences of added sugar consumption.[56]

1.4.2 Fiber

A study by van de Laar et al. found that low lifetime fiber intake is associated with arterial stiffness.[57] The study followed 373 participants in the Netherlands starting from age 13 to 36.[57] Fiber intake was assessed throughout the 24-year period using a dietary history interview involving participants recalling their intake from the past month.[57] When the participants were 36 years old, ultrasonography was performed on 3 major arteries including the brachial, carotid, and femoral arteries.[57] From their measurements, they calculated arterial stiffness variables similar to AIx.[57] The association they found between low lifetime fiber intake and increased arterial stiffness was mainly attributed to lower consumption of fruits, vegetables, and whole grains.[57] There were no relations between lifetime fiber intake and other fiber-related CVD risk factors, such as BP and blood lipids, but the authors stated that this could be due to the young and healthy population studied.[57]

Similar to added sugar, studies involving fiber consumption and endothelial function have looked at postprandial effects rather than dietary patterns. Two acute

studies varied meal composition and measured postprandial FMD.[58,59] One of these studies used four breakfast meals comprised of varying amounts of fiber and fat to see if fiber consumption neutralizes postprandial endothelial dysfunction in healthy adolescents.[58] They found the high-fat, low-fiber meal resulted in a significant decrease in FMD four hours after the consumption of the meal.[58] This effect was not observed in the high-fat, high-fiber meal, which was attributed to fiber limiting fat absorption.[58] This was evidenced by decreased triglyceride levels in the high-fat, high-fiber meal compared to the high-fat, low-fiber meal.[58] While these results were promising, further analysis comparing the meals showed that the trends for the two high-fat meals with differing amounts of fiber were not significantly different.[58] Furthermore, it is difficult to apply these findings to adults given that the population was comprised of adolescents.[58] The other acute study compared endothelial function using type II diabetics who received two different meals with varying fiber content.[59] The meals included either brown rice (high-fiber) or white rice (low-fiber).[59] The assessment used for endothelial function was similar to FMD, but instead of assessing changes in artery diameter, total blood flow during reactive hyperemia was assessed.[59] After the 8-week intervention, the authors found an improvement in endothelial function in the brown rice group.[59] The opposite was seen in the white rice group (5.8% decreased from baseline).[59] The authors mentioned that it is difficult to compare dietary interventions in diabetics with a healthy population due to the difference in glycemic response.[59]

1.4.3 Glycemic Index

The relation between GI and CVD has been evaluated using the GI of the entire diet as well as the GI of individual meals. A meta-analysis by Jenkins et al.

looked at the relation between the GI of the diet as a whole and the incidence of cardiovascular events.[50] This study found that a high GI diet was associated with an increased risk of a cardiovascular event in adults ages 35 to 70 years.[50] This relation was observed in participants with and without preexisting CVD.[50] Another meta-analysis looked at both GI and GL with CVD risk factors and all-cause mortality.[51] The study found that high GI diets are associated with increased CVD risk factors and mortality in healthy populations while high GL diets are associated with severe heart disease and all-cause mortality in diseased populations.[51] Although the two dietary variables were associated with different outcomes, the general recommendation by the authors is to decrease both the GI and GL of the diet to achieve improved cardiovascular health.[51]

A substance's GI has been evaluated in the context of the carbohydrates of which it is comprised. A study by Gaesser et al. looked at the effect of cereal fiber on postprandial endothelial function in a healthy adult population.[60] This was done by using four meals with varying fiber and GI: a high-fiber/high-GI meal, a high-fiber/low-GI meal, a low-fiber/low-GI meal, and a low-fiber/high-GI meal.[60] Consumption of the high-fiber/low-GI meal resulted in an increased FMD value compared to the baseline measurement.[60] The high-fiber/low-GI meal was the only meal to have a statistically significant impact on FMD.[60] Other outcomes included the two low-GI meals improving the glycemic response and the high-fiber/low-GI meal decreasing insulin production.[60]

Associations between GI and arterial stiffness have been researched using methods similar to the previously discussed acute studies involving endothelial function. A study by Kelsch et al. looked at the effect of a high-GI meal and a low-GI

meal on PWV after three hours of sitting.[61] After both meals, PWV increased after sitting.[55] There was no difference in PWV between the two meals.[61] This relation was likely observed due to the study population being young, healthy adults.[61] A different study used four meals varying in GI and GL that assessed AIx.[62] The low-GI, low-GL meal resulted in a reduction in AIx four hours after the meal compared to baseline.[62] The findings of this study differ from those of Kelsch et al. in that a low-GI meal resulted in different arterial stiffness outcomes than its high-GI counterparts.[61,62] These conflicting results could be due to differing methods.[61,62] In both studies, the subjects remained sedentary after the consumption of the meal, but measurements were taken at different times (three hours vs four hours after consumption).[61,62] In addition, one study used PWV while the other used AIx.[61,62] More work is needed in this area to evaluate the effect of GI and GL on measures of arterial stiffness.

1.4.4 Summary

Epidemiological studies have shown that although total carbohydrate intake has no association with CVD outcomes, the type of carbohydrate and carbohydrate-related measurements do.[40,50,52] High amounts of added sugar consumption has been associated with increased CVD mortality and CVD biomarkers.[52,53] In addition, consumption of a beverage with a high amount of added sugar has resulted in acute endothelial dysfunction.[56] Although a few studies looked at both added sugar intake and arterial stiffness, none have studied arterial stiffness as a primary outcome.[54,55] Lifetime intake of dietary fiber has been shown to decrease AIx, but there are no data to support a relation between fiber intake and PWV.[57] Fiber-containing meals have been shown to improve FMD both following a meal and after an eight-week intervention.[58,59] Diets with a high GI have been tied to CVD risk factors as well as

decrements in postprandial endothelial function in the absence of fiber.[50,51,60] The relation between GI and arterial stiffness has been inconsistent across studies.[61,62] The current literature on types of carbohydrates and their relations with arterial stiffness and endothelial function has focused primarily on the postprandial response rather than regular dietary intake. It is not known if associations between different types of carbohydrate and measures of vascular function exist in young healthy adults which may be a precursor to more established CVD later in life.

1.5 Aims and Hypotheses

Aim: Examine the relation between indices of carbohydrate intake with measures of vascular function and blood pressure in healthy young adults.

Hypothesis 1: A higher intake of added sugar will be positively associated with PWV, AIx, and BP and negatively associated with FMD.

Hypothesis 2: A higher intake of dietary fiber will be negatively associated with PWV, AIx, and BP and positively associated with FMD.

Hypothesis 3: A diet with a higher GI will be associated with a greater PWV, AIx, and BP and lower FMD compared to a diet with a lower GI.

Chapter 2

METHODS

2.1 Participant Recruitment and Screening

Healthy men and women between the ages of 18 and 45 years were recruited to participate in this cross-sectional study. Participants were recruited through university-based communication systems and flyers. After obtaining written informed consent, enrolled participants completed a laboratory screening visit to determine eligibility. Exclusion criteria included obesity (BMI > 30 kg/m^2), high blood pressure (SBP ≥ 140 and/or DBP ≥ 90 mmHg), pregnancy, use of tobacco products, or current diagnosis or history of chronic diseases such as diabetes, heart disease, or kidney disease.

2.2 Screening Visit

During the screening visit, a medical history questionnaire was completed, participant height and weight were measured, and seated BP after 10 minutes of rest was measured in triplicate and averaged. This study was approved by the Institutional Review Board of the University of Delaware (ID# 1008199). Participants were also given an ambulatory BP monitor (Oscar 2, SunTech Medical, Morrisville, NC).

2.3 Dietary Intake Assessment

A 3-day diet record was used to assess habitual dietary intake. Participants recorded their food and beverage intake during three days between their screening visit and their study visit. Two of these days were weekdays and the other one was a weekend day. The 3-day diet record was returned during the vascular testing visit and was analyzed using the Nutrition Data System for Research (NDSR 2018 and 2019, University of Minnesota, Minneapolis, MN, USA). The NDSR variable “Glycemic

Index Glucose Reference" was used to represent glycemic index. The NDSR variable "Added Sugars by Available Carbohydrate" was used to represent added sugar intake. The NDSR variable "Total Dietary Fiber" was used to represent dietary fiber intake.

2.4 Ambulatory Blood Pressure Monitoring

Participants were asked to wear an ambulatory BP monitor for a 24-hour period prior to their testing visit. They were instructed to wear the BP cuff on their upper arm midway between the elbow and the shoulder. BP was taken every 20 minutes during the day and every 30 minutes during the night. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and pulse pressure (PP) were recorded.

2.5 Vascular Testing Visit

Participants were instructed to fast the night prior to their two-hour vascular testing visit and to not consume any caffeine or alcohol at least 12 hours prior to their visit. Participants were also asked to not exercise for 24 hours before the visit. At the beginning of their visit, a venous blood sample was drawn and later analyzed to provide a lipid panel (total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglyceride levels) and blood glucose value.

Arterial stiffness was assessed by carotid to femoral pulse wave velocity (PWV) using the Sphygmocor XCEL system (ATCOR Medical, Sydney, Australia). With the participant in a supine position, a tonometer was placed on the carotid artery and a blood pressure cuff was placed on the upper thigh to simultaneously capture the carotid and femoral pressure waveforms. The distance between these two sites was measured as the distance from the top of the thigh cuff to the sternal notch minus the

distance from the carotid artery to the sternal notch. PWV was calculated as the measured distance divided by the average measured time delay between the initial upstroke of 12 consecutive corresponding carotid and femoral waveforms.

Augmentation index (AIx), a measure of wave reflection, was also assessed using the Sphygmocor XCEL system (ATCOR Medical, Sydney, Australia). A brachial cuff placed on the left arm was used to capture a brachial waveform, which in turn was used to synthesize a central aortic waveform. AIx was calculated as the ratio between augmented pressure and central pulse pressure and expressed as a percent: $AIx = (P_2-P_1)/(P_s-P_d)$, where P_1 and P_2 are the first and second shoulders of systolic pressure, respectively, P_s is peak systolic pressure, and P_d is end diastolic pressure.

Endothelial function was assessed using brachial artery flow-mediated dilation (FMD) according to published guidelines.[20] During this test, participants were supine with their right arm supported at heart level, perpendicular to their body, with a small BP cuff on their forearm approximately 3 cm distal to the antecubital crease. Longitudinal images of the brachial artery were recorded using an ultrasound (GE P5; GE Healthcare, Waukesha, WI, USA). A one-minute recording of the brachial artery was obtained before inflating the blood pressure cuff to 200 mmHg for five minutes. Then, the cuff was deflated, reactive hyperemia ensued, and the recording continued for two minutes. Ultrasound images were transmitted to a National Instruments IMAQ PCI-1411 image acquisition board at a frequency of 30 frames/second, and brachial artery diameter was determined using Quipu 3.6.0 software. The value for FMD is reported as the percent change of the diameter of the brachial artery from baseline to the peak diameter during reactive hyperemia.

2.6 Statistical Analysis

Data were checked for outliers and assessed for normality prior to analysis. Differences in baseline characteristics between men and women were assessed using independent samples t test. Associations between the dietary intake and vascular measurements were assessed by Pearson bivariate and partial correlations, the latter of which controlled for age and sex. Analyses were performed using SPSS (IBM, version 28) and significance was set at $p<0.05$. Data are reported as mean and standard deviations (SD).

Chapter 3

RESULTS

3.1 Participant Characteristics

In this study, there were a total of 55 participants of which 21 were men and 34 were women. Average baseline participant characteristics are shown in **Table 1**. A majority of the participants were non-Hispanic Whites. As expected, the average participant had a normal BMI and had a BP in the normotensive range although men had a higher SBP and DBP compared to women. Women had higher HDL levels; however, there were no differences in total cholesterol or LDL levels. All lab values were within normal limits for both men and women.

Table 1. Screening characteristics of the study population.

Characteristic	All (n=55)	Men (n=21)	Women (n=34)	P value
Age, years	26.7 ± 6.1	27.5 ± 5.8	26.1 ± 6.2	0.42
Race, n (W/B/A)	44/2/9	14/1/6	30/1/3	
Ethnicity, n (NH/H)	51/5	20/2	31/3	
BMI, kg/m^2	23.5 ± 3.0	24.2 ± 2.9	23.0 ± 3.0	0.14
Screening SBP, mmHg	113 ± 12	117 ± 10	110 ± 12	0.01
Screening DBP, mmHg	69 ± 9	74 ± 9	67 ± 9	<0.01
Screening MAP, mmHg	84 ± 9	88 ± 8	81 ± 9	<0.01
Screening HR, bpm	65 ± 8	63 ± 8	67 ± 7	0.15
Total Cholesterol, mg/dL	160 ± 27	162 ± 30	159 ± 26	0.75
HDL Cholesterol, mg/dL	55 ± 12	48 ± 11	60 ± 10	0.01
LDL Cholesterol, mg/dL	89 ± 26	96 ± 26	84 ± 25	0.19
Triglycerides, mg/dL	80 ± 37	91 ± 51	73 ± 20	0.18
Blood Glucose, mg/dL	88 ± 6	87 ± 6	88 ± 7	0.53

A, Asian; B, Black; BMI, body mass index; DBP, diastolic blood pressure; H, Hispanic; HDL, high density lipoprotein; HR, heart rate; LDL, low density lipoprotein; MAP, mean arterial pressure; NH, non-Hispanic; SBP, systolic blood pressure; W, White. P values represent the difference between men and women. Values are displayed as mean ± SD unless otherwise stated.

3.2 Nutrient Intake

Average dietary intakes are shown in **Table 2**. Participants consumed around 2,000 kcal/d on average. Men consumed significantly more energy than women. This was also true of carbohydrates and protein, but not fat. Each macronutrient fell within its Acceptable Macronutrient Distribution Range (AMDR). Men and women consumed similar percentages of macronutrients except for fat, where women had a higher percentage. Women exceeded the recommendation for added sugar as a percent of energy (>10%), but not in grams (<50 g/d).[2] Neither men or women met the fiber recommendation of 37 g/d and 25 g/d, respectively.[3] Men consumed significantly more potassium, phosphorus, and iron.

Table 2. Habitual dietary data for the study population.

Nutrient	All (n=55)	Men (n=21)	Women (n=34)	P value
Energy, kcal/day	2039 ± 553	2312 ± 675	1870 ± 384	<0.01
Carbohydrate, g/day	237.9 ± 75.7	270.9 ± 87.0	217.5 ± 60.6	0.01
% Energy	46.4 ± 10.2	48.0 ± 12.8	45.3 ± 8.1	0.33
Protein, g/day	88.3 ± 35.0	107.7 ± 45.0	76.3 ± 19.6	<0.01
% Energy	17.4 ± 5.0	18.7 ± 5.2	16.6 ± 4.9	0.13
Fat, g/day	81.5 ± 29.6	87.7 ± 39.2	77.6 ± 21.6	0.22
% Energy	34.5 ± 7.9	31.7 ± 8.5	36.2 ± 7.2	0.04
Glycemic Index	58.8 ± 4.3	58.3 ± 4.4	59.1 ± 4.3	0.53
Added Sugar, g/day	48.4 ± 27.3	48.8 ± 26.6	48.2 ± 28.1	0.95
% Energy	9.6 ± 5.3	8.5 ± 4.3	10.3 ± 5.9	0.23
Total Fiber, g/day	22.7 ± 8.7	24.8 ± 10.8	21.4 ± 7.1	0.16
Sodium, mg/day	3210.1 ± 1082.1	3565.4 ± 1270.8	2990.7 ± 898.7	0.06
Potassium, mg/day	2772.2 ± 890.8	3141.7 ± 1148.3	2544.0 ± 599.0	0.01
Phosphorus, mg/day	1307.7 ± 372.0	1493.0 ± 409.0	1193.3 ± 300.0	<0.01
Calcium, mg/day	943.4 ± 363.2	1033.1 ± 433.1	888.1 ± 306.5	0.15
Magnesium, mg/day	335.3 ± 111.4	369.2 ± 134.2	314.3 ± 90.6	0.08
Iron, mg/day	14.7 ± 5.3	17.3 ± 5.2	13.0 ± 4.7	<0.01

Values are displayed as mean ± SD. P values represent the difference between men and women.

3.3 Hemodynamics

The BP and vascular measures including arterial stiffness and endothelial function are reported in **Table 3**. Twenty-four-hour PP was significantly higher in men compared to women. Twenty-four-hour SBP was also higher in men compared to women, though the difference did not reach statistical difference. Twenty-four-hour HR was significantly higher in women compared to men. There were no other differences between men and women for any of the other BP or vascular measurements.

Table 3. Vascular measurements and 24-hour ambulatory blood pressure monitoring.

Characteristic	All (n=55)	Men (n=21)	Women (n=34)	P value
PWV, m/s	5.6 ± 0.7	5.6 ± 0.7	5.6 ± 0.7	0.82
AIx, %	5.4 ± 11.9	2.4 ± 13.1	7.3 ± 10.8	0.14
FMD, %	7.8 ± 2.7	7.6 ± 2.8	7.9 ± 2.7	0.78
24-h SBP, mmHg	114 ± 10	118 ± 8	112 ± 11	0.06
24-h DBP, mmHg	67 ± 7	68 ± 6	66 ± 8	0.46
24-h MAP, mmHg	83 ± 7	84 ± 6	82 ± 8	0.24
24-h PP, mmHg	48 ± 7	50 ± 6	46 ± 6	0.04
24-h HR, bpm	70 ± 9	66 ± 8	73 ± 9	<0.01

AIx, augmentation index; DBP, diastolic blood pressure; FMD, flow mediated dilation; HR, heart rate; MAP, mean arterial pressure; PP, pulse pressure; PWV, pulse wave velocity; SBP, systolic blood pressure; Values are displayed as mean ± SD. P values represent the difference between men and women.

3.4 Pulse Wave Velocity

The relations between PWV and the carbohydrate-related dietary variables of GI, added sugar, and dietary fiber are shown in **Figure 1**. There was a negative association between added sugar and PWV only when not accounting for age and sex. There were no statistically significant associations between GI or dietary fiber and PWV.

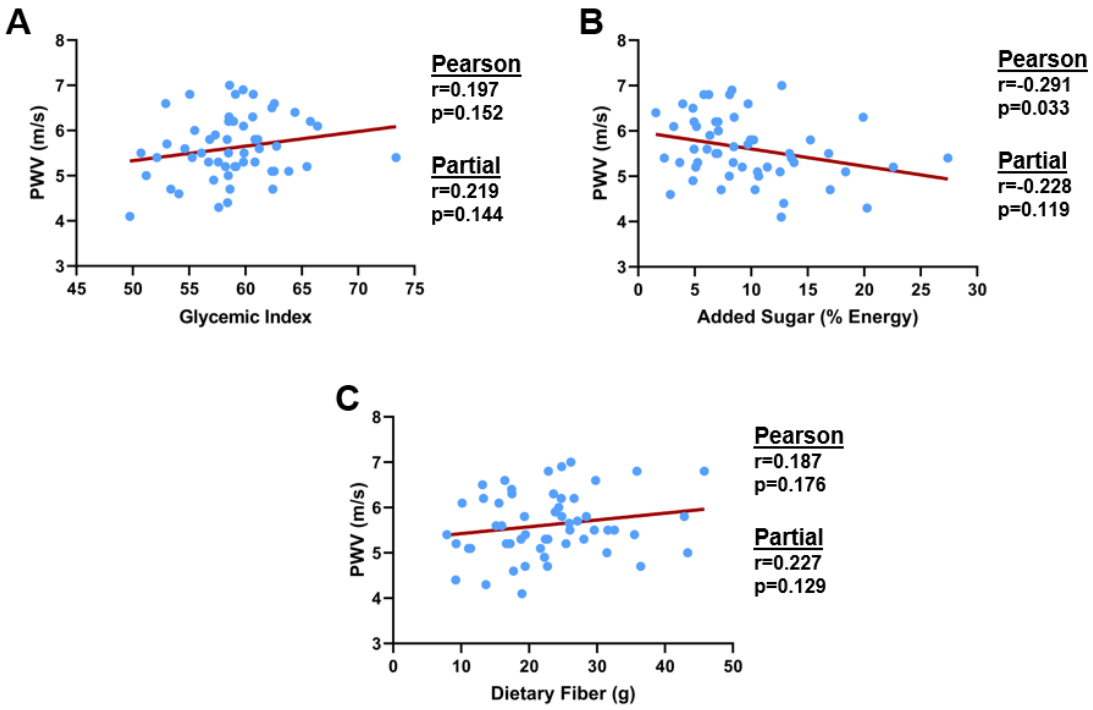

Figure 1. Associations between various indices of carbohydrate intake and pulse wave velocity (PWV). A) glycemic index and PWV, B) added sugar and PWV, and C) dietary fiber and PWV.

3.5 Augmentation Index

The relations between augmentation index (AIx) and the carbohydrate-related dietary variables of glycemic index, added sugar, and dietary fiber are shown in **Figure 2**. There were no statistically significant associations between GI, added sugar, or dietary fiber and AIx.

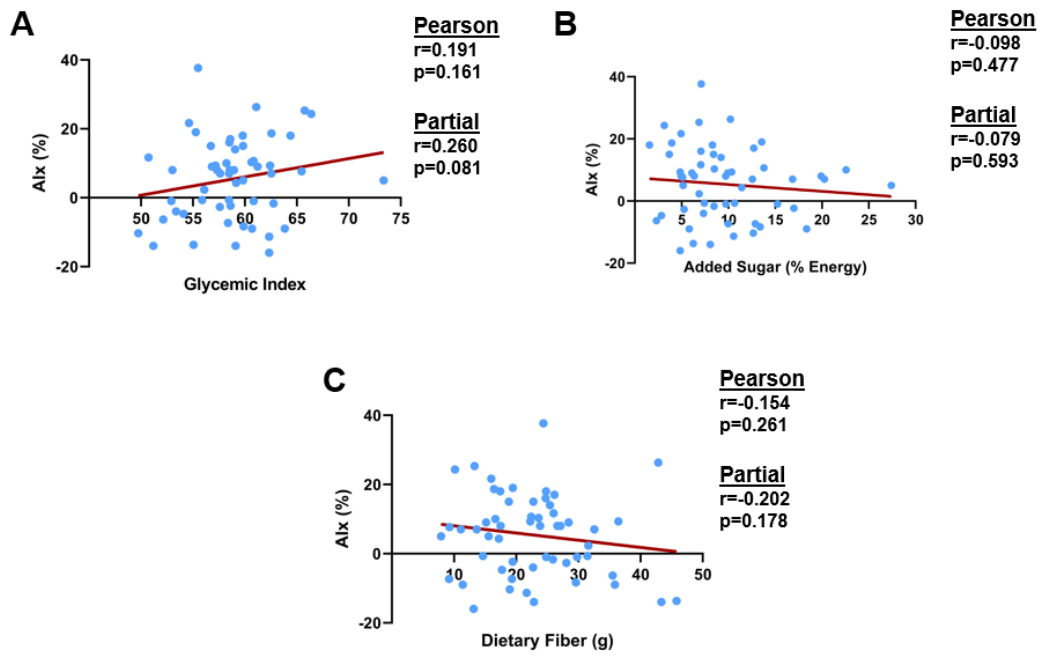

Figure 2. Associations between various indices of carbohydrate intake and augmentation index (AIx). A) glycemic index and AIx, B) added Sugar and AIx, and C) dietary Fiber and AIx.

3.6 Brachial Artery Flow-Mediated Dilation

The relations between brachial artery flow-mediated dilation (FMD) and the carbohydrate-related dietary variables of glycemic index, added sugar, and dietary fiber are shown in **Figure 3**. There were no statistically significant associations between glycemic index, added sugar, or dietary fiber and FMD.

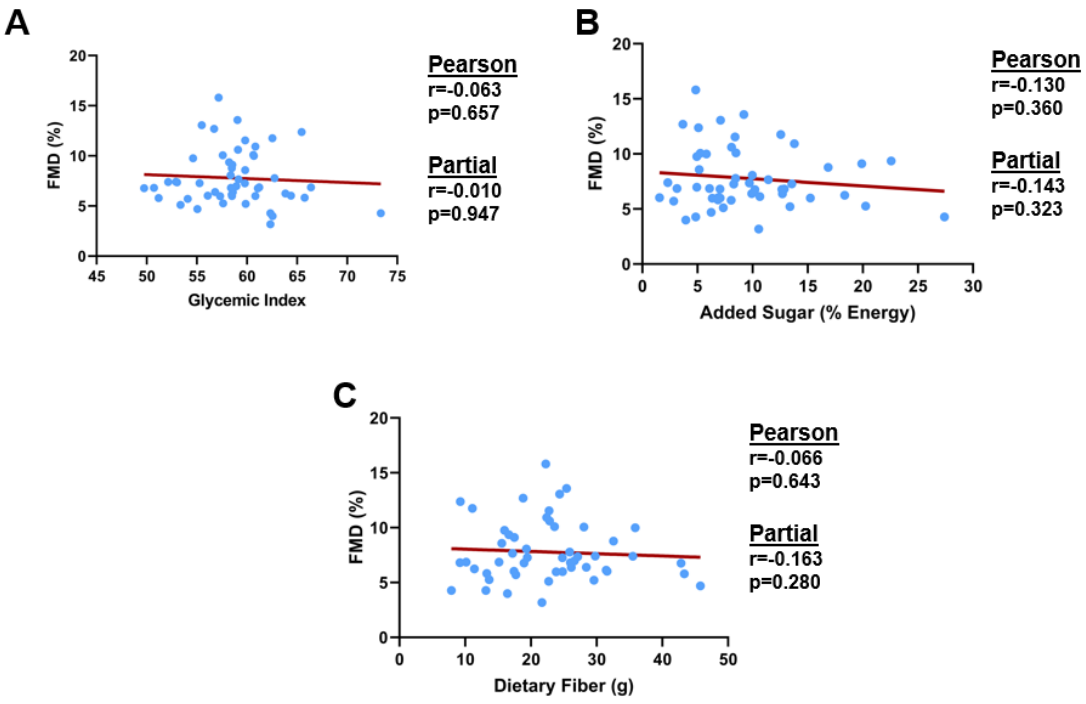

Figure 3. Associations between various indices of carbohydrate intake and flow-mediated dilation (FMD). A) glycemic index and FMD, B) added sugar and FMD, and C) dietary fiber and FMD.

3.7 24-Hour Blood Pressure

The relations between 24-hour ambulatory SBP and DBP with the carbohydrate-related dietary variables of GI, added sugar, and dietary fiber are shown in **Figure 4**. There was an association between SBP and dietary fiber intake such that as dietary fiber increased, SBP increased. This association was lost when age and sex were controlled for. There were no other statistically significant associations between GI, added sugar, or dietary fiber and 24-hour SBP. There were no statistically significant associations between glycemic index, added sugar, or dietary fiber and 24-hour DBP.

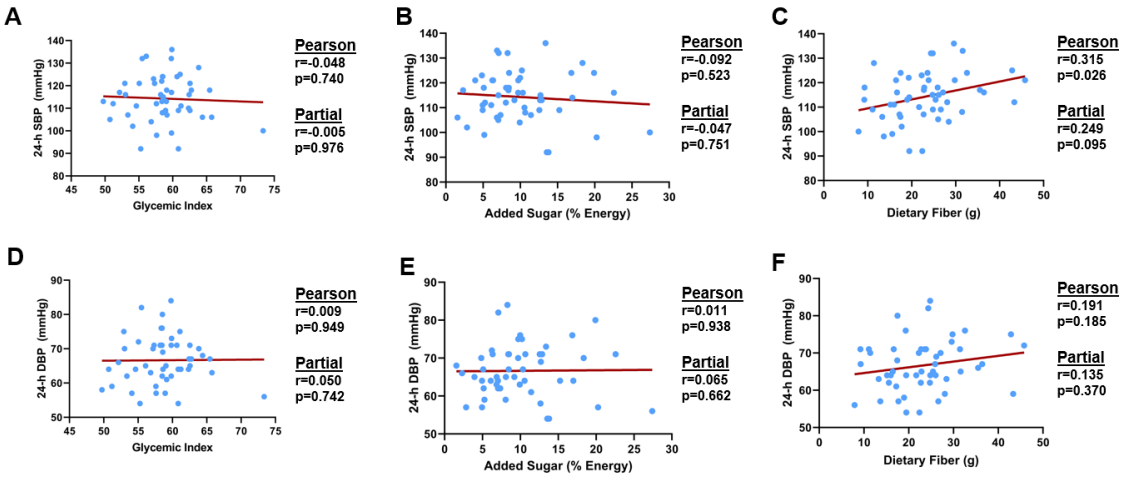

Figure 4. Associations between various indices of carbohydrate intake and blood pressure. A) glycemic index and SBP, B) added sugar and SBP, C) dietary fiber and SBP, D) glycemic index and DBP, E) added sugar and DBP, F) dietary fiber and DBP.

The relations between 24-hour ambulatory MAP and PP with the carbohydrate-related dietary variables of GI, added sugar, and dietary fiber are shown in **Figure 5**. There were no statistically significant associations between GI, added sugar, or dietary fiber and 24-hour MAP. There was a positive association between PP and dietary fiber intake only when not accounting for age and sex. There were no other statistically significant associations between GI, added sugar, or dietary fiber and 24-hour PP.

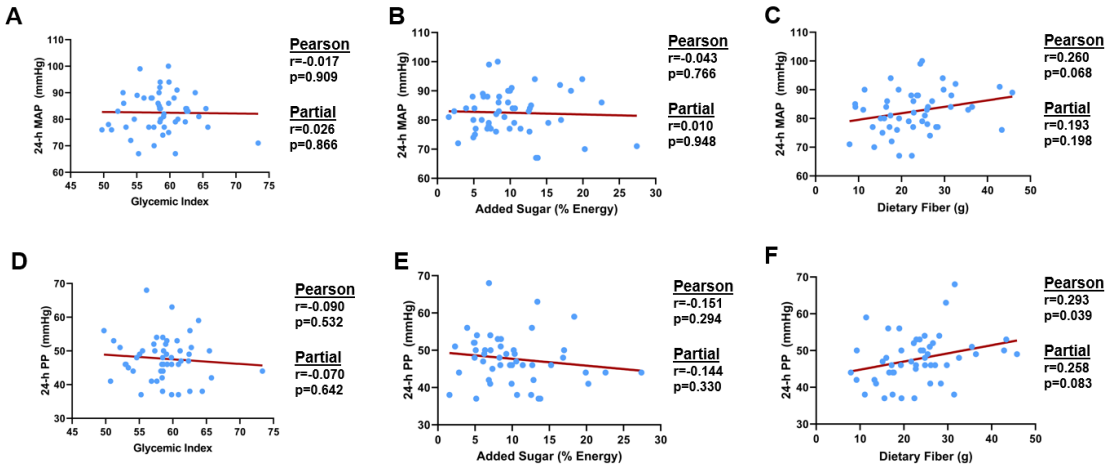

Figure 5. Associations between various indices of carbohydrate intake and blood pressure. A) glycemic index and MAP, B) added sugar and MAP, C) dietary fiber and MAP, D) glycemic index and PP, E) added sugar and PP, F) dietary fiber and PP.

Chapter 4

DISCUSSION

The aim of this study was to examine the relations between indices of carbohydrate intake and measures of vascular function in healthy young adults. We examined added sugar intake, dietary fiber intake and the GI in their habitual diets. Both fiber intake and GI were not significantly related to measures of arterial stiffness, wave reflection, or endothelial function. Of the vascular measures, added sugar was only associated with PWV. This result suggests that added sugar intake is negatively associated with increased arterial stiffness, which is in contrast to our hypothesis. When examining BP, the only significant relations were between fiber intake and SBP as well as fiber and PP. This suggests that higher dietary fiber is positively associated with BP which was in contrast to our hypothesis. These significant relations were lost when controlling for age and sex.

The association between added sugar intake and PWV prior to controlling for age and sex was negative, which would suggest that a diet high in added sugar was associated with decreased arterial stiffness. After controlling for age and sex, this relation was no longer significant. It should be mentioned that all participants had a PWV in the normal range that was not different between men and women. Typically, it is not until a PWV is > 10 m/s that vessels are considered stiff, and this is somewhat age dependent.[63] We had hypothesized that a greater added sugar intake would be associated with a faster PWV. The basis for this hypothesis was evidence from previous studies suggesting that added sugar could play a role in the development of CVD. Added sugar intake has been positively associated with CVD, specifically CVD mortality, when intakes exceeded 20% of total energy.[52] This relation was observed in

a systematic review of observational studies which included studies of healthy participants over the age of 20.[52] At intakes below 20% of total energy, there was no association between added sugar intake and CVD-related variables. Notably, this association was dependent on the source of added sugar as consumption of foods and beverages that contained sufficient nutrients other than added sugar did not increase mortality risk.[52] In our study, average added sugar intakes fell just below the recommended upper limit of 10% of energy (9.6 ± 5.3%).[45] Notably, average intakes for the women in the study were just above the recommendation (10.3 ± 5.9%) although not statistically different from the men. Although average intakes were close to or above the recommendation, they rarely exceeded the 20% threshold established to be positively associated with increased CVD mortality risk.[52] Indeed, the added sugar intake of our sample could be too low to observe any negative associations with vascular health. The systematic review also only included longitudinal studies and used CVD mortality as an outcome, which differs greatly from our study which was cross-sectional and had CVD risk factors as outcomes.

The population studied and the study designs used in previous research that focused on CVD risk factors as outcomes differ from the methods used in our study. Many of these studies were acute or chronic interventions, used a wider age range or older participants. For example, higher added sugar intake has been previously positively associated with an increase in BP, but specifically in older women.[64] Several studies have used a young, healthy population but utilized a dietary intervention, such as a study which found that FMD decreased one hour following the consumption of a high-added sugar beverage in a sample of young, healthy men.[56] The authors attributed their finding to acute hyperglycemia causing metabolic stress which

decreased NO availability.[56] Although postprandial hyperglycemia does not occur exclusively after added sugar consumption, high-added sugar foods and beverages may lack micronutrients which prevent hyperglycemia. Another study found that the consumption of blueberries alongside an energy-dense meal improved the glycemic response compared to the same meal without blueberries.[65] However, there was no difference in PWV, AIx, FMD, or BP between the two meals.[65] The authors attributed these findings to studying a population with metabolic syndrome.[65] Indeed, other studies that featured a healthy population saw improved postprandial FMD after the consumption of a meal high in phytochemicals.[65,66,67] These findings relate to the systematic review which looked at CVD mortality as the authors mentioned that the association between added sugar intake and CVD outcomes depended on the inclusion of other nutrients.[52] Our findings that there were no associations between added sugar intake and CVD risk factors after controlling for age and sex could be due to looking at added sugar independent of nutrient density. A future study could look at the relations between added sugar intake from specifically energy-dense food compared to PWV, AIx, FMD, and BP. Another direction could be observing these relations over a longer period of time as our study was cross-sectional.

In our study, most of the vascular outcomes were not associated with fiber intake. The two variables that were significantly related to fiber intake were SBP and PP. Higher SBP and PP are risk factors for CVD and may indicate poorer vascular health. The Amsterdam Growth and Health Longitudinal Study found that lower consumption of a fiber rich diet in youth was associated with increased carotid artery stiffness in adulthood, but not AIx.[57] It may be that if our participants were followed prospectively for a number of years, those with a lower fiber intake may develop

stiffer arteries and present with greater wave reflection. We did find that these two associations were no longer significant after controlling for age and sex. Additionally, these relations were not significant when looking at men and women separately. Differences between sexes are likely due to men having a significantly higher ambulatory PP and a nearly significantly greater SBP. Although not significant, men did consume more fiber than women, which is not unexpected due to their greater energy intake. It should be noted that the average fiber intake for both men and women fell below the recommendations, thus the range of intakes may not have been sufficient to find significant relations between fiber and BP and vascular health.[45] Furthermore, this study also tracked consumption of different food groups. They found that those who consumed fewer fruits, vegetables, and whole grains throughout their life also tended to have stiffer arteries.[57] Hence, the prospective study design allowed them to monitor dietary intake across 24 years, which gave them far more information than collected in the singular study visit in our study.

The food source of fiber may play a role in its associations with vascular measures, similar to added sugar. Evans et al. analyzed 28 studies that compared fiber intake to BP.[68] Additionally, they analyzed the studies in separate groups depending on the type of fiber consumed.[68] A BP lowering effect was seen with β-glucans, a fiber commonly found in oats and barley.[68] A more recent study found that an oat bran supplement lowered BP in a population with hypertension.[69] Another systematic review looked at psyllium, another type of fiber supplementation.[63] They found that psyllium supplementation reduced SBP, especially in those with a higher baseline BP.[63] Both β-glucans and psyllium are soluble fibers.[63,68] Both of the reviews and the oat bran study included participants with high BP and obesity, which suggests that

those with an elevated BP and/or obesity may be more responsive to fiber interventions.[68,69] As mentioned previously, our study also did not look at the food sources of fiber, which may have been significant given the previous evidence that specific types of fiber lower BP.[63,68,69] Furthermore, out participants had a normal BP. Similar to our data on added sugar, future directions in this area could look at food sources with the same vascular outcomes as well as changes over time.

The prior findings that diet GI was positively associated with CVD risk factors in older, diseased populations did not carry over to a young, healthy population.[50] This relation was only approaching significance when looking at AIx after controlling for age and sex ($p=0.081$). As previously mentioned, several studies reported an increase in arterial stiffness following the consumption of a high-GI meal compared to a low-GI meal when evaluating AIx, but not PWV.[61,62] We report similar findings although all of our associations between GI and measures of vascular function were not significant.

Intervention studies have used high-GI and low-GI diets in the range of 45 to 65.[70] Generally, diets with GI values less than 55 were designated as a low-GI group and diets with GI values greater than 70 were designated as a high-GI group.[70] Only one participant in our sample had a diet that exceeded a GI of 70. This could mean that the range of GI values in our sample was too narrow to observe any associations between an increased diet GI and vascular outcomes. Notably, there were several participants below the low-GI threshold which is where relatively healthier vascular outcomes were expected to be seen. Interestingly, low-GI (<55) diets all fell below the trendline for the associations between GI and FMD. Previous studies that compared GI and FMD used the GI of a meal rather than diet GI, making it difficult to compare the

results.[54] The use of diet GI may be more applicable when designing an intervention study as the diet GI of the average young, healthy adult may not feature the extremes needed to see associations between diet GI and CVD risk factors.

Few studies featuring vascular outcomes have used diet GI as a dietary variable. A systematic review and meta-analysis of studies comparing diet GI with BP found that a lower GI diet was consistently associated with decreased DBP and inconsistently associated with decreased SBP.[70] However, the authors mentioned that a diet could have a low GI for several different reasons as there are multiple factors that could affect the GI of an individual food.[70] The observed beneficial associations between low-GI diets and BP could be due to the high soluble fiber content and low intake in sweetened drinks found in these diets. This is consistent with studies examining added sugar and fiber intake on BP. While sweetened drinks may be high in added sugar, added sugar intake may not be fully representative of the detrimental associations of these beverages with vascular health as other foods are high in added sugar. Similarly, specific types of fiber have been shown to decrease BP, but the same effects have not been observed in all types of fiber.

Chapter 5

CONCLUSIONS

5.1 Strengths and Limitations

The strengths of this study include the use of tools chosen to assess dietary intake and vascular function. NDSR is considered the gold standard for analyzing diet records.[71] The use of ambulatory BP monitoring gives a more complete view of participants' BP than a single BP measure in the laboratory setting. This study was also novel in that it assessed several measures of vascular function in conjunction with diet. Previous studies had either used acute interventions with FMD, PWV, or AIx, or assessed usual dietary intake but only looked at long-term CVD outcomes, such as mortality. The major limitation of this study is its observational nature. Thus, it is impossible to determine any causation in the relations that were studied. In addition, the aforementioned novelty also provided difficulty in comparing results to prior research.

5.2 Future Directions

Future observational studies regarding the carbohydrate-related variables used in this study may benefit from looking at specific sources of added sugar and fiber. Future interventional studies using the vascular measures from this study could involve the use of groups on either side of the recommendations for added sugar and fiber as well as diets above and below the thresholds for high-GI and low-GI since PWV, AIx, and FMD have rarely been used as outcomes in studies with similar designs.

5.3 Conclusions

Dietary intakes of different types of carbohydrates were associated with indicators of cardiovascular health in a young, healthy population. Added sugar was negatively associated with PWV and fiber was positively associated with 24-hour SBP and PP. These associations were in opposition to the hypothesized relations. However, they were no longer significant after controlling for age and sex, suggesting that these were confounding variables. Added sugar consumption may have to greatly exceed the recommendations for there to be an association with poor vascular health, which did not occur in our study. Similarly, fiber recommendations may have to be met to observe positive associations with vascular outcomes. The food sources of added sugar and fiber could also play a role in their relations with vascular health. Finally, the GI of the diet was not associated with any of the vascular outcomes. Diet GI may be more appropriate for intervention studies so that more extreme values can be captured. The findings of this study suggest that different types of carbohydrates did not have a deleterious effect on vascular health in our healthy young subjects.

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