THE ACUTE EFFECTS OF A HIGH GLYCEMIC INDEX MEAL COMBINED WITH
PROLONGED SITTING ON VASCULAR FUNCTION: A RANDOMIZED CROSSOVER TRIAL

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ABSTRACT

Elizabeth Kelsch: The Acute Effects of a High Glycemic Index Meal Combined with Prolonged Sitting on Vascular Function: A Randomized Crossover Trial
(Under the direction of Lee Stoner)

Chronic prolonged sitting and consumption of high glycemic index (GI) meals are known to increase the risk of cardiovascular diseases. However, it is currently unknown whether prolonged sitting and consumption of a high GI meal have a combined negative acute effect on local and central arterial stiffness (AS). Therefore, 18 young, healthy participants (21.7±2.5 y, 70% F, 25.5±6.1 kg/m^2) were randomized to: 3h sitting with a high GI beverage (HGI) or a low GI beverage (LGI). Prolonged sitting (3 hours) and consumption of a HGI beverage increased central (brachial-femoral) AS, in the LGI (0.27 m/s) and the HGI (0.45 m/s) condition (p=0.027). Peripheral (femoral-ankle) PWV increased (0.211 m/s) regardless of the level of GI consumed. Carotid-femoral PWV was not influenced by prolonged sitting or HGI meal consumption. Sitting increases central and peripheral AS in young, healthy individuals. Consuming a HGI meal has a moderate detrimental effect on central (brachial-femoral) PWV.

KEY WORDS: Arterial stiffness; pulse wave velocity; high glycemic; near infra-red spectroscopy; sedentary; inactivity

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LIST OF ABBREVIATIONS

AIx   Augmentation Index

ANS   Autonomic Nervous System

APL   Applied Physiology Laboratory

AS   Arterial Stiffness

AUC   Area under the curve

BF   Blood Flow

BP   Blood Pressure

CGM   Continuous Glucose Monitor

CO   Cardiac Output

cSBP   Central Systolic Blood Pressure

CV   Cardiovascular

CVD   Cardiovascular disease

ET-1   Endothelin-1

GI   Glycemic index

Hb   Hemoglobin

HbO_2 Oxygenated Hemoglobin

H-GI   High glycemic index

HHb   Deoxygenated Hemoglobin

HR   Heart Rate

HRV   Heart Rate Variability

L-GI   Low glycemic index

MAP	Mean Arterial Pressure
NIRS	Near Infrared Spectroscopy
NO	Nitric Oxide
PRU	Peripheral Resistance Units
PWA	Pulse Wave Analysis
PWV	Pulse Wave Velocity
SB	Sedentary Behavior
SRS	Spatially Resolved Spectroscopy
SV	Stroke Volume
T2D	Type 2 diabetes
tHb	Total Hemoglobin
TSI	Tissue Saturation Index

DEFINITION OF TERMS

Arterial Stiffness — Describes distensibility, compliance, and elastic modulus of the arterial vascular system

Flow Mediated Dilation — Any vasodilatation of an artery following an increase in luminal blood flow and internal-wall shear stress

Glycemic Index — A relative ranking of carbohydrate in foods according to how they affect blood glucose levels

High Glycemic Index — Simple carbohydrate; in the present study, GI of 100

Low Glycemic Index — Index Complex carbohydrate; in the present study, GI of 19

Postprandial period — The period of time following consumption of a meal

Prolonged Sitting — Acute bout of sitting lasting for >30 minutes

Pulse Wave Velocity — Velocity Measures arterial stiffness, speed of the pressure wave

Shear Stress — Velocity Measures arterial stiffness, speed of the pressure wave

Turbulent Blood Flow — Nonlaminar blood flow

CHAPTER I: INTRODUCTION

Chronic sedentary behavior, defined as very low intensity behaviors (≤1.5 metabolic equivalents) in a seated, reclined or supine posture[122] has been directly tied to the development of cardiovascular disease (CVD). This is a concern because the majority of American adults are sitting for 70% of their day[1], and CVD cost the US $555 billion in medical bills, prescriptions, home-care, and other services in 2016[2]. While the chronic associations between repeated exposure to prolonged sitting and CVD are well known, the mechanism by which an acute bout of sitting leads to cardiovascular decline is less clear. Additionally, sitting is typically accompanied by other poor lifestyle choices, such as the consumption of high glycemic index (GI) meals[124]. Regular dietary exposure to high GI meals has also been linked to the development of CVD[66,107]. Prolonged sitting following consumption of a high GI food is common in today's society[1,66]; however, the combined acute effect of both prolonged sitting and consumption of a high GI meal on the cardiovascular system is unclear.

It has been shown that sitting for three hours results in decreased blood flow to the legs and subsequent leg endothelial dysfunction[3,4]. Current unpublished findings from our laboratory demonstrate that this local impairment translates to modest (0.3 m/s) central cardiovascular impairments, specifically increased central arterial stiffness. Additionally, high GI meals, which induce postprandial hyperglycemia, have been linked to an increase in arterial stiffness[52] and endothelial dysfunction via increased oxidative stress[46,50-51]. To date, no studies have investigated the combined acute effects of prolonged sitting and high GI meal consumption on the local and central cardiovascular system.

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The long-term goal from this study is to elucidate the acute effects of prolonged sitting and consumption of a high GI meal, which will allow for a better understanding of the mechanism linking these chronic exposures to CVD. This will either lead to the need for research of strategies to reduce the dose of sedentary behavior and high GI consumption, or a need for greater understanding surrounding the specific positive contributions healthy lifestyle choices have on "protecting" against these stimuli. While both sedentary behavior and repeated consumption of HGI foods are associated with metabolic, vascular, and cognition dysfunction, this study focused on central and peripheral vascular function, via pulse wave velocity (PWV). To achieve this, this study compared a HGI meal versus a low glycemic index (LGI) meal during a bout of 3 hours of sitting and explored the effects on central and peripheral PWV in a young, active, healthy population. We also looked at perfusion to the medium gastrocnemius to assess blood pooling in the lower extremities.

Aim: To examine the acute effects of prolonged sitting with consumption of a low or high glycemic index meal on local and central cardiovascular health

Twenty healthy, young and physically active (>90 min/week) participants were recruited. Using a randomized cross-over design, each participant completed two conditions: prolonged sitting (3 hours) plus low GI meal consumption (L-GI), and prolonged sitting plus high GI meal consumption (H-GI). The primary outcome was central arterial stiffness (AS), and the secondary outcome was local (leg) arterial stiffness.

1. Compared to L-GI, does H-GI result in greater increase in central AS?

2. Compared to L-GI, does H-GI result in a greater increase in leg AS?

Research Hypotheses:

1. During prolonged sitting, H-GI will result in a greater increase in central AS.

2. During prolonged sitting, H-GI will result in a greater increase in leg AS.

ASSUMPTION AND DELIMITATIONS

Delimitations:

1. Women were studied during the follicular phase of their menstrual cycle in order to control for fluctuations in reproductive hormones.

2. Participants were matched for risk factors to avoid confounding effects.

3. All participants had similar dietary intake prior to and during both testing sessions

4. All participants were between the ages of 18 and 35.

5. All participants engaged in regular physical activity (>90 min/week Mod-Vig intensity)

6. Researchers encouraged no movement throughout the protocol.

Limitations

1. Only two NIRS probes were available for use, and were only able to measure the Hb in the tissue directly beneath the probe.

2. The 3-hour protocol may have under estimated what is needed to evoke the desired physiological changes for the purpose of this study.

CHAPTER II - REVIEW OF LITERATURE

INTRODUCTION TO TOPIC

Adults in the U.S. spend approximately 50-70% of their waking hours sitting[110]. The American workplace has seen an 83% increase in sedentary desk jobs since 1950 111]. Recent findings have shown that prolonged sitting is linked to vascular impairment, via endothelial dysfunction and arterial stiffness, and has been associated with cardiovascular diseases (CVD), metabolic syndrome, and all-cause mortality[3,11,72]. Additionally, the Western diet, which commonly includes highly refined sugars with a high GI, has been shown to increase risk for developing CVD[42,99].

In order to understand the association between chronic exposure to prolonged sitting and high GI foods and the acute response, we need to assess the known acute effects of these two stimuli separately. The following review will outline the current findings in the literature concerning prolonged sitting and high GI meals. The effects of prolonged sitting will be broken down by blood flow, vascular function, and central cardiovascular effects. The effects of a high GI meal will be broken down by hormone response, the effect of glucose and insulin on the local vasculature, and central CV effects. A proposed mechanism for the combined acute effects of sitting and consumption of a high GI meal will then be explored (figure 1).

CONSIDERATION 1: VASCULAR RESPONSES TO PROLONGED SITTING

Chronic effects of prolonged sitting

Prolonged sitting has been a topic of growing interest due to the gradual shift in our world's work environment. The prevalence of sedentary behavior and increased time spent

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sitting, common to many professions, has been associated with the development of CVD[3,4,17–20]. Many studies have linked chronic prolonged sitting with the development of type II diabetes, due to the acute effect of decreased insulin sensitivity[21]. There is now enough evidence to conduct meta-analyses exploring the association between sedentary time and disease incidence, concluding that prolonged sedentary time is independently associated with deleterious health outcomes regardless of physical activity[11]. More specifically, these deleterious health outcomes include impaired physical function[112], CVD risk-factors[113,114], T2DM risk-factors[115], and mortality[11,116].

Acute effects of prolonged sitting

During a period of prolonged sitting, blood will pool in the lower limbs due to the lack of muscular contractions facilitating venous return to the heart[3,4,25]. The lack of muscle pump will impair the uptake of insulin by the muscle, and could lead to insulin resistance. Blood pooling will lead to reduced overall leg blood flow, and in turn, reduced shear stress. Since shear stress in a vessel is essential for maintaining endothelial health[22], impaired shear stress leads to impaired endothelial function. Additionally, the biomechanics of the sitting position has been shown to reduce shear stress by 45% when compared to straight legs in a supine position[4]. Overall, prolonged sitting is associated with an acute impairment in aortic shear stress and subsequently aortic endothelial function leading to aortic arterial stiffness, which could potentially lead to cardiometabolic disease in the future.

Blood Flow

In the context of sitting, it is known that long bouts of uninterrupted sitting leads to a decrease in lower limb blood flow[3]. There are several proposed mechanisms for this physiological response. Increased hydrostatic pressure from gravitational forces that act on the limb vasculature when sitting, as well as lack of venous return via muscle pump, cause blood to pool in the legs, which causes a decrease in total blood flow to the legs. Another proposed

mechanism is adrenergic vasoconstriction due to the increased muscle sympathetic nerve activity that is elicited in the seated position[23]. It was also theorized that circulating vasoconstrictor factors may increase leg vascular resistance leading to decreased blood flow[3]. One study found that after 6 hours of sitting, the ability of resistance vessels in the leg to vasodilate was impaired by 40%[3]. This suggests that nitric oxide (NO), a vasodilator, may be contributing to decreased lower limb blood flow.

Vascular Function

All blood vessels have an inner layer composed of endothelial cells that secrete nitric oxide (NO), among other molecules[24]. NO is secreted in response to shear stress in the vessel[3]. Shear stress is the force produced by blood physically moving along the endothelial cells and is proportional to blood flow. Shear stress contributes to endothelial health[3]. As shear stress increases, via increased blood flow, NO is released causing the vessel to dilate, which leads to increased blood flow and shear stress, maintaining endothelial health.

Decreased shear stress has been noted as the source of vascular dysfunction during prolonged sitting[3,4,25]. A decrease in shear stress has been linked to the production of endothelin-1 (ET-1)[26]. Endothelial cells express ET-1, which leads to the formation of vascular reactive oxygen species, reduced NO availability, and impaired endothelium-dependent dilation[22,27–31]. Therefore, it is known that prolonged sitting leads to decreased blood flow to the legs, decreased leg vascular shear stress, impaired vasodilator abilities, and impaired endothelial function. However, these local impairments also translate to whole-body cardiovascular effects and other systems of the body due to the interaction between the cardiovascular system and the central nervous system and endocrine system.

One additional measure of vascular function is arterial stiffness. Arterial stiffness (AS) refers to the dispensability of artery walls, determined by both structure and function of the vessel, and has been noted as an independent predictor of CVD[32]. Typically, AS is measured

in the aorta, so it is considered a measure of central cardiovascular health. AS has been shown to increase with sedentary behavior independent of age and disease[33], as well as after a short bout of sitting[34–36,108,120]. Studies have also shown that endothelial NO and shear stress are related to arterial stiffness (AS)[37,38]. Since AS is dependent on structure and function of the vessel, if endothelial function is impaired, there will be subsequent effects on AS. Therefore, we conclude that prolonged sitting has a negative effect on central cardiovascular health in addition to local vascular health.

CONSIDERATION 2: ACUTE RESPONSES TO A HIGH GLYCEMIC INDEX MEAL

Other lifestyle factors influence CVD disease risk, including the consumption of high glycemic index meals. Glycemic index (GI) quantifies the glycemic response following the consumption of a carbohydrate-rich food. Foods with a high GI are accompanied with an elevation of postprandial plasma glucose levels, rather than the gradual glucose response that is seen after consuming a low GI meal[42,43]. Hyperglycemia in the postprandial state has been linked to oxidative stress, which, in conjunction with nitric oxide (NO) synthesis disruption, leads to macrovascular endothelial dysfunction[44,45]. There is increasing evidence that the postprandial state is a contributing risk factor for the development of atherosclerosis in health adults[46,47].

Hormone Responses

Blood glucose begins to rise approximately 10 minutes after the start of a meal[47]. Following consumption of a high GI meal or food, blood glucose rises significantly and puts the body in a state of postprandial hyperglycemia. The beta cells of the pancreas respond by releasing insulin into the bloodstream. The release of insulin leads to the mobilization of GLUT4 receptors from inside cells to the cellular membrane to allow for glucose uptake. The hyperglycemia response to a high GI meal is followed by a hypoglycemic state, due to the secretion of insulin by the beta cells of the pancreas, as seen in recreationally active

males[48,49]. This oscillation in blood glucose levels has been associated with oxidative stress and endothelial dysfunction[50,51], and was found to have more negative effects of endothelial function than constant hyperglycemia in both normal and type 2 diabetic populations[51].

Local cardiovascular effects from glucose

Acute hyperglycemia has been shown to cause endothelial dysfunction within minutes or hours[47,53–55]. The proposed mechanism for this impaired endothelial function is that acute hyperglycemia increases the production of free radicals, which reduces NO availability, and inhibits vasodilation[53,55–57]. Endothelium-derived NO is considered the most potent vasodilator[58]. The production of free radicals from hyperglycemia that leads to endothelial dysfunction is known as oxidative stress. Oxidative stress can elicit changes in DNA, proteins, lipids, and give rise to various disease states[59].

Local cardiovascular effects from insulin

Insulin is an endothelial-dependent vasodilator in skeletal muscle vasculature[60–63]. This has been shown to aid in insulin-mediated glucose uptake in skeletal muscle[61,64]. When insulin is secreted by the beta cells of the pancreas, it facilitates blood flow to skeletal muscle via capillary recruitment and vasodilation to allow glucose to be taken up more readily. The mechanism behind insulin-mediated vasodilation is via stimulation of nitric oxide production in endothelial cells[65]. After the consumption of a high GI meal, postprandial hyperglycemia triggers hyperinsulinemia[66]. Hyperinsulinemia is a higher-than-normal blood insulin concentration, and is linked to the development of coronary heart disease[67–69].

Central CV

The acute effects of consuming a high glycemic meal are harmful on local cardiovascular components. However, the acute effects of a high glycemic index meal on central cardiovascular measures are unclear. It is known that chronically, high GI meals contribute to CVD[47,50,70,71]. It has been shown that chronic postprandial hyperglycemia, a

direct result from consumption of a high GI meal, doubles the risk for cardiovascular mortality[15]. Since chronic consumption of high GI meals significantly contribute to CVD risk and mortality, the acute response in the central CV system should be studied in order to identify potential mechanisms to link acute response to chronic disease.

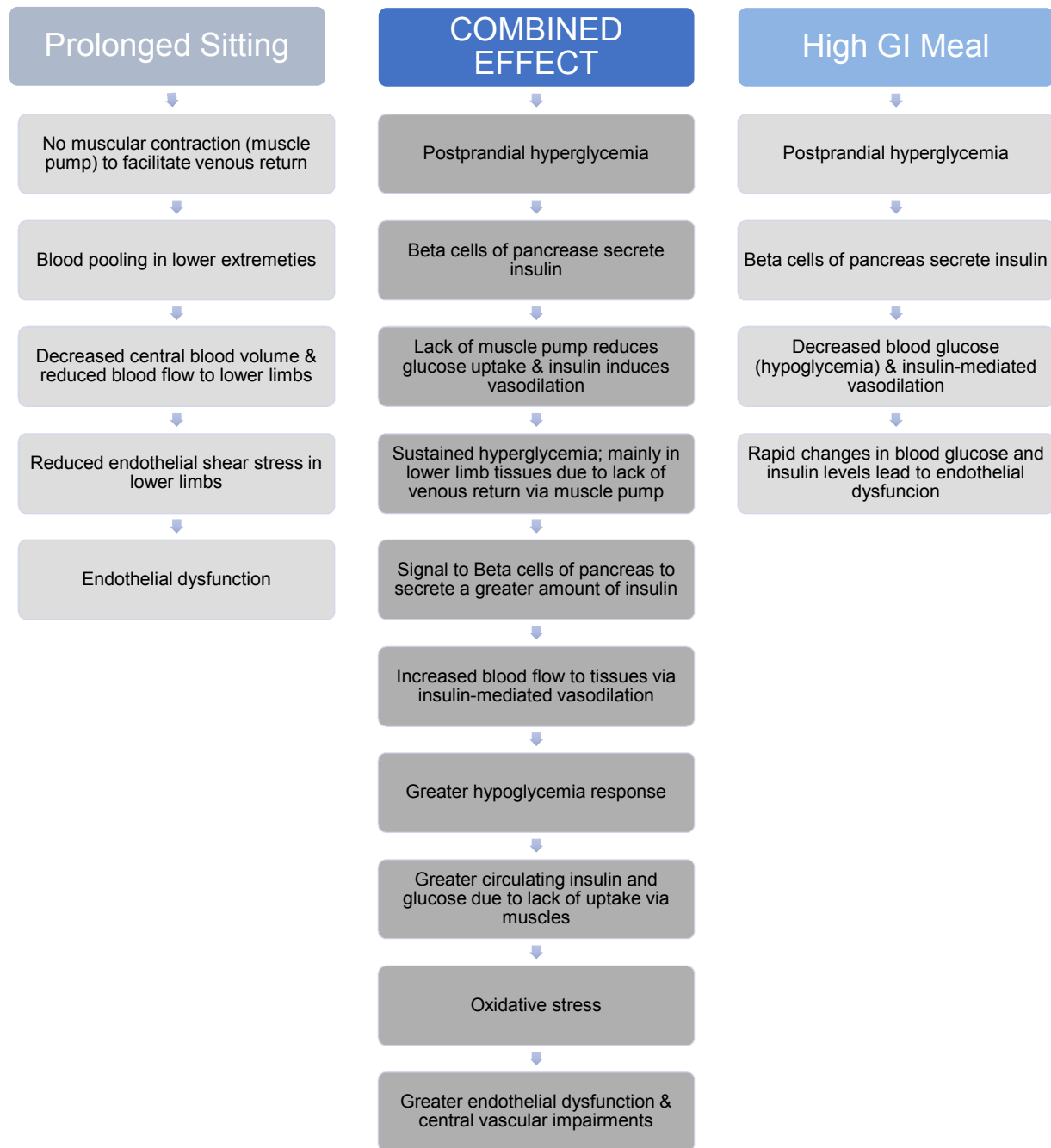| Prolonged Sitting	COMBINED EFFECT	High GI Meal
No muscular contraction (muscle pump) to facilitate venous return	Postprandial hyperglycemia	Postprandial hyperglycemia
Blood pooling in lower extremeties	Beta cells of pancrease secrete insulin	Beta cells of pancreas secrete insulin
Decreased central blood volume & reduced blood flow to lower limbs	Lack of muscle pump reduces glucose uptake & insulin induces vasodilation	Decreased blood glucose (hypoglycemia) & insulin-mediated vasodilation
Reduced endothelial shear stress in lower limbs	Sustained hyperglycemia; mainly in lower limb tissues due to lack of venous return via muscle pump	Rapid changes in blood glucose and insulin levels lead to endothelial dysfuncion
Endothelial dysfunction	Signal to Beta cells of pancreas to secrete a greater amount of insulin	
	Increased blood flow to tissues via insulin-mediated vasodilation	
	Greater hypoglycemia response	
	Greater circulating insulin and glucose due to lack of uptake via muscles	
	Oxidative stress	
	Greater endothelial dysfunction & central vascular impairments	

Figure 1. *The proposed mechanism for the individual and combined effects of prolonged sitting and high GI meal on the cardiovascular system*

IMPLICATIONS/WHY IS THIS STUDY NEEDED?

It is known that both prolonged sitting and consumption of a high GI meal have acute negative effects on local cardiovascular measures. However, it is unknown how these two stimuli interact and what their combined effect on local vasculature is, and it is unknown how the central cardiovascular system is affected as a result. Currently, no studies have observed the effects of a high GI meal and prolonged sitting on the cardiovascular system in young, healthy adults. If this gap in knowledge is elucidated, it will contribute to the larger picture of how sedentary behavior interacts with other lifestyle factors and how healthy adults are affected. This will either lead to the need for research of strategies to reduce the dose of sedentary behavior and high GI consumption, or a need for greater understanding surrounding the specific positive contributions healthy lifestyle choices have on "protecting" against these stimuli.

METHODOLOGICAL CONSIDERATIONS

The following section will outline proposed methodology in order to test the proposed mechanism. To date, there has been no research looking into the effects of prolonged sitting and consumption of a high glycemic index meal on the local and central cardiovascular measures. Two major considerations in the study design were external validity and internal validity. External validity refers to the extent to which study results can be generalized to a population. Internal validity refers to how well a study controlled for confounding variables, and allows a cause-effect relationship to be established. The appropriate participants and study design were used to control external validity, and the appropriate instrumentation was used to control internal validity.

STUDY DESIGN CONSIDERATIONS

The gold standard study design for research design is a randomized controlled trial. However, this study design would require participants to be divided into two groups and there may be confounding variables between the two groups. A cross-sectional study design could

also be appropriate, but we would not be able to deem causality, so it is not the most optimal. Therefore, the best study design for the desired outcome was a double-blind randomized crossover design with two experimental conditions (L-GI and H-GI) and the primary outcome being central cardiovascular health via aortic pulse velocity (PWV). A crossover design allows participants to act as their own control, which reduces the chance for confounders.

MEASUREMENT CONSIDERATIONS

Vascular Function

Vascular health can be measured using endothelial function, central blood pressure, pulse wave analysis, and arterial stiffness. Endothelial function can be measured using ultrasound flow mediated dilation (FMD) of the brachial artery, but this requires a highly trained ultrasound technician. Additionally, previous research has found that prolonged sitting plays a role in the impairments of lower limb vascular health (via arterial stiffness), but it does not translate to brachial FMD[3,22,72]. Therefore, measuring endothelial function is not feasible for this experimental design.

Arterial stiffness (AS) is a common measurement for vascular health, and more feasible than other measures. AS is directly related to cardiovascular disease risk[73]. Several methods have been introduced to assess AS including pulse wave velocity (PWV), ultrasound-derived indicators, pulse pressure, MRI-derived indicators, and pulse wave analysis (PWA)[73,74]. The gold standard measurement for AS is carotid-femoral PWV[75–77]. PWV can be assessed with applanation tonometry, the gold standard, using the PulsePen, the SphygmoCor device, or the Complior device [78,79], as well as ultrasound, MRI, or the Vicorder device. The vicorder device (SMT Medical), has been shown to be a valid and reliable measure of PWV [78–82]. Like the other measures, the vicorder is a non-invasive assessment of PWV. However, the vicorder is less intrusive and requires very little operator training[80]. The Vicorder device is able to measure central aortic Pulse Wave Velocity (PWVa). Arterial stiffness and AIx are independent

risk factors for coronary artery disease [83]. PWA is a reliable and valid measure for arterial

stiffness and endothelial function [77,84,85]. According to Vlachopoulos *et al*., a >1 m/s change

in PWV correlated to a 14% increase in CV events, 15% increase in CV mortality, and a 15%

increase in all-cause mortality[86]. Similarly, a 10% increase of Aix correlated to a 32% increase

in CV mortality, and a 38% increase in all-cause mortality[86].

Blood Pooling

Throughout a bout of sitting, blood pools in the lower limbs. It is necessary to measure

this pooling to ensure we are getting the predicted response from sitting, as well as be able to

contextualize pooling among the other vascular responses. As time spent sitting increases,

hemoglobin is expected to rise. Previous work from this lab showed a 24% increase in tHb from

10 to 90 minutes of sitting in a young, sedentary population[121]. Blood pooling in the calf was

measured by looking at tissue perfusion assessed using near infrared spectroscopy (NIRS)

(PortaMon, Artinis, Medical Systems). NIRS is a reliable and valid method for assessing tissue

perfusion [89]. A NIRS probe was placed on the medial gastrocnemius of the right leg to

determine the total amount of hemoglobin, as well as oxygenated and deoxygenated, in the

tissue directly underneath.

Blood Glucose

Blood glucose was assessed as dependent measures of the metabolic system. The two

methods for measuring blood glucose levels are a finger prick or venous draw and testing via a

glucometer, or a continuous glucose monitor (CGM). Using a glucometer will allow blood

glucose levels to be attained immediately, whereas most CGMs require information to be

downloaded to a computer before viewing. However, using a CGM allows continuous readings

of blood glucose levels, whereas a finger prick reading from a glucometer only provides a one-

time reading. Using a CGM will enable us to find potential trends in glucose levels. A continuous

glucose monitor (iPro2, Medtronics) was used throughout the protocol on each experimental

day to measure real-time interstitial glucose. The CGM provided relevant information on hyper and hypoglycemia, as well as glucose variability [88].

Meal Intervention

The literature was searched extensively to determine the most appropriate high and low GI beverage choices. This is represented in **Table 1.** Most studies matched for macronutrient content and the average low GI used was ~36 and the high GI ~80 [16,49,51,93–104].

Table 1. Low and High GI meals used in literature

Author	Low GI meal	GI	High GI meal	GI	Matched
Kaviani 2016	Boiled lentils, tomato sauce and Canola oil	26	Instant mashed potato, egg whites and white bread	76	Macronutrient content
Ludwig 1999	Veggie omelet & fruit	N/A	Instant oatmeal with sweetener (glucose plus artificial sweetener) + milk treated with 2 drops of lactase to increase the GI of the milk sugar	N/A	N/A
Nilsson 2007	Spaghetti	58	White wheat bread	100	Starch (g/serving)
Clark 2006	Psyllium ready-to-eat cereal	56	Farina	64	N/A
Vega-Lopez 2015	N/A/	N/A	500 mL of glucose OR 96g of commercial white bread (pepperidge farm)	N/A	50g carbs each
Bailey 2017	Muesli, semi-skimmed milk, apple and peaches	47	Cornflakes, skimmed milk, white bread and strawberry jam	78	Macronutrient content, energy, and fluid
Little 2010	Lentils	26	Instant mashed potato, egg whites and white bread	76	Macronutrient content
Cocate 2011	All Bran cereal, fat-free strawberry yogurt, grape juice, multi-grain bread, margarine, and apple	28	Corn flake cereal, whole milk, sports drink (high carbohydrate, water, and electrolytes replenisher), white bread, and margarine	79	Macronutrient content
Jamurtas 2011	Dried apricots	30	White bread with strawberry jam	70	1.5 g of carbohydrate per 1 kg of body mass
Keller 2016	37 g isomaltulose	32	28g maltodextrin + 9g sucrose	90	N/A
Kaplan 2000	300 mL lemon beverage (290 mL water and 10 mL lemon juice) sweetened with 23.7 mg sodium saccharin) (Hermesetas Original; JL Freeman Inc, Boucherville, Canada)	N/A	Glucose: 300 mL lemon beverage (290 mL water and 10 mL lemon juice) containing 50 g glucose (Dextrose monohydrate; Bio-Health, Dawson Traders Ltd, Toronto)	N/A	N/A
Nilsson 2009	Glucose solution (50 g glucose suspended in 450 ml water): divided into 6 portions over 150minutes	N/A	Glucose solution (50 g glucose suspended in 450 ml water): consumed in 10-12 minutes prior to protocol	N/A	N/A
Jentjens	Galactose solution	25	Glucose solution	10	aCHO = 75g

2003				0	
Moore 2009, 2010	Bran flakes cereal, semi-skimmed milk, water	30	Corn flakes cereal, semi-skimmed milk, water	72	1g/kg body weight of carbohydrate
Visvanathan 2004	379 ml Apple & Cherry Juice, a commercially available preservative-free fruit juice (Wild about Fruit Company Pty Ltd, Wandin, Victoria, Australia; (Foster-Powell et al. 2002))	43	50g glucose, 359mL water & 20 ml bottled lemon squeeze (Berri Ltd, Victoria, Australia; 99·9% lemon juice with 25 mg carbohydrate/L)	100	N/A

POPULATION/SAMPLING

Research participants were young adults (18-35), free of disease and similar in physical activity participation status (>90 minutes/week of moderate-vigorous exercise). A young, healthy population avoids the potential for age-related cardiovascular impairments. Using young subjects minimizes potential confounding variables, since younger people are typically healthier. This increases the internal validity of the study and allow for more direct conclusions to be inferred from our sample. An active sample (>90 minutes/week of moderate-vigorous exercise) was used to induce a greater effect from prolonged sitting since subjects are adapted to regular physical activity. This is considered due to a previous study from our laboratory (unpublished), and lack of major findings from sedentary subjects. Ideally a generalizable sample would be used, but practically, especially at this early stage, it is more feasible to recruit a homogenous group. Special considerations for female participants were accounted for such that they were tested during the first 7-10 days of the follicular phase of the menstrual cycle when hormone levels are relatively constant in comparison to the luteal phase. In order for both testing days to occur during this timeframe, we scheduled female participants so that their first experimental visit occurred at start of the menstrual cycle, and their second visit was toward the middle or end of their cycle.

STATISTICAL CONSIDERATIONS

The independent variables for the study were time (pre, 10, 90, 170, post) and high or low GI intervention. The main dependent variables were PWV, AIx, and TSI%. Potential covariates for PWV was MAP, and accelerometer fidgeting data was a covariate for blood pooling data. For this study, a repeated measures analysis was performed. First, we considered a multivariate test, but with many time points for assessing PWV, it is risky to run a multivariate approach. This is because if a subject is missing one time point, they will be dropped from the entire analysis through listwise deletion. However, we wanted a statistical test that will only drop that one time point. Thus, we considered use of a mixed linear model. A mixed linear model handles repeated measures and missing data (by dropping that one missing time point). Additionally, we recognized that many subjects will start at different intercepts. The best statistical test that can handle these considerations is a mixed linear model. T-tests were performed to compare across time points or condition for all variables.

OTHER CONSIDERATIONS

Sex As A Biological Factor

Men and women have different trajectories for acquisition of CVD risk factors over the course of a lifetime. We are not adequately powered to draw sex-specific conclusions from these results. However, the sexes can be stratified for sensitivity analysis.

Ethnicity/Race

While this study attempted to recruit a racially diverse sample, this study was not adequately powered to determine whether there were race/ethnicity and cardiovascular impairment risk interactions.

Generalizability

We were not able to generalize our results, as we selected a homogenous sample in order to minimize confounding variables. However, we would be able to apply our results to young, healthy, active individuals.

Potential Challenges & Alternative Strategies

As with any research study, there may be challenges with proper functioning of equipment and recruitment of participants. For 1+ year prior to commencement of data collection, all research assistants involved became well versed with the usage of laboratory equipment. Additionally, all channels of media were used to recruit participants in an attempt to complete scheduling in advance of data collection commencement.

Since sensitive cardiovascular and metabolic measures were taken in this study, participation in exercise should be controlled. Current literature is inconsistent, but was summarized to find that most pre-testing guidelines included a 24-hour refrain from exercise. Therefore, this approach was taken for this study.

SUMMARY

It is known that both prolonged sitting and consumption of a high GI meal have acute negative effects on local cardiovascular structure and function. However, it is unknown how these two stimuli interact and what their combined effect on central and local vasculature in young health adults. Currently, no studies have observed the effects of a high GI meal and prolonged sitting on the cardiovascular system in young healthy individuals.

As previously stated, prolonged sitting acutely elicits decreased blood flow to the legs, decreased leg vascular shear stress, impaired vasodilator abilities, impaired endothelial function, and increased arterial stiffness. Based on the literature, these effects last until sitting is interrupted with activity [4]. Consumption of a high GI meal acutely elicits endothelial

dysfunction, oxidative stress, and hyperinsulinemia. The response of the combined stimuli is unknown, but the predicted response is augmented arterial stiffness and vascular dysfunction.

The sample population of interest was primarily composed of college students and employees with desk jobs. Due to the nature of their environment, they are exposed to large amounts of prolonged sitting and Western diet choices. Understanding how these stimuli may be effecting this population is necessary to piece together chronic disease that develops later in life, most likely from these behaviors. This study aimed to provide clarity to the ability of young health individuals to respond to these stressors, and potential mechanisms for future chronic disease development. What aspect of repeat acute exposure to these stressors is leading to chronic disease in later years?

CHAPTER III: METHODOLOGY

This study is reported in accordance with CONSORT (Consolidated Standards of Reporting Trials) guidelines. Ethical approval was obtained from the University of North Carolina at Chapel Hill institutional review board, and all participants provided written informed consent prior to participating in the study.

PARTICIPANTS

Participants were recruited from the University of North Carolina at Chapel Hill and the surrounding area and screened prior to enrollment in the study. Recruitment took place between October and February. Testing took place between November and March. Twenty participants, between 18 and 35 years old, were identified and scheduled for participation. Exclusion criteria included: less than 90 minutes of structured exercise per week, any known cardio-metabolic disorders, pregnant women, smokers, currently taking medication known to affect cardiovascular function. Healthy, moderately active individuals were recruited to ensure a homogenous sample size and enhance the internal validity of the study. Participants signed an informed consent form prior to participation in the study.

EXPERIMENTAL DESIGN

This study was a double-blinded randomized crossover design with two experimental conditions: consumption of a low glycemic index beverage (LGI) and consumption of a high glycemic index beverage (HGI) preceded by a familiarization session. Both conditions were matched with a 3-hour period of sitting. Three hours was chosen based on previous studies [4,22,90,91]. Additionally, the peak postprandial glucose concentration time is estimated to be

~2 hours post-meal consumption[92]. Three hours ensures that we capture the peak

postprandial glucose response. Testing began between 6:00 and 10:00 AM in the

Cardiometabolic Lab. Refer to figure 1 for a complete overview of the study design.
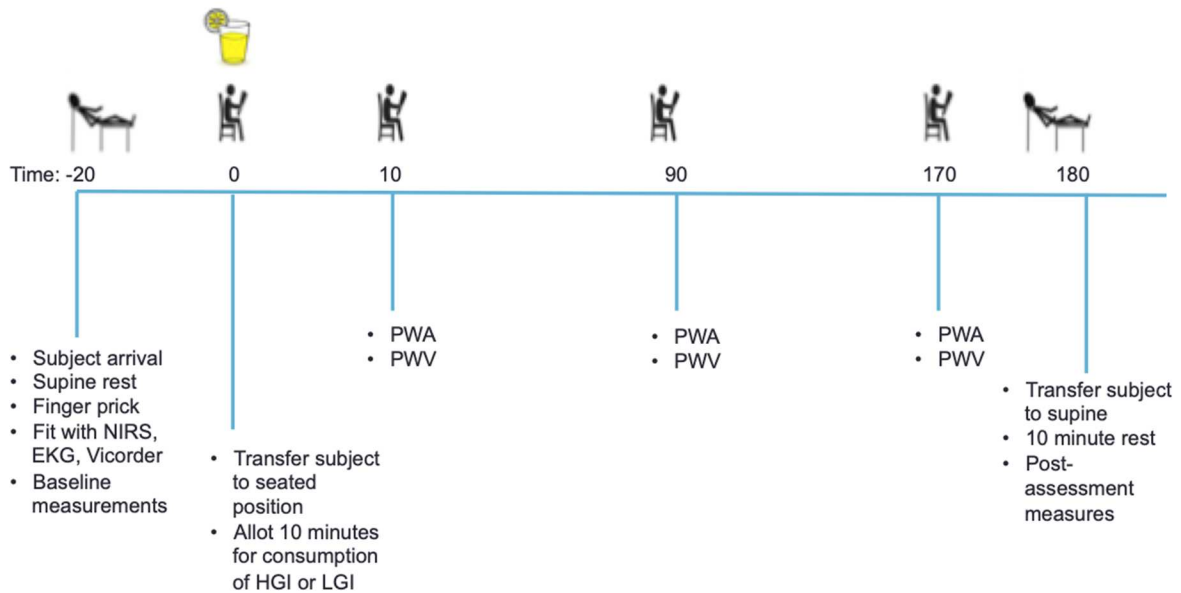
Figure 2. Protocol timeline for experimental measures

Familiarization Visit

Participants reported to the Cardiometabolic Lab 12-24 hours prior to their first

experimental day. Upon arrival, they signed the informed consent document and completed a

medical history questionnaire. Subsequently, height, via stadiometer (Perspective Enterprises,

Portage, Michigan), and weight, via calibrated scale (Health o meter; Pelstar LLC, McCook, IL)

were measured. Participants were then given a food log for them to complete for the 24 hours

leading up to each experimental day. Each participant was fitted with an accelerometer

(ActiSleep +; ActiGraph LLC, Fort Walton Beach, FL) on their right ankle to covary for

spontaneous movement during the subsequent experimental visit as well as ensure abstinence

from exercise 24h prior to the experimental visits. The accelerometer was worn until their

completion of the study. A continuous glucose monitor (iPro2, Medtronic, Northridge CA) was then inserted into the participant's site of choice: abdomen (approximately 5cm lateral from the umbilicus), lower back above left or right hip, or the posterior side of the upper arm. The continuous glucose monitor (CGM) was worn 12-24 hours prior to the first experimental day until their completion of the study. Participants were instructed to perform a finger prick to calibrate the CGM one hour after leaving the lab and once before bed (glucometer: Contour Next One; Ascensia Diabetes Care US Inc., Parsippany, NJ). Before leaving, the participant was given a pre-determined frozen meal to be consumed 12 hours prior to their experimental visits (Lean Cuisine; Nestle, Vevey, Switzerland).

Visit 2 and 3

Participants arrived to the Cardiometabolic lab (22.36 ±2°C, 26.09 ± 8.94% humidity) between 6:00 and 10:00 AM, with 2-7 days between experimental visits. Participants arrived overnight fasted from food, alcohol, and caffeine (at least 12 hours) and having abstained from exercise for at least 24 hours prior to the visit. Upon arrival, height and weight were recorded and a finger prick was performed followed by 20 minutes of quiet rest in the supine position. During the 20 minute rest, the participant was fitted with a NIRS probe on the medial gastrocnemius of the right leg, 3-lead ECG, a non-invasive blood pressure device (NIBP; ADInstruments) and the Vicorder device. After 20 minutes, baseline measurements were attained in the supine position. ECG and NIRS data were collected continuously until the completion of the study visit. Vicorder measurements were taken in triplicate: brachial blood pressure, central PWA, carotid-femoral PWV, brachial-femoral PWV, brachial-ankle PWV, and femoral-ankle PWV.

After baseline measurements were obtained, the participants were passively transferred to a seated position (Armedica AM353 Hi-lo Treatment Table; Tiger Medical, TIGER#TM83695). The chair height and angle were measured and recorded to match for the second experimental

visit. Following re-positioning, participants were allotted 10 minutes to consume either a high GI (glucose; GI: 100) or low GI (fructose; GI: 19) beverage (1g/kg BW + 300mL water) flavored with lemon juice ad libitum. The 3-hour sitting protocol commenced following the consumption of the beverage [4,22,90,91]. During the 3 hours, participants were asked to sit completely still while watching a low stimulus documentary (pre-screened by EK and KB). PWV and PWA measurements were recorded in triplicate at 10, 90, and 170 minutes. After 3 hours (180 minutes) of sitting, the participants were passively transferred to the supine position and PWV and PWA was reassessed after 10 minutes of rest. Blood volume may change throughout the course of the sitting protocol due to filtration of the blood in the kidneys and insensible water loss through perspiration and respiration. This may cause between 100-250 ml of water loss in a period of 3 hours. For this purpose, water intake was monitored during both testing sessions. In addition, participants were instructed to refrain from using the restroom during the study because standing and walking to the restroom would alter CV mechanisms. There were no instances of participants getting up to use the restroom at any point during the study.

EXPERIMENTAL MEASURES

Primary And Secondary Outcome: Central And Peripheral PWV

Central arterial stiffness was assessed by measuring carotid-femoral and brachial-femoral PWV using the Vicorder device (SMT Medical), and lower limb arterial stiffness was assessed via femoral-ankle PWV. The Vicorder device, has been shown to be a valid and reliable measure of PWV [78–82]. The Vicorder device is able to measure PWA and central aortic Pulse Wave Velocity (PWVa), a measure of arterial stiffness, which is an independent risk factor for coronary artery disease [83]. PWA is a reliable and valid measure for arterial stiffness and endothelial function [77,84,85]. According to Vlachopoulos *et al*., a >1 m/s change in carotid-femoral PWV correlated to a 14% increase in CV events, 15% increase in CV mortality,

and a 15% increase in all-cause mortality[86]. A clinically meaningful change for the lower limb is currently unknown.

Blood pressure cuffs were placed on the dominant arm, upper thigh, and ankle for PWV and PWA measurements. The bottom of each cuff was marked with a permanent marker on the skin in the event of cuff movement. A specialized device was used to measure the distance for PWV measurements: all measurements were recorded in cm. For carotid-femoral PWV, the distance was measured from the sternal notch to the top of the femoral cuff. An additional 2.5cm was added to the distance to represent the distance as if measured to the middle of the cuff (this was to decrease measurement error as the middle of the cuff in not marked and would be difficult to guess by simply looking). For femoral-ankle PWV, the distance was measured from the top of the femoral cuff to the top of the ankle cuff. For brachial-ankle PWV, the distance from the top of the brachial cuff to the top of the ankle cuff was measured. When transferred to a seated position, all cuffs were checked to ensure exact placement remained from the supine position. All PWV and PWA measurements were taken in triplicate in the supine position and repeated at minute 10, minute 90, and minute 170 of the sitting protocol. When the participant was transferred to the supine position after 180 minutes, PWV and PWA measurements were repeated following a 10-minute supine rest.

Control Measures

ECG, NIBP, NIRS, and the continuous glucose monitor (CGM) collected measurements continuously throughout the three hours. ECG and NIBP measures allowed for continuous monitoring of hemodynamics. NIRS was used in order to assess blood pooling in the calf because this is the site for blood pooling and the gastrocnemius has been shown to be as sensitive as the soleus[125]. The CGM, which had been inserted 12-24 hours prior, was checked for proper adherence to the skin. Glucose data was extracted from the CGM and AUC and glucose variability were calculated (variability = highest glucose value – lowest glucose

value for each subject over 3 hours). For NIRS placement, the participant was asked to perform a calf raise in order for the researcher to identify the largest muscle belly circumference of the right medial gastrocnemius. This place was marked and ultrasound was used to find the thickest muscle belly with no large vasculature for the placement of a NIRS probe. The placement was measured from the popliteal fossa to the heel to ensure the same placement for the second visit. The NIRS probe was secured to the skin with double-sided tape and a light shield. SRS tHb and TSI% data were extracted from the NIRS output. NIRS SRS tHb is a validated estimate of total hemoglobin under the area of interest[125]. A 3-lead ECG was then attached to the participant at the right arm, left arm, and left lower sites. The pointer and middle fingers of the non-dominant hand were then measured to determine sizing for proper finger cuffs for the non-invasive blood pressure (NIBP) device. The cuffs were secured on the middle phalanx and attached to the NIBP wrist unit. The device was set to sample for alternating 30 minute periods on each cuff, beginning with the pointer finger. This was to diminish the discomfort for the participant. Although NIBP data was collected continuously over the 3 hours, 30 second averages were taken at each time point of interest for analysis purposes.

RANDOMIZATION

Using online randomization software (www.randomizer.org) participants were randomized into either CON or GI intervention for the first visit. The randomization for all participants was performed prior to the first participant visit of the study and referred back to prior to each participant's familiarization session. Prior to the enrollment of the first participant, the high and low GI solutions were covered and marked as "A" and "B" by an unaffiliated research assistant. The blinding was not revealed until all statistical analysis was completed at the end of the study.

SAMPLE SIZE

Calculations to determine minimum sample size were based on the primary central vascular health outcome, aortic pulse wave velocity (PWV). Previous research has reported that prolonged sitting reduces lower limb vascular health and endothelial function between 57-80%[25]. Based on a PWV of 6.2 m/s, which was predicted for healthy participants <30 y[105], a 57% decrease in PWV would be 3.5 m/s. For the current study, we opted to sample based on a conservative change score of 1 m/s. We also used a conservative usual error of 1 m/s. Using magnitude-based inference, to estimate the sample size required to detect the smallest detrimental (or beneficial) effect in a cross-over study, with the maximum chances of a type 1 and 2 error set at 5% (i.e. very unlikely), approximately seventeen participants were required. In order to account for drop out or missing data, twenty participants were recruited for the study. Participants were recruited from undergraduate classes at UNC as well as through email, flyer posting, and word of mouth. In order to improve adherence to intervention protocols, specifically the pre-testing guidelines, we informed the participant that the accelerometer and CGM would provide information concerning all activity and food/beverage consumption.

QUALITY CONTROL

At the conclusion of the study a random selection of 10% of the Vicorder data sets (e.g. from 3 participants) were re-entered by an independent observer. No data points differed, so no further action was taken.

DATA MANAGEMENT AND STATISTICAL ANALYSIS

Data sheets were stored in a locked drawer and in digital form on two study computers. All statistical analyses were performed using Jamovi (2018, Version 0.9). The α level was set a priori for all statistical procedures at $\alpha=0.05$. Raw data are presented as mean [standard deviation] and mixed model data are presented as mean [95% confidence interval]. Supine (Pre vs. Post) and sitting (min 10, 70 and 170) date were analyzed separately.

Continuous glucose data was averaged over time and analyzed using a paired samples t-test. Glucose AUC and variability was also calculated to compare to PWV variability using a Pearson's R correlation. For all other outcomes, the effects of time (pre vs. post and 10 vs. 90 vs. 170) and condition (A vs. B) were analyzed using linear mixed models with fixed effects of condition and random effect of time and participant. Using a mixed model is appropriate given the correlated data (repeated measures) with various distributional assumptions and its ability to accommodate missing data. A condition-by-time interaction term was included in regression models to examine between condition differences across time points. All models were adjusted for baseline values. PWV outcomes were adjusted for baseline values and MAP, as MAP is a confounder of PWV. Effect sizes were calculated as Cohen's d, where <0.20 is considered to be a small, > 0.20 to < 0.50 a moderate, and > 0.60 a large effect. For the mixed models Cohen's d was calculated as the effect of condition (β) or time (β) from linear mixed models divided by the baseline SD.

CHAPTER IV: RESULTS

PARTICIPANTS

Participants were young (23 ± 3 years), moderately active (280 ± 175 minutes/week), normal height and weight (172.6 ± 8.7 cm; 72.8 ± 15 kg; BMI: 24.3 ± 3.7 kg/m^2), and otherwise healthy men and women (33% female). Participants self-identified as Caucasian (n=14), African American (n=2), and Middle-Eastern (n=4). All participants conformed to pretesting guidelines. Participant characteristics are illustrated in table 2.

Eighteen of the twenty participants completed the study and were used for analysis. Figure 3 illustrates the CONSORT diagram for participant recruitment and drop out. All data was collected and analyzed successfully apart from two missing data points from NIRS in the LGI condition for participant 17.
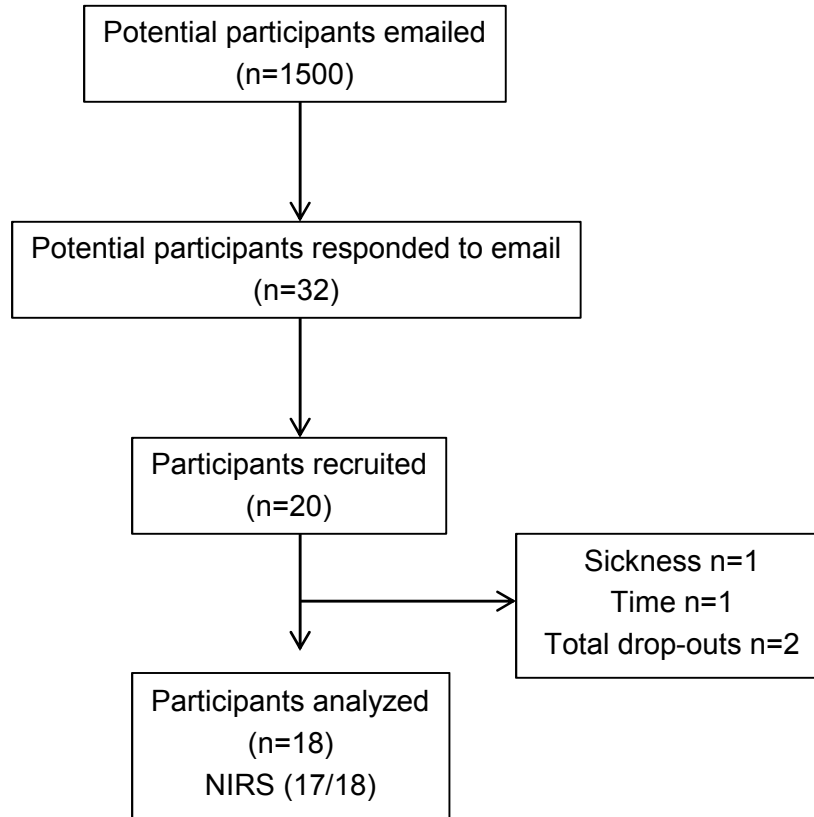
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                    ┌─────────────────────────────────────┐
                    │   Potential participants emailed     │
                    │            (n=1500)                  │
                    └─────────────────────────────────────┘
                                     │
                                     ▼
                    ┌─────────────────────────────────────┐
                    │ Potential participants responded     │
                    │         to email (n=32)              │
                    └─────────────────────────────────────┘
                                     │
                                     ▼
                    ┌─────────────────────────┐
                    │  Participants recruited  │
                    │         (n=20)           │            ┌──────────────────────┐
                    └─────────────────────────┘            │   Sickness n=1       │
                              │        └───────────────────▶│   Time n=1           │
                              ▼                              │ Total drop-outs n=2  │
                    ┌─────────────────────────┐            └──────────────────────┘
                    │  Participants analyzed   │
                    │         (n=18)           │
                    │       NIRS (17/18)       │
                    └─────────────────────────┘
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Figure 3. CONSORT diagram. *NIRS: near infrared spectroscopy*

BASELINE DATA

Baseline data is reported in Table 2. There were no significant differences in baseline hemodynamic measures between testing days (table 2). Baseline tHb data are not reported as values as they are expressed relative to baseline. In order to achieve the most precise results, baseline measures were set as covariates when analyzing all supine primary, secondary, and ancillary measures. Adherence to pre-assessment guidelines were confirmed through accelerometer and blood glucose data. There was no significant difference between total MVPA (moderate-vigorous physical activity) during the 12-24 hours prior to experimental visits (HGI: 42.2, LGI: 52.4 minutes, p=0.079). Additionally, the AUC for blood glucose data 12 hours prior to experimental visits was similar between LGI and HGI (64,330 ± 19.9 and 65,936 ± 14.3, respectively, p<0.05).

Table 2. Participant Characteristics and Hemodynamic Measures at Baseline (mean ± SD)

Variable	Total	LGI	HGI	P value (effect size)
HR (BPM)		58.51 ± 10.68	58.47 ± 11.55	0.972 (0.004)
MAP (mmHg)		83.56 ± 9.44	82.41 ± 7.97	0.535 (0.131)
DBP (mmHg)		58.39 ± 5.65	57.92 ± 5.36	0.647 (0.086)
SBP (mmHg)		121.72 ± 12.20	122.12 ± 9.89	0.814 (-0.036)
PP (mmHg)		63.33 ± 9.35	64.26 ± 7.65	0.609 (-0.109)
cSBP (mmHg)		114.81 ± 12.73	114.03 ± 8.34	0.934 (0.072)
cPP (mmHg)		55.86 ± 9.68	56.91 ± 6.65	0.392 (-0.127)
pp/cPP (mmHg)		1.14 ± 0.10	1.05 ± 0.29	0.239 (0.404)
AIx (%)		11.36 ± 5.11	11.83 ± 6.20	0.740 (-0.083)
SEVR (%)		179.94 ± 47.99	170.91 ± 33.69	0.380 (0.218)
SV (mL)		57.28 ± 8.82	56.97 ± 15.42	0.651 (0.024)
CO (L/min)		3.49 ± 0.84	3.56 ± 1.19	0.918 (-0.074)
TPR (PRU)		3.76 ± 0.99	4.00 ± 1.33	0.413 (-0.211)

PA: physical activity; HR: heart rate; MAP: mean arterial pressure; DBP: diastolic blood pressure; SBP: systolic blood pressure; PP: pulse pressure; cSBP: central systolic blood pressure; cPP: central pulse pressure; pp/cPP: pulse pressure/central pulse pressure; AIx: augmentation index; SEVR: subendocardial viability ratio; SV: stroke volume; CO: cardiac output; TPR: total peripheral resistance; PRU: peripheral resistance units

STIMULUS: CONTINUOUS BLOOD GLUCOSE

Figure 4 illustrates the average blood glucose (mg/dL) across the three hours between the conditions. Overall, HGI had a higher average value (107 ± 19 mg/dL) compared to LGI (92 ± 4 mg/dL), and a larger area under the curve (AUC) (19429 ± 98) than LGI (16498 ± 19). For average values, there was a large significant effect (p<0.001, d=0.983). Additionally, for AUC values, there was a large significant effect (p<0.001, d=1.01). Across the 3-hour postprandial period, the highest glucose value observed was 98.76 mg/dL for the LGI condition and 138.94 mg/dL for the HGI condition. The lowest glucose value observed was 86.76 mg/dL for the LGI condition and 78.5 mg/dL for the HGI condition. Average glucose variability was 71.56 ± 26.60 mg/dL for the HGI condition and 28.71 ± 19.72 mg/dL for the LGI condition.
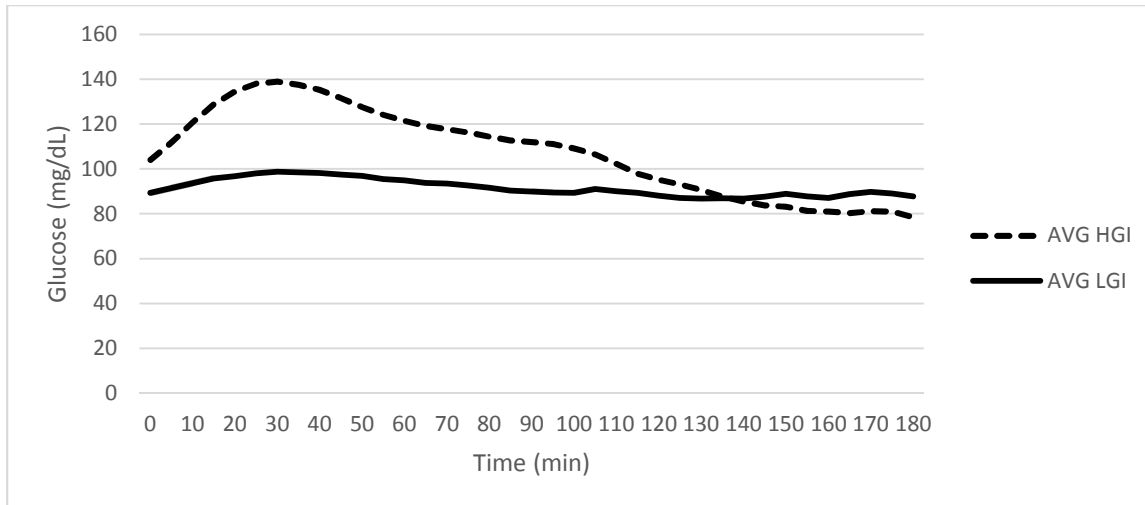
Figure 4. Average blood glucose response to high and low GI beverage for all subjects over 3 hours of sitting

PRIMARY OUTCOME: CENTRAL PULSE WAVE VELOCITY

There was no significant time x condition interaction for central (carotid-femoral) PWV in the supine position (table 3). However, there was a significant time x condition interaction for bfPWV (p=0.027) (figure 5). PWV increased by 0.27 m/s in the LGI condition (95% CI: 0.2517, 0.669) and 0.45 m/s in the HGI condition (95% CI: 0.068, 0.485).

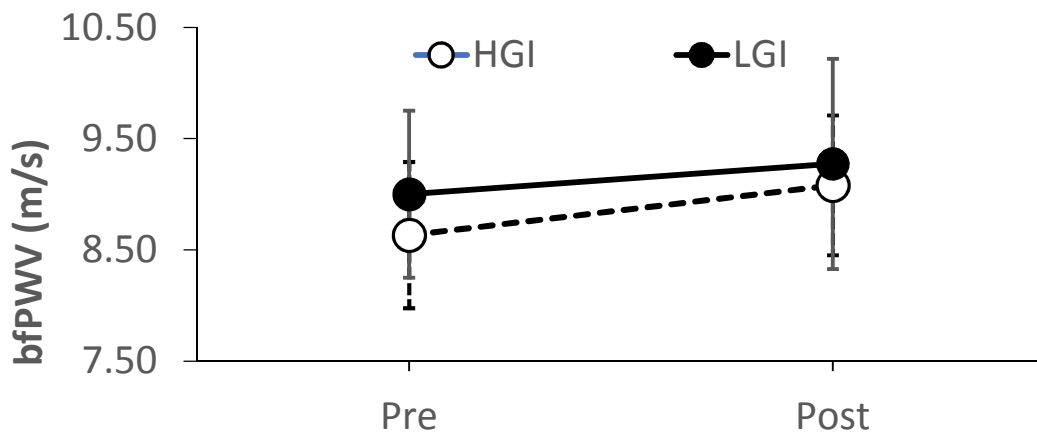

Figure 5. Mean brachial-femoral PWV supine measurements before and after three hours of uninterrupted sitting (bars represent SD). *bfPWV: brachial-femoral pulse wave velocity; HGI: high glycemic index; LGI: low glycemic index*

Table 3. Supine Arterial Stiffness Measures

Time	Condition		cfPWV *m/s*	bfPWV *m/s*	faPWV *m/s*
Pre	HGI	X	6.08	8.63	6.75
		SD	0.95	0.66	0.83
	LGI	X	6.11	9.00	6.96
		SD	1.08	0.75	0.77
Post	HGI	X	6.13	9.08	7.13
		SD	0.97	0.63	1.11
	LGI	X	5.98	9.27	7.67
		SD	1.24	0.95	1.15
Interaction		*P*	*0.223*	***0.027***	*0.178*
Time		*P*	*0.628*	***0.001***	***0.004***
		ES	*-0.060*	*0.470*	*0.380*
Condition		*P*	*0.254*	*0.078*	*0.180*
		ES	*-0.190*	*-0.300*	*0.230*

cfPWV: carotid-femoral pulse wave velocity; bfPWV: brachial-femoral pulse wave velocity; faPWV: femoral-ankle pulse wave velocity; HGI: high glycemic index; LGI: low glycemic index

SECONDARY OUTCOME: PERIPHERAL PWV

There was no significant time x condition interaction for lower limb (femoral-ankle) PWV in the supine (Table 2) or seated position. However, there was a significant effect for time (p=0.004, d=0.38; 95% CI: 0.219, 0.897) in the supine condition (figure 6).
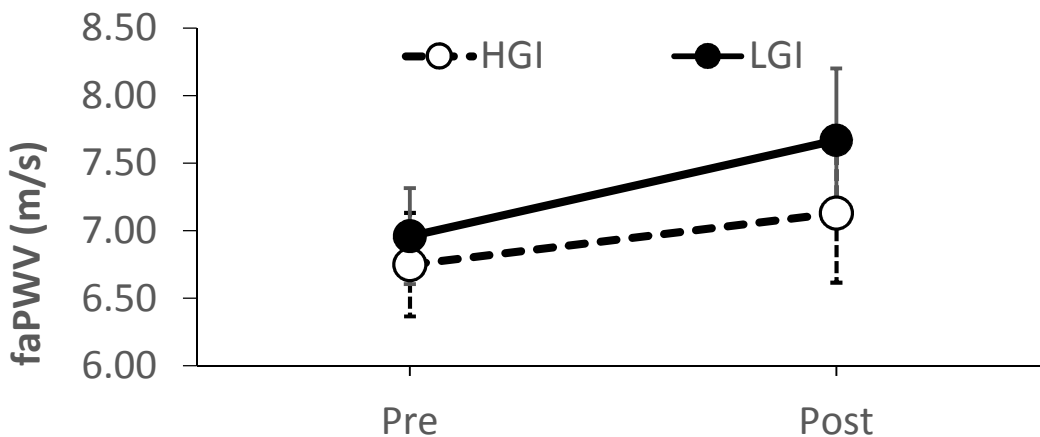

Figure 6. Mean femoral-ankle PWV supine measurements before and after three hours of uninterrupted sitting (bars represent SD). *faPWV: femoral-ankle pulse wave velocity; HGI: high glycemic index; LGI: low glycemic index*

ANCILLARY ANALYSIS

There were no significant interaction or condition effects for AIx or cSBP. There was a significant time effect for augmentation index (AIx). AIx decreased by 2.93% (p=0.02, d=-0.32; 95% CI: -5.05, -0.814) in the supine position (table 4).

Table 4. Hemodynamic measures and NIRS output. *cSBP: central systolic blood pressure; Aix: augmentation index; CO: cardiac output; TPR: total peripheral resistance; PRU: peripheral resistance units; HR: heart rate; BPM: beats per minute; SRS tHb: spatially resolved spectroscopy total hemoglobin; TSI: tissue saturation index; HGI: high glycemic index; LGI: low glycemic index*

			cSBP	AIx	CO	TPR	HR	SRS tHb	TSI
			mm Hg	*%*	*L*	*PRU*	*BPM*	*mm*	*%*
Pre	HGI	X	114.03	11.83	3.56	4.00	61.70	49.61	57.98
		SD	8.34	6.20	1.19	1.33	11.51	22.94	54.41
	LGI	X	114.81	11.36	3.49	3.76	60.71	56.84	58.68
		SD	12.73	5.11	1.19	1.33	11.51	22.94	54.41
Post	HGI	X	112.69	8.58	3.62	3.85	61.74	52.16	52.29
		SD	8.22	4.52	1.00	1.17	11.58	16.36	52.96
	LGI	X	114.72	8.75	3.68	3.90	68.75	55.92	58.09
		SD	10.90	3.21	0.89	0.89	16.20	20.66	51.29
Interaction		*P*	*0.615*	*0.710*	*0.710*	*0.421*	*0.050*	*0.586*	*0.226*
Time		*P*	*0.581*	***0.015***	*0.501*	*0.976*	*1.000*	*0.818*	*0.138*
		ES	*-0.066*	*-0.320*	*0.120*	*-0.005*	*0.308*	*0.028*	*-0.179*
Condition		*P*	*0.387*	*0.859*	*0.982*	*0.644*	*0.233*	*0.074*	*0.123*
		ES	*0.148*	*-0.030*	*0.006*	*-0.116*	*0.304*	*0.305*	*0.262*

There were no significant condition x time interaction effects or time effects in the NIRS output. There were significant condition effects for SRS tHb (p=0.02), and TSI% (p=0.001) (table 4).

SUPPLEMENTAL ANALYSIS

All measures made in the seated position (10, 90, 170 minutes) are included in Appendix B. In the seated position, there was a significant condition effect for MAP (p=0.001, d=0.71), DBP (p=0.001, d=0.73), and SBP (p=0.001, d=0.86), taken from the NIBP device, with all values being greater in the HGI condition. There was a significant condition effect for SV (p=0.001, d=-1.35), CO (p=0.001, d=-1.07), and TPR (p=0.001, d=-0.94) (appendix B). There was no correlation between glucose and changes in bfPWV (r=0.205, p=0.238; 95% CI: 0.504, -0.138),

and a weak-moderate correlation between glucose variability and changes in bfPWV (r=0.400, p=0.017; 95% CI: 0.647, 0.077) (figure 7).
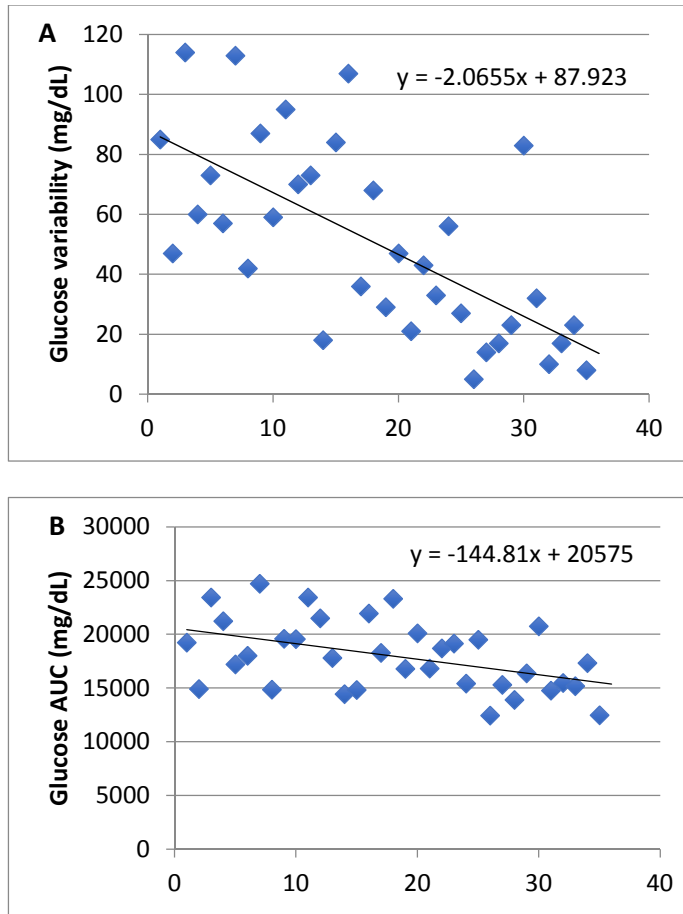

Figure 7. *Correlation between glucose variability bfPWV (A) and glucose AUC and*

bfPWV (B). AUC: area under the curve; HGI: high glycemic index; LGI: low glycemic index

CHAPTER V: DISCUSSION

The purpose of this study was to examine the peripheral and central cardiovascular response to prolonged sitting following the consumption of a low and high GI meal. It was hypothesized that prolonged sitting and the consumption of a high GI meal would elicit greater detriments in both central and peripheral PWV. The expected glucose stimulus was achieved, with the LGI meal eliciting a small, gradual increase in blood glucose, and the HGI meal eliciting a large increase in blood glucose for ~30 minutes following consumption. Prolonged sitting (3 hours) and consumption of a high GI beverage increased central (brachial-femoral) arterial stiffness, with a slightly greater detriment in the HGI condition. However, carotid-femoral PWV was not influenced by prolonged sitting or HGI meal consumption. Peripheral (femoral-ankle) PWV increased regardless of the level of GI consumed. Collectively, this data demonstrates that in young health adults a single bout of prolonged uninterrupted sitting combined with a high glycemic index beverage can negatively affect markers of central and peripheral cardiovascular function, but there are some inconsistencies in the findings.

LIMITATIONS AND STRENGTHS

To better contextualize the discussion, the limitations and strengths of the current study must be stated. First, our study results lack generalizability as we used a group of healthy, active, young adults. However, we elected to recruit a homogenous group of healthy, active, young adults to minimize the confounding influence of age and CVD. Although the sample was healthy, it is unknown how much previous exposure they had to these stimuli. Second, participants were all required to be active for at least 90 minutes per week, however it is unknown what lifestyle choices were made outside of that. Future studies should collect

accelerometer and food log data on participants for an extended period of time in order to gather more data concerning lifestyle choices. Third, we were unable to determine sex interactions due to being inadequately powered, but we are confident that the experimental conditions were well controlled in that fluctuations in estrogen in females would not affect our results.

Although this study has a few limitations, it also has several strengths including the use of the Vicorder device. The Vicorder measures PWV and PWA in a non-intrusive manner with little operator training, ease of use, and low user error. This also allowed for a comprehensive assessment of cardiovascular measures. Second, we were adequately powered to conduct this study. Third, trial conditions were standardized for potential confounders such as diet, physical activity, and baseline values. Finally, this study is a double blind crossover design, which reduces bias in our results.

COMPARISON TO LITERATURE

This study found that prolonged sitting (3 hours) and consumption of a high GI beverage increased central (brachial-femoral) arterial stiffness, with a moderately (d=0.50) greater detriment in the HGI condition. However, carotid-femoral PWV was not influenced by prolonged sitting or HGI meal consumption. There was a moderate (d=0.41) increase in peripheral (femoral-ankle) PWV slightly regardless of the level of GI consumed. Although the results were not clinically significant (>1m/s), it does support the current literature[108,120-121]. That is, the established <1m/s clinical value is for chronic changes, repeatedly exposing the aorta to a 0.45 m/s increase in stiffness following a high GI meal may accumulate to significant cardiovascular burden over time[123]. This offers an additional insight into a young, healthy person's ability to respond to a high dose of sugar.

Prolonged sitting resulted in a significant increase in central (brachial-femoral) PWV, with a moderately greater change (0.18 m/s) in the high GI condition. Two other studies, plus

one additional unpublished paper [personal communication] have reported increased central

PWV with an acute bout of prolonged sitting [108,120]. The current study is the first to

investigate changes in central PWV with prolonged sitting and a high glycemic index meal. The

findings indicate that high GI consumption exacerbates the detriments from prolonged sitting,

and should be further explored. However, the carotid-femoral PWV was not significantly

impacted by prolonged sitting or a high GI meal, which was predicted based on previous

literature [3, 108]. A potential reason is that the brachial-femoral measurement incorporates the

subclavian artery. Since the subclavian artery is slightly smaller than the aorta, it may be more

responsive to change in a healthy population, which could explain the differences in

measurement responses. While no studies have directly compared the reliability of brachial-

femoral PWV versus carotid-femoral PWV, it is plausible that the brachial-femoral PWV confers

greater reliability and sensitivity. For the current study we did consistently find greater signal

strength for the brachial-femoral measurement. The responsiveness of carotid-femoral and

brachial-femoral PWV should be explored further to understand their reliability in this population.

Prolonged siting resulted in a significant increase in peripheral (femoral-ankle) PWV,

regardless of GI beverage. These results contribute to the existing literature that prolonged

sitting acutely impairs the structure and function of lower limb vascular health [3-4,22,25], with a

decrease in shear stress being the proposed mechanism [22]. Shear stress has been shown to

decrease across a bout of prolonged uninterrupted sitting due to a decrease in blood flow to the

limb caused by blood pooling in the legs (shown by the increase in perfusion). However, in the

current study, tHb did not increase (indicative of blood pooling) in the LGI condition. This could

be attributed to the subjective feeling of cold many subjects experienced during the bout of

sitting, potentially causing local vasodilation, facilitating venous return. This may not have been

seen in the HGI condition, because HGI consumption is associated with greater insulin

secretion, which is a vasodilator. However, the data is inconclusive in supporting this as there

were no significant changes in CO. The potential blood pooling is caused by lack of venous return due to lack of muscle pump and gravitational forces. A decrease in shear stress is associated with endothelial function impairment. Findings from this study, an increase in lower limb arterial stiffness due to sitting, are in line with current literature. Current literature assessing the effects of acute prolonged sitting (3-8 h) on endothelial function, using flow-mediated dilation (FMD), reported a 48-71% decline in leg FMD [3-4,25,72,117-119]. Impairment to the vasculature of the leg due to prolonged sitting is of clinical relevance since evidence supports that the leg vasculature is vulnerable to atherosclerosis [4]. However, consumption of a high GI meal did not further impair peripheral vasculature. This suggests that not all vasculature responds the same to the same stimulus, and this should be explores in other areas of the body.

The proposed mechanism explaining cardiovascular detriments from prolonged sitting begins with blood pooling in the lower limbs. This leads to a decrease in venous return, and therefore a decrease in cardiac output. We did not find any changes in CO throughout the 3-hour sitting protocol, but that may be attributed to the young, healthy sample that was used. A decrease in CO would result in decreased pressure on baroreceptors, increasing sympathetic nervous system activity and therefore increasing total peripheral resistance (TPR). We did not find any changes in TPR throughout the protocol either, which would support the lack of change in CO. The theorized increase in CO and TPR would increase the central hemodynamic load on the cardiovascular system, explaining the pathway from peripheral to central impairments from prolonged sitting. Possible explanations for the lack of support for this mechanism from our results could be the sample chosen is better able to compensate with an adverse stimulus, or measurement error. It is possible that the NIBP (non invasive blood pressure) used to measure CO and TPR was variable based on positional placement.

Prolonged sitting resulted in a significant reduction in AIx, regardless of GI beverage. AIx is a measure of systemic arterial stiffness derived from the ascending aortic pressure waveform [106]. A reduction in AIx after prolonged uninterrupted sitting could be attributed to the increase in blood pooling, leading to a reduction in venous return and therefore a reduction in stroke volume and subsequently AIx. These results have been found previously in our lab[121]. The results showed a slight reduction in stroke volume from minute 10 to minute 170 in the high GI beverage condition, but a fluctuation in stroke volume between minute 10 and 90 and minute 90 and 170 in the low GI beverage condition. These findings suggest that AIx may not be telling us about vascular health during sitting, but rather autonomic function. It also suggests that the blood may in fact be pooling, and the NIRS measurement is not adequately reflecting this pooling.

IMPLICATIONS

Prior to this study, it was understood that prolonged sitting and high GI consumption have detrimental chronic and acute effects on the cardiovascular system[3-4,108]. However, it was unknown how the two stimuli interacted acutely to effect the central and local cardiovascular system. The data herein supports the interaction between prolonged sitting and high GI consumption and their effects on the cardiovascular system in a young, healthy population. There seem to be greater changes in arterial stiffness at the level of the heart rather than the local leg vasculature. Due to the common nature of prolonged sitting and high GI consumption in everyday life, particularly in offices, this information is useful and pertinent. If the mechanism behind acute exposure to prolonged sitting and high GI consumption and subsequent cardiovascular detriments are elucidated, the chronic effects leading to CVD can be better understood. Future studies addressing prolonged sitting and its interaction with other lifestyle factors on cardiovascular function are warranted, perhaps focusing on extending the generalizability of the finding to an actual office worker.

Table 5. Implications

What did we know?

- Chronic prolonged sitting is correlated with CVD
- Western diet (highly composed of high GI foods/beverages) is correlated with CVD
- Acute prolonged sitting impairs cardiovascular structure and function (central and local) in healthy and diseased populations
- Acute consumption of high GI foods/beverages elicits impairments to the vasculature (oxidative stress) and unstable glucose/insulin response

What did we not know?

- Acute effects of prolonged sitting and HGI beverage on CV system in young, healthy adults

What have we learned?

- Acute prolonged sitting (3 hours) impairs lower limb vascular structure in young, healthy adults
- Augmentation index is impaired over 3 hours of sitting in young, healthy adults
- High GI beverage does not exacerbate these effects

Why is this new information useful?

- We now have a better understanding of how young, healthy adults respond to these combined stimuli

What do we need to know next?

- Does a larger glucose dose elicit an impaired response in this population?
- Home or work-based studies are needed to determine these effects in a real-life scenario.
- Middle-aged populations need to be observed as they are the "middle ground" between the young healthy population, and the diseased population

CONCLUSIONS

The purpose of this study was to determine the effects of prolonged sitting (3 hours), with or without a high GI beverage, on central (carotid-femoral and brachial-femoral) and local (femoral-ankle) arterial stiffness in young, health adults. The findings suggest that, in this population, prolonged sitting does not contribute to significant detriments on central arterial stiffness, but it does increase local stiffness regardless of high or low glycemic index beverage consumption. This suggests that local detriments could impair central cardiovascular function, either with a larger stimulus or in less healthy population. Further studies are necessary to investigate the interaction between local impairments and central cardiovascular function.

APPENDIX A – ETHICAL APPROVAL

University of North Carolina at Chapel Hill
Consent to Participate in a Research Study
Adult Participants

Consent Form Version Date: _7/26/2018_____
IRB Study # 17-2532
Title of Study: Prolonged Sitting With or Without a High Glycemic Index Meal: Acute Effects on Vascular and Cerebrovascular Function in Healthy Adults
Principal Investigator: Elizabeth Kelsch
Principal Investigator Department: Exercise and Sport Science
Principal Investigator Phone number: (919) 962-0396
Principal Investigator Email Address: ekelsch@live.unc.edu
Co-Investigators: Katie Burnet, Dr. Erik Hanson
Faculty Advisor: Lee Stoner
Faculty Advisor Phone Number: (919) 962-0534
Faculty Advisor Email Address: stonerl@email.unc.edu

_____ _____

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.
You may choose not to participate, or you may withdraw your consent to be in the study, for any reason, without penalty.

Research studies are designed to obtain new knowledge. This new information may help people in the future.   You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies. Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study.

You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
To examine the acute effects of sitting and consumption of a high glycemic index (HGI) meal on cardiovascular health, cerebrovascular health, cognition, and the proposed mechanisms within the body that may lead to the observed effects. Findings from this study may contribute to public health policy concerning sedentary behavior and the Western diet.

You are being asked to be in the study because you are between the ages of 18-40 years.

Are there any reasons you should not be in this study?

You should not be in this study if you have a BMI greater than 30 kg/m^2, you have known cardiovascular or metabolic diseases, you smoke tobacco, or you are pregnant (urine pregnancy test will be given).

How many people will take part in this study?
There will be approximately 20 people in this research study.

How long will your part in this study last?
Should you wish to participate in the study, you will be required to attend the Applied Physiology Laboratory at University of North Carolina at Chapel Hill on three occasions. The first visit will last approximately 30 minutes, and the following visits approximately 4 hours.

What will happen if you take part in the study?
Visit One: Participants will complete report to the UNC Applied Physiology Laboratory where informed consent will be obtained. Participants will be screened for participation in the study, which will include a medical history questionnaire. During this familiarization day, participants will be given a food log for them to complete for the 24 hours leading up to the first experimental day until their completion of the study. Each subject will be fitted with an accelerometer (ActiSleep +; ActiGraph LLC, Fort Walton Beach, FL) on their ankle to covary for spontaneous movement as well ensure abstinence from exercise 24h prior to the experimental visits. The accelerometer will be worn at least 24 hours prior to the first experimental day until their completion of the study. A continuous glucose monitor (iPro2, Medtronic, Northridge CA) will then be inserted into the participant's abdomen, approximately 5cm lateral from the umbilicus. The continuous glucose monitor (CGM) will be worn the day prior to each experimental visit, until the cessation of each experimental visit. The participants will receive a standardized frozen meal to consume 12 hours prior to each experimental visit.

Visit Two: For the first experimental visit, participants will report to the Applied Physiology Laboratory between 6 and 10am in rest in the supine position for 20 minutes. During supine rest, the participant will be fitted with near infrared spectroscopy (NIRS) devices on the prefrontal cortex and the medial gastrocnemius, a 3-lead electrocardiogram, the Vicorder device, and a non-invasive blood pressure device (NIBP) on the wrist. Subsequently, cognitive function will be assessed using a Stroop test, Trail Making test, and a memory test; noninvasive measures of vascular health will be performed using the Vicorder device. The participants will then be passively transferred to a sitting position, where (s)he will be allotted 10 minutes to consume either the control (CON) or high glycemic index (HGI) beverage. The participant will then remain seated for 180 minutes while watching a low-stimulus documentary. Brain and calf blood flow and tissue oxygenation will be monitored continuously with NIRS. After 10, 90 and 170 minutes of sitting vascular function and cerebrovascular blood flow will be measured. The participants will then be passively transferred to the supine position after 180 minutes of sitting. Following 10 minutes of quiet rest, vascular health will be re-measured and cognitive function will be reassessed. If the participant needs to use the restroom, this will be recorded, and they will be asked to repeat this movement for the subsequent visit.

Visit Three: Participants will return for follow up testing within 10 days after visit two. The experimental procedures will be identical to visit 2. The participant will be randomly assigned to either HGI or CON for visits two and three. The beverage will be administered by an undergraduate research assistant to allow for double blindedness.

What are the possible benefits from being in this study?
There is no direct benefit to participants.

What are the possible risks or discomforts involved from being in this study?
While in this study, a finger prink will need to administered to obtain blood glucose levels, which may be uncomfortable. The insertion of a continuous glucose monitor could cause discomfort. A Urine Pregnancy test provided by the study will be obtained for all women of child-bearing potential, which could also cause some discomfort. The device we will use to monitor blood flowing to your calf and brain also possesses a small risk of eye damage/irritation, and skin heating/irritation. There may be uncommon or previously unknown risks. You should report any problems to the researcher.

How will information about you be protected?
Hard copies of any identifiable information will be stored in a locked file cabinet within an access-controlled laboratory in Fetzer Hall (Applied Physiology Lab) at the University of North Carolina at Chapel Hill campus. Only members of the research team will have access to the cabinet. Any electronic files with identifiable information will be kept separate in password-protected files on password-protected computers that will be accessible to only members of the research team. Upon completion of the study, all data will be transferred to an electronic storage device, files will become password protected, and all hard copies will be shredded.
Participants will not be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies (for example, the FDA) for purposes such as quality control or safety.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be receiving a vascular health report for taking part in this study.

Will it cost you anything to be in this study?
It will not cost you anything to be in this study.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any

time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions about the study, complaints, concerns, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

What if you have questions about your rights as a research participant?
All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject, or if you would like to obtain information or offer input, you may contact the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

Consent Form Version Date: _8/18/2018_____
IRB Study # 17-2532
Title of Study: Prolonged Sitting With or Without a High Glycemic Index Meal: Acute Effects on Vascular and Cerebrovascular Function in Healthy Adults
Principal Investigator: Elizabeth Kelsch

Participant's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_____ _____
Signature of Research Participant   Date

_____
Printed Name of Research Participant

                                                                              _____
_____ Date
Signature of Research Team Member Obtaining Consent

_____
Printed Name of Research Team Member Obtaining Consent

Table 1. Seated Hemodynamic Measures

Seated			HR	MAP	DBP	SBP	PP	cSBP	cPP	pp/cPP	AIx	SEVR	SV	CO	TPR
			bpm	mm Hg	mm Hg	mm Hg	mm Hg	mm Hg	mm Hg	mm Hg	%	%	ml	L/min	PRU
HGI	10	X	68.26	82.19	58.33	120.94	63.15	112.53	55.31	1.11	9.97	9.97	174.44	114.72	7.44
		SD	11.84	10.28	8.31	10.52	7.47	9.99	9.66	0.29	7.18	7.18	36.52	20.47	1.93
	90	X	72.16	82.78	58.94	118.92	59.53	111.17	51.72	1.10	8.97	8.97	174.83	102.09	7.41
		SD	12.26	7.38	7.13	10.19	10.13	8.59	8.13	0.10	5.75	5.75	41.50	17.99	2.47
	170	X	70.73	84.31	61.53	121.53	60.00	112.97	51.69	1.16	8.86	8.86	180.67	103.66	7.00
		SD	13.55	9.31	7.63	9.61	6.90	9.42	6.50	0.29	4.12	4.12	41.60	12.28	1.75
LGI	10	X	69.85	86.78	63.39	125.33	61.94	117.53	54.97	1.13	8.75	8.75	189.36	112.88	7.75
		SD	12.58	10.07	7.72	10.35	7.23	12.92	8.90	0.11	4.61	4.61	34.74	15.86	1.71
	90	X	73.80	88.83	64.22	125.17	60.94	119.67	55.33	1.11	9.64	9.64	170.19	106.41	7.69
		SD	13.05	8.50	7.30	9.85	6.69	9.64	6.40	0.07	4.28	4.28	32.24	15.59	1.58
	170	X	72.85	85.53	62.28	125.11	62.83	115.36	52.64	1.19	10.03	10.03	169.89	102.12	7.16
		SD	12.65	7.39	7.95	11.04	9.63	8.78	6.21	0.12	4.69	4.69	34.06	12.58	1.39
Interaction		P	0.980	0.976	0.098	0.057	0.459	0.257	0.054	0.268	0.977	0.308	0.308	0.006	0.448
Time		P	0.015	0.516	0.603	0.474	0.282	0.609	0.099	0.217	0.985	0.985	0.168	0.002	0.190
		ES	0.196	0.037	0.088	0.005	-0.084	-0.066	-0.212	0.127	0.008	0.008	-0.144	-0.367	-0.168
Condition		P	0.110	0.105	0.001	0.001	0.001	0.305	0.001	0.165	0.569	0.760	0.760	0.960	0.756
		ES	0.272	0.708	0.727	0.862	0.175	0.865	0.235	0.096	0.051	0.051	-0.008	0.055	0.189

HR: heart rate; MAP: mean arterial pressure; DBP: diastolic blood pressure; SBP: systolic blood pressure; PP: pulse pressure; cSBP: central systolic blood pressure; cPP: central pulse pressure; pp/cPP: pulse pressure/ central pulse pressure; AIx: augmentation index; SEVR: systemic vascular resistance; SV: stroke volume; CO: cardiac output; TPR: total peripheral resistance; HGI: high glycemic index; LGI: low glycemic index

Table 2. Seated NIRS data

Seated			tHb_2 µM	tHb_3 µM	HbO2_2 µM	HbO2_3 µM	HHb_2 µM	HHb_3 µM	SRS tHb mm	TSI %
HGI	10	X	52.84	53.09	25.76	26.24	27.08	26.86	60.81	55.08
		SD	56.26	62.39	31.55	36.82	25.03	25.87	29.96	51.53
	90	X	44.28	44.29	20.26	19.93	24.01	24.20	53.05	51.00
		SD	48.54	51.49	28.39	29.42	20.42	22.01	25.61	54.02
	170	X	52.69	53.13	24.59	25.61	28.09	27.52	60.53	56.40
		SD	47.23	52.04	26.78	29.68	20.84	22.78	25.53	53.27
LGI	10	X	40.29	42.61	17.44	18.84	22.84	23.76	54.22	50.20
		SD	59.11	63.14	33.78	36.74	25.60	26.66	34.67	51.85
	90	X	49.46	50.43	21.89	23.41	27.56	27.02	61.22	57.27
		SD	56.04	61.58	31.69	36.50	24.84	25.45	27.82	51.29
	170	X	38.64	41.60	16.94	18.72	21.70	22.88	55.34	48.89
		SD	45.72	50.13	26.25	28.92	19.74	21.50	24.77	54.02
Interaction		P	0.924	0.989	0.954	0.984	0.774	0.922	0.955	0.542
Time		P	0.638	0.851	0.459	0.802	0.857	0.915	0.919	0.981
		ES	-0.091	-0.053	-0.121	-0.064	-0.048	-0.033	0.039	0.012
Condition		P	0.013	0.052	0.017	0.041	0.012	0.079	0.020	0.001
		ES	0.423	0.329	0.407	0.347	0.425	0.297	0.395	0.655

tHb: total hemoglobin; HbO2: oxygenated hemoglobin; HHb: deoxygenated hemoglobin; SRS tHb: spatially resolved spectroscopy total hemoglobin; TSI: tissue saturation index; HGI: high glycemic index; LGI: low glycemic index

APPENDIX C – PARTICIPANT LOGS

Food log given to participants to record intake 24 hours prior to each experimental visit

24-Hour Food Log – Visit 1

Time	Place	Meal	Food/Beverage Item	Details/Ingredients/Preparation	Amount
Example: 8:00AM	Home	Breakfast	Brown sugar instant oatmeal	Made with water, nothing else added	2 packets

Sleep diary completed by participants upon arriving for each experimental visit

Karolinska Sleep Diary

Bedtime (hr.):_____

Time of awakening (hr.):_____

Time falling asleep?_____

How did you sleep?

Very poorly-1   2   3   4   5-very well

Feeling refreshed after wakening?

Not at all-1   2   3   4   5-Completely

Calm Sleep?

Very restless-1   2   3   4   5-very calm

Slept throughout the allotted time?

Woke up much to early-1   2   3   4   5-yes

Ease of waking up?

Very difficult-1   2   3   4   5-very easy

Ease of falling asleep?

Very difficult-1   2   3   4   5-very easy

Amount of dreaming?

None-1   2   3   4   5-much

Number of awakenings?_____

REFERENCES

1. Owen N, Sparling PB, Healy GN, Dunstan DW, Matthews CE. Sedentary behavior: Emerging evidence for a new health risk. Mayo Clin. Proc. 2010.

2. Cardiovascular Disease: A Costly Burden For America Projections Through 2035. [cited 2018 Jun 1]; Available from: https://healthmetrics.heart.org/wp-content/uploads/2017/10/Cardiovascular-Disease-A-Costly-Burden.pdf

3. Restaino RM, Holwerda SW, Credeur DP, Fadel PJ, Padilla J. Impact of prolonged sitting on lower and upper limb micro- and macrovascular dilator function. Exp Physiol [Internet]. NIH Public Access; 2015 [cited 2017 Sep 15];100:829–38. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25929229

4. Morishima T, Restaino RM, Walsh LK, Kanaley JA, Fadel PJ, Padilla J, et al. Prolonged sitting-induced leg endothelial dysfunction is prevented by fidgeting. Am J Physiol Hear Circ Physiol. 2016;311:H177-82.

5. Rubin J, Nambi V, Chambless LE, Steffes MW, Juraschek SP, Coresh J, et al. Hyperglycemia and Arterial Stiffness: the Atherosclerosis Risk in the Communities Study. [cited 2018 Jun 11]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3936879/pdf/nihms552781.pdf

6. Kawano H, Motoyama T, Hirashima O, Hirai N, Miyao Y, Sakamoto T, et al. Hyperglycemia Rapidly Suppresses Flow-Mediated Endothelium- Dependent Vasodilation of Brachial Artery. J Am Coll Cardiol [Internet]. 1999 [cited 2018 May 31];34:146–54. Available from: https://ac.els-cdn.com/S0735109799001680/1-s2.0-S0735109799001680-main.pdf?_tid=6b1253f0-4cc9-42a1-be3a-95a465d9021a&acdnat=1527788138_f600f3dd85b3c95e3edda052df5aac4c

7. Akbari CM, Saouaf R, Barnhill DF, Newman PA, LoGerfo FW, Veves A. Endothelium-dependent vasodilatation is impaired in both microcirculation and macrocirculation during acute hyperglycemia. J Vasc Surg. 1998;

8. Matthews CE, Chen KY, Freedson PS, Buchowski MS, Beech BM, Pate RR, et al. Amount of Time Spent in Sedentary Behaviors in the United States. 2003 [cited 2017 Dec 7]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3527832/pdf/nihms-209987.pdf

9. McCarthy M, Edwardson CLL, Davies MJJ, Henson J, Bodicoat DHH, Khunti K, et al. Fitness Moderates Glycemic Responses to Sitting and Light Activity Breaks. Med Sci Sports Exerc. 2017;2216–22.

10. The Price of Inactivity [Internet]. [cited 2018 Jun 2]. Available from: http://www.heart.org/HEARTORG/HealthyLiving/PhysicalActivity/StartWalking/The-Price-of-Inactivity_UCM_307974_Article.jsp#.WxLkc9OUsWp

11. Biswas A, Oh PI, Faulkner GE, Bajaj RR, Silver MA, Mitchell MS, et al. Sedentary Time and Its Association With Risk for Disease Incidence, Mortality, and Hospitalization in Adults A Systematic Review and Meta-analysis. [cited 2017 Nov 19]; Available from: http://dssimon.com/MM/ACP-sedentary/Sendentarytime.pdf

12. Edwardson CL, Gorely T, Davies MJ, Gray LJ, Khunti K, Wilmot EG, et al. Association of Sedentary Behaviour with Metabolic Syndrome: A Meta-Analysis. PLoS One [Internet]. 2012 [cited 2017 Nov 19];7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3325927/pdf/pone.0034916.pdf

13. Wilmot EG. Sedentary time in adults and the association with diabetes, cardiovascular disease and death: systematic review and meta-analysis. [cited 2017 Nov 19]; Available from: http://getamericastanding.org/pdfs/research/36.pdf

14. Heart Disease and Stroke Cost America Nearly $1 Billion a Day in Medical Costs, Lost Productivity | CDC Foundation [Internet]. [cited 2018 Jun 2]. Available from: https://www.cdcfoundation.org/pr/2015/heart-disease-and-stroke-cost-america-nearly-1-billion-day-medical-costs-lost-productivity

15. Singh SK. Post-prandial hyperglycemia. Indian J Endocrinol Metab [Internet]. Wolters Kluwer -- Medknow Publications; 2012 [cited 2017 Oct 4];16:S245-7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23565390

16. Keller J, Kahlhöfer J, Peter A, Bosy-Westphal A. Effects of Low versus High Glycemic Index Sugar-Sweetened Beverages on Postprandial Vasodilatation and Inactivity-Induced Impairment of Glucose Metabolism in Healthy Men. 2016 [cited 2018 May 3]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5188457/pdf/nutrients-08-00802.pdf

17. Hamilton MT, Hamilton DG, Zderic TW. Role of Low Energy Expenditure and Sitting in Obesity, Metabolic Syndrome, Type 2 Diabetes, and Cardiovascular Disease. 2655 [cited 2017 Dec 7]; Available from: http://diabetes.diabetesjournals.org/content/56/11/2655.full-text.pdf

18. Katzmarzyk PT, Church TS, Craig CL, Bouchard C. Sitting Time and Mortality from All Causes, Cardiovascular Disease, and Cancer. Med Sci Sport Exerc [Internet]. 2009 [cited 2017 Dec 7];41:998–1005. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19346988

19. Bellettiere J, Winkler EAH, Chastin SFM, Kerr J, Owen N, Dunstan DW, et al. Associations of sitting accumulation patterns with cardio-metabolic risk biomarkers in Australian adults. | Jt Dr Progr Public Heal [Internet]. [cited 2017 Sep 19]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5491133/pdf/pone.0180119.pdf

20. Thorp AA, Healy GN, Owen N, Salmon J, Ball K, Shaw JE, et al. Deleterious Associations of Sitting Time and Television Viewing Time With Cardiometabolic Risk Biomarkers. Aust Diabetes [Internet]. 2004 [cited 2017 Sep 19]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2809275/pdf/zdc327.pdf

21. Brocklebank LA, Falconer CL, Page AS, Perry R, Cooper AR. Accelerometer-measured sedentary time and cardiometabolic biomarkers: A systematic review. Prev Med (Baltim) [Internet]. 2015 [cited 2017 Nov 19];76:92–102. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25913420

22. Padilla J, Fadel PJ. Prolonged sitting leg vasculopathy: contributing factors and clinical implications Running Title: Sitting leg vasculopathy. Am J Physiol Hear Circ Physiol [Internet]. 2017 [cited 2017 Oct 4]; Available from:

http://ajpheart.physiology.org/content/ajpheart/early/2017/07/19/ajpheart.00326.2017.full
.pdf

23. Ray CA, Rea RF, Clary MP, Mark AL. Muscle sympathetic nerve responses to dynamic one-
legged exercise: effect of body posture. [cited 2018 Jun 2]; Available from:
https://www.physiology.org/doi/pdf/10.1152/ajpheart.1993.264.1.H1

24. Vane JR, Botting RM. Secretory functions of the vascular endothelium. J Physiol Pharmacol
[Internet]. 1992 [cited 2018 Jun 10];43:195–207. Available from:
http://www.ncbi.nlm.nih.gov/pubmed/1493252

25. Restaino RM, Walsh LK, Morishima T, Vranish JR, Martinez-Lemus LA, Fadel PJ, et al.
Endothelial dysfunction following prolonged sitting is mediated by a reduction in shear
stress. [cited 2018 Jun 2]; Available from:
https://www.physiology.org/doi/pdf/10.1152/ajpheart.00943.2015

26. Morawietz H, Talanow R, Szibor M, Rueckschloss U, Schubert A, Bartling B, et al.
Regulation of the endothelin system by shear stress in human endothelial cells. J
Physiol [Internet]. 2000 [cited 2018 Jun 2];525:761–70. Available from:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2269966/pdf/tjp0525-0761.pdf

27. Amiri F, Virdis A, Fritsch Neves M, Iglarz M, Seidah NG, Touyz RM, et al. Endothelium-
restricted overexpression of human endothelin-1 causes vascular remodeling and
endothelial dysfunction. Circulation. 2004;110:2233–40.

28. Dong F, Zhang X, Wold LE, Ren Q, Zhang Z, Ren J. Endothelin-1 enhances oxidative
stress, cell proliferation and reduces apoptosis in human umbilical vein endothelial cells:
role of ET B receptor, NADPH oxidase and caveolin-1. Br J Pharmacol [Internet]. 2005
[cited 2018 Jun 2];145:323–33. Available from:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1576147/pdf/145-0706193a.pdf

29. Duerrschmidt N, Wippich N, Goettsch W, Broemme H-J, Morawietz H. Endothelin-1 Induces
NAD(P)H Oxidase in Human Endothelial Cells. Biochem Biophys Res Commun
[Internet]. Academic Press; 2000 [cited 2018 Jun 2];269:713–7. Available from:
https://www.sciencedirect.com/science/article/pii/S0006291X00923544

30. Pernow J, Shemyakin A, Böhm F. New perspectives on endothelin-1 in atherosclerosis and
diabetes mellitus. Life Sci [Internet]. 2012 [cited 2018 Jun 2];91:507–16. Available from:
http://www.ncbi.nlm.nih.gov/pubmed/22483688

31. Vanhoutte PM, Shimokawa H, Feletou M, Tang EHC. Endothelial dysfunction and vascular
disease - a 30th anniversary update. Acta Physiol [Internet]. 2017 [cited 2018 Jun
2];219:22–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26706498

32. Mattace-Raso FUS, Van Der Cammen TJM, Hofman A, Van Popele NM, Bos ML,
Schalekamp MADH, et al. Arterial stiffness and risk of coronary heart disease and
stroke: The Rotterdam Study. Circulation. 2006;113:657–63.

33. Bohn L, Ramoa A, Silva G, Silva N, Abreu SM, Ribeiro F, et al. Sedentary Behavior and
Arterial Stiffness in Adults with and without Metabolic Syndrome. Int J Sport Med
[Internet]. 2017 [cited 2018 Jun 2];38:396–401. Available from: https://www.thieme-

connect.com/products/ejournals/pdf/10.1055/s-0043-101676.pdf

34. Ford ES, Kohl HW, Mokdad AH, Ajani UA. Sedentary behavior, physical activity, and the metabolic syndrome among U.S. adults. Obes Res. 2005;13:608–14.

35. Horta BL, Schaan B. Objectively measured physical activity and secentary-time are associated with arterial stiffness in Brazilian young adults. Atherosclerosis [Internet]. 2015 [cited 2018 Jun 2]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4678284/pdf/main.pdf

36. García-Hermoso A, Notario-Pacheco B, Recio-Rodríguez JI, Martínez-Vizcaíno V, Rodrigo De Pablo E, Belio JFM, et al. Sedentary behaviour patterns and arterial stiffness in a Spanish adult population e The EVIDENT trial for the EVIDENT Group f. Atherosclerosis [Internet]. 2015 [cited 2018 Jun 2];243:516–22. Available from: https://www.atherosclerosis-journal.com/article/S0021-9150(15)30151-9/pdf

37. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature [Internet]. Nature Publishing Group; 1999 [cited 2018 Jun 10];399:601–5. Available from: http://www.nature.com/articles/21224

38. Woodman CR, Price EM, Harold Laughlin M, Harold M. Shear stress induces eNOS mRNA expression and improves endothelium- dependent dilation in senescent soleus muscle feed arteries. [cited 2018 Jun 10]; Available from: https://www.physiology.org/doi/pdf/10.1152/japplphysiol.00408.2004

39. Cui J, McQuillan PM, Blaha C, Kunselman AR, Sinoway LI. Limb venous distension evokes sympathetic activation via stimulation of the limb afferents in humans. Am J Physiol Heart Circ Physiol [Internet]. American Physiological Society; 2012 [cited 2018 Jun 9];303:H457-63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22707559

40. Mueller PJ. New Investigator Award Of The Neural Control And Autonomic Regulation Physical (in)activity-dependent alterations at the rostral ventrolateral medulla: influence on sympathetic nervous system regulation. 2009 [cited 2018 Jun 9]; Available from: https://www.physiology.org/doi/pdf/10.1152/ajpregu.00101.2010

41. Moffitt JA, Foley CM, Schadt JC, Laughlin MH, Hasser EM. Attenuated baroreflex control of sympathetic nerve activity after cardiovascular deconditioning in rats. Am J Physiol [Internet]. 1998 [cited 2018 Jun 9];274:R1397-405. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9612408

42. Bailey. Effects of breaking up prolonged sitting following low and high GI breakfast consumption on gluciose and insulin concentrations.

43. de Vegt F, Dekker JM, Jager A, Hienkens E, Kostense PJ, Stehouwer CDA, et al. Relation of Impaired Fasting and Postload Glucose With Incident Type 2 Diabetes in a Dutch Population. JAMA [Internet]. American Medical Association; 2001 [cited 2017 Dec 7];285:2109. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.285.16.2109

44. Loader J, Montero D, Lorenzen C, Watts R, Méziat C, Reboul C, et al. Acute hyperglycemia

impairs vascular function in healthy and cardiometabolic diseased participants: Systematic review and meta-analysis. Arterioscler Thromb Vasc Biol. 2015;35:2060–72.

45. Zilversmit DB. Atherogenesis: a postprandial phenomenon. Circulation [Internet]. American Heart Association, Inc.; 1979 [cited 2017 Dec 7];60:473–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/222498

46. Ceriello A. The post-prandial state and cardiovasculardisease: relevance to diabetes mellitus. 2000; Available from: http://onlinelibrary.wiley.com/doi/10.1002/%28SICI%291520-7560%28200003/04%2916:2%3C125::AID-DMRR90%3E3.0.CO;2-4/epdf

47. Ceriello A. The possible role of postprandial hyperglycemia in the pathogenesis of diabetic complications. Diabetologia [Internet]. 2003 [cited 2018 Jun 3];46. Available from: https://link.springer.com/content/pdf/10.1007%2Fs00125-002-0931-5.pdf

48. Stevenson E, Williams C, Nute M. The influence of the glycaemic index of breakfast and lunch on substrate utilisation during the postprandial periods and subsequent exercise. Br J Nutr [Internet]. 2005 [cited 2018 Jun 3];93:885–93. Available from: https://www.cambridge.org/core/services/aop-cambridge-core/content/view/C097754EE00E3A96B7B239FDE3BD56FA/S0007114505001340a.pdf/influence_of_the_glycaemic_index_of_breakfast_and_lunch_on_substrate_utilisation_during_the_postprandial_periods_and_subsequent

49. Cocate PG, Pereira LG, Marins JC, Cecon PR, Bressan J, Alfenas RC. Metabolic responses to high glycemic index and low glycemic index meals: a controlled crossover clinical trial. Nutr J [Internet]. 2011 [cited 2018 May 3];10:1. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3027095/pdf/1475-2891-10-1.pdf

50. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol J-P, et al. Activation of Oxidative Stress by Acute Glucose Fluctuations Compared With Sustained Chronic Hyperglycemia in Patients With Type 2 Diabetes. [cited 2017 Nov 20]; Available from: http://pmmp.cnki.net/Resources/CDDPdf/evd%5C200801%5CJAMA%5C

51. Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, et al. Oscillating Glucose Is More Deleterious to Endothelial Function and Oxidative Stress Than Mean Glucose in Normal and Type 2 Diabetic Patients. Diabetes [Internet]. 2008 [cited 2017 Nov 20];57. Available from: http://diabetes.diabetesjournals.org/content/diabetes/57/5/1349.full.pdf

52. Brownlee M. The Pathobiology of Diabetic Complications A Unifying Mechanism. Banting Lect [Internet]. 2005 [cited 2018 Jun 3]; Available from: http://diabetes.diabetesjournals.org/content/diabetes/54/6/1615.full.pdf

53. Giugliano D, Marfella R, Coppola L, Verrazzo G, Acampora R, Giunta R, et al. vascular effects of acute hyperglycemia in humans are reversed by L-arginine.Avidence for reduced availability of nitric oxide during hyperglycemia. Circulation. 1997;95:1783–90.

54. Title LM, Cummings PM, Giddens K. Oral Glucose Loading Acutely Attenuates Endothelium-Dependent Vasodilation in Healthy Adults Without Diabetes: An Effect Prevented by Vitamins C and E. J Am Coll Cardiol [Internet]. 2000 [cited 2018 Jun 3];36:2185–91. Available from: https://ac.els-cdn.com/S0735109700009803/1-s2.0-

S0735109700009803-main.pdf?_tid=bf7b4a5c-2748-4041-8c50-f764afea6c1e&acdnat=1528061929_4c2ef2b5a623a706bbad0052b320317f

55. Bohlen HG, Lash JM. Topical hyperglycemia rapidly suppresses EDRF-mediated vasodilation of normal rat arterioles. [cited 2018 Jun 3]; Available from: https://www.physiology.org/doi/pdf/10.1152/ajpheart.1993.265.1.H219

56. Bucala R, Tracey KJ, Cerami A. Advanced Glycosylation Products Quench Nitric Oxide and Mediate Defective Endothelium-dependent Vasodilatation in Experimental Diabetes. [cited 2018 Jun 3]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC295094/pdf/jcinvest00057-0060.pdf

57. Tesfamariam B, Cohen RA. Free radicals mediate endothelial cell dysfunction caused by elevated glucose. [cited 2018 Jun 3]; Available from: https://www.physiology.org/doi/pdf/10.1152/ajpheart.1992.263.2.H321

58. Moncada S, Higgs A. The L-Arginine-Nitric Oxide Pathway. N Engl J Med [Internet]. 1993 [cited 2018 Jun 3];329:2002–12. Available from: http://www.nejm.org/doi/abs/10.1056/NEJM199312303292706

59. Kumsta C, Thamsen M, Jakob U. Effects of Oxidative Stress on Behavior, Physiology, and the Redox Thiol Proteome of Caenorhabditis elegans. Antioxid Redox Signal [Internet]. [cited 2018 Jun 3];14:1023–37. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3052275/pdf/ars.2010.3203.pdf

60. Cleland SJ, Petrie JR, Ueda S, Elliott HL, Connell JM. Insulin-mediated vasodilation and glucose uptake are functionally linked in humans. Hypertension. 1999;33:554–8.

61. Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P. Nitric Oxide Release Accounts for Insulin's Vascular Effects in Humans. J Clin Invesr [Internet]. 1994 [cited 2018 Jun 4];94:2511–5. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC330085/pdf/jcinvest00490-0341.pdf

62. Steinberg H 0, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin-mediated Skeletal Muscle Vasodilation Is Nitric Oxide Dependent A Novel Action of Insulin to Increase Nitric Oxide Release Key words: euglycemic hyperinsulinemic clamp * leg blood flow * methacholine chloride * sodium nitroprusside * vascular resistance. 1994 [cited 2018 Jun 4];94:1172–9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC295191/pdf/jcinvest00021-0270.pdf

63. Abramson LI, Schkloven N, Margolis MN, Mirsky IA. INFLUENCE OF MASSIVE DOSES OF INSULIN ON PERIPHERAL BLOOD FLOW IN MAN'. [cited 2018 Jun 4]; Available from: https://www.physiology.org/doi/pdf/10.1152/ajplegacy.1939.128.1.124

64. Laakso M, Edelman S V, Brechtel G, Baron AD. Decreased Effect of Insulin To Stimulate Skeletal Muscle Blood Flow in Obese Man A Novel Mechanism for Insulin Resistance. Clin Invest [Internet]. 1990 [cited 2018 Jun 4]; Available from: https://pdfs.semanticscholar.org/848f/3971b97a88caf244775384d9dc186a0bcee8.pdf?_ga=2.131494863.1712467190.1528122001-804241867.1528122001

65. Muniyappa R, Montagnani M, Koh KK, Quon MJ. Cardiovascular Actions of Insulin. [cited

2018 Jun 4]; Available from:
https://watermark.silverchair.com/edrv0463.pdf?token=AQECAHi208BE49Ooan9kkhW_
Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAc4wggHKBgkqhkiG9w0BBwagggG7MIIBtwIBADC
CAbAGCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQMLpVhpeRtR_N8F2e4AgEQgI
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mcgjNESrsc5AVrccaDRYTmijq5XaUNJdjFaWll5LCE5WpqFKXQNgOkjX1xcBTeCkdtS6-
ZebDJ9WEb213nIKJMQ-KcbfsZem0

66. Dickinson S, Colagiuri S, Faramus E, Petocz P, Brand-Miller JC. Human Nutrition and
Metabolism Postprandial Hyperglycemia and Insulin Sensitivity Differ among Lean
Young Adults of Different Ethnicities. J Nutr [Internet]. 2002 [cited 2018 Jun
4];132:2574–9. Available from:
https://watermark.silverchair.com/4w0902002574.pdf?token=AQECAHi208BE49Ooan9k
khW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAdIwggHOBgkqhkiG9w0BBwagggG_MIIBuwIB
ADCCAbQGCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQM8j9YJkjBhvlpLcvUAgE
QgIIBhRRELDdUwLfiSLHfgm_cIkz3gelePagMQjuwQmKu5q

67. Zavaroni I, Bonini L, Gasparini P, Barilli AL, Zuccarelli A, Dall'Aglio E, et al.
Hyperinsulinemia in a normal population as a predictor of non-insulin-dependent
diabetes mellitus, hypertension, and coronary heart disease: the Barilla factory revisited.
Metabolism [Internet]. 1999 [cited 2018 Jun 4];48:989–94. Available from:
http://www.ncbi.nlm.nih.gov/pubmed/10459563

68. Vague P, Raccah D, Scelles V. Hypofibrinolysis and the insulin resistance syndrome. Int J
Obes Relat Metab Disord [Internet]. 1995 [cited 2018 Jun 4];19 Suppl 1:S11-5. Available
from: http://www.ncbi.nlm.nih.gov/pubmed/7550531

69. Steiner G, Lewis GF. Hyperinsulinemia and Triglyceride-Rich Lipoproteins. [cited 2018 Jun
4]; Available from:
http://diabetes.diabetesjournals.org/content/diabetes/45/Supplement_3/S24.full.pdf

70. Ceriello A. Postprandial Glucose Regulation and Diabetic Complications. 2004;164:2090–5.

71. Bonora E, Muggeo M. Postprandial blood glucose as a risk factor for cardiovascular disease
in Type II diabetes: the epidemiological evidence. Diabetologia [Internet]. 2001 [cited
2018 Jun 4];44:2107–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11793012

72. Thosar SS, Bielko SL, Wiggins CC, Wallace JP. Differences in brachial and femoral artery
responses to prolonged sitting. [cited 2018 Jun 10]; Available from:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4290438/pdf/12947_2014_Article_544.pd
f

73. Oliver JJ, Webb DJ. Noninvasive Assessment of Arterial Stiffness and Risk of
Atherosclerotic Events ATVB In Focus Noninvasive Assessment of Atherosclerosis: from

Structure to Function Noninvasive Assessment of Arterial Stiffness and Risk of Atherosclerotic Events. Arter Thromb Vasc Biol [Internet]. 2003;23:554–66. Available from: http://atvb.ahajournals.org/content/23/4/554%5Cnhttp://atvb.ahajournals.org//subscriptions/%5Cnhttp://atvb.ahajournals.org/

74. Mackenzie IS, Wilkinson IB, Cockcroft JR. QJM Assessment of arterial stiffness in clinical practice. [cited 2018 Jun 9]; Available from: https://watermark.silverchair.com/950067.pdf?token=AQECAHi208BE49Ooan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAaMwggGfBgkqhkiG9w0BBwagggGQMIIBjAIBADCCAYUGCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQMURkY-skWVDY5qo_YAgEQgIIBVjbx7_oSCmC0bk-8L45B2V3w_YHFLK2pRVUj-IMhfVD5Z5ucyzZ-NPL83KN2BbSf50SvbaO6xWsIDigAHF6ETYk-FmpjV1upPew2HnmwPh41xWQXhTxwkylp2y3KsdQdpZvMy2oPJQkiE4dAkh3qug7Ftg8lsKZ7Q7qudvat8jPiGq0qYDYyjrvKgOXpD10Hyve4Jv-4wtcRAk-ZxUbWt-LXUL22_sOXNs00x5oHHPbP41KdsY0dExA4qS89xVjR5LJ2yNz95HvBreF1mNo1073I9BGxRVCQ4-WxQSizY5HwMNkWirNDfbyGVm7e17QH9uDAXYlUzVMh09NT5BFnNks3EM8VKkKqERLlWv60C1dW75J1sDYNF3TyNp5onbfLp9J8MIlvdkBZiq25wC-kik78xiMAd4K80_lqhLHYfgcfGetXe5rx8rGC5i-3EMrFbtda9Fvhyg

75. Mancia G. 2013 ESH/ESC Guidelines for the management of arterial hypertension. Eur Heart J [Internet]. 2013 [cited 2018 Jun 4];34. Available from: https://watermark.silverchair.com/eht151.pdf?token=AQECAHi208BE49Ooan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAbEwggGtBgkqhkiG9w0BBwagggGeMIIBmgIBADCCAZMGCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQM4sf8oCB1xlP9l-yvAgEQgIIBZH00_TRbWhdmbV0EpEkP4z6_NpgSeYNNNyx6H-fQK8-LU2r7

76. Laurent S, Cockcroft J, Bortel L Van, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. [cited 2018 Jun 4]; Available from: https://watermark.silverchair.com/ehl254.pdf?token=AQECAHi208BE49Ooan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAbIwggGuBgkqhkiG9w0BBwagggGfMIIBmwIBADCCAZQGCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQMsabHNhUFW3WWrPfNAgEQgIIBZdB2_Qvma5ziaQkiavY6-ZjdwhDhbYsglmEqPDNRbMuYyP_GYy6HFj6QsFVBeMwn6K3A-SZCdbeA-ytyYj6d2akNx0jK6DpwUuCaqCJ5vwZGOibd-ct-whHBWI2zK4IkL0kuOdpw-wQ41ZiI2qrcuY99uUgUFvJq2ln_nQJA9tGKIKfzJEIVDJpukNyUn2HfD8q7mNihtd5vFeDf5igoG3ijEu4raE40LzIyj-2s-sEUoAFiuICM9DTr-b-31-g_y0WezqzHWQXd66p50c27ZYgUl6ejycuA_jRhYc4K2pLE7h9JCat8rZoqe570tuTh-uMrHWLQhG2VEQQb6680MFJhoCJ5uxJxaMwlm557W-JxxybONJ5J0iTL8uVkhkO2WpzKe4PBFZrYfwwVCg4M2PVkeC6ELG7aRqlOQNRAsRP39SC8hH6_RJ789wdIiNNPOBeo6wHBAYWbBWiqOiBYkB8fqQh5iw

77. Weber T, O'Rourke MF, Lassnig E, Porodko M, Ammer M, Rammer M, et al. Pulse waveform characteristics predict cardiovascular events and mortality in patients undergoing coronary angiography. J Hypertens [Internet]. 2010 [cited 2017 Nov 20];28:797–805. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20164805

78. Kis E, Cseprekál O, Kerti A, Salvi P, Benetos A, Tisler A, et al. Measurement of pulse wave velocity in children and young adults: a comparative study using three different devices.

Hypertens Res [Internet]. 2011 [cited 2018 Jun 4];34:1197–202. Available from: https://www.nature.com/articles/hr2011103.pdf

79. Kracht D, Shroff R, Baig S, Doyon A, Jacobi C, Zeller R, et al. Validating a New Oscillometric Device for Aortic Pulse Wave Velocity Measurements in Children and Adolescents. Am J Hypertens [Internet]. 2011 [cited 2018 Jun 4];24:1294–9. Available from:https://watermark.silverchair.com/24_12_1294.pdf?token=AQECAHi208BE49Ooan 9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAaYwggGiBgkqhkiG9w0BBwagggGTMIIBjwI BADCCAYgGCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQMsT0Lt__h585dsH4GA gEQgIIBWZ4lBneFtGKTm2d2VA- X0vdBd3REGTAltrqcwFKUUN1v8Bh1p5go5ub0GMbL6cLw0J4PTLfsl6Pns4HM2rMGR5 U8JQq0MmzcCmrxg7Fs0_QuDdwqnWMblHf_rwN3svi6h3VuEdiqYTnmfBWQCqoEbNIf qBXTNT5xPTUUoqhlvPXsLOf4LEq6cxTLJwfK6CjieooZypGRrmKjpk6hvs5_ZctIv- h8EytDiuSMdaFqKyAQp8ATwQwH4EecgyLNgEBDxHFQPOsioMMf73InkVFbBww1X4Z ik888pdNAWDdaaHGQYW6wJs-rmEsYqmrtqt6Ykk_s0fJYjQGvEmyTh4AtyvFd7- _dJYrXjjHKInV_sphtAMZPfnDgRVmab7R_HHWZAnGg6Y9RddO5YDcJuT9BuxZ_oSSN rIwh3dkvt6zLqmBNP3KoHfAfeJZYkloOdjhD0J94IrLnOqjCvg

80. Hickson SS, Butlin M, Broad J, Avolio AP, Wilkinson IB, Mceniery CM. Validity and repeatability of the Vicorder apparatus: a comparison with the SphygmoCor device. Hypertens Res [Internet]. 2009 [cited 2018 Jun 4];32154:1079–85. Available from: https://www.nature.com/articles/hr2009154.pdf

81. Redheuil A, Bensalah M, Kachenoura N, Bruguiere E, Azarine A, Perdrix L. Measuring aortic distensibility with cmr using central pressures estimated in the magnet: comparison with carotid and peripheral pressures. 2011 [cited 2018 Jun 4]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3106581/pdf/1532-429X-13-S1- P27.pdf

82. Pucci G, Cheriyan J, Hubsch A, Hickson SS, Gajendragadkar PR, Watson T, et al. Evaluation of the Vicorder, a novel cuff-based device for the noninvasive estimation of central blood pressure. J Hypertens [Internet]. 2013 [cited 2018 Jun 4];31:77–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23079681

83. Weber T, Auer J, O'Rourke MF, Kvas E, Lassnig E, Berent R, et al. Arterial Stiffness, Wave Reflections, and the Risk of Coronary Artery Disease. Circulation. 2004;109:184–9.

84. Papaioannou TG, Karatzis EN, Karatzi KN, Gialafos EJ, Protogerou AD, Stamatelopoulos KS, et al. Hour-to-hour and week-to-week variability and reproducibility of wave reflection indices derived by aortic pulse wave analysis: implications for studies with repeated measurements. J Hypertens [Internet]. 2007 [cited 2017 Nov 20];25:1678–86. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17620966

85. Stoner L, Young JM, Fryer S. Assessments of Arterial Stiffness and Endothelial Function Using Pulse Wave Analysis. Int J Vasc Med Artic ID [Internet]. Hindawi Publishing Corporation; 2012 [cited 2017 Nov 20];903107. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3361177/pdf/IJVM2012-903107.pdf

86. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of Cardiovascular Events and All-Cause Mortality With Arterial Stiffness A Systematic Review and Meta-Analysis. JAC [Internet]. [cited 2018 Jun 4];55:1318–27. Available from: https://ac.els-

cdn.com/S0735109710002809/1-s2.0-S0735109710002809-main.pdf?_tid=02e2c3ec-dd47-4fe3-9268-65e64f3aea46&acdnat=1528143514_fa14fde470fe3e097331a71578113efa

87. Stauss HM, Stauss HM. Heart rate variability. [cited 2017 Nov 20]; Available from: http://ajpregu.physiology.org/content/ajpregu/285/5/R927.full.pdf

88. Schnell O, Barnard K, Bergenstal R, Bosi E, Garg S, Guerci B, et al. Role of Continuous Glucose Monitoring in Clinical Trials: Recommendations on Reporting. [cited 2017 Nov 20]; Available from: http://online.liebertpub.com/doi/pdf/10.1089/dia.2017.0054

89. Cui X, Bray S, Bryant DM, Glover GH, Reiss AL. A quantitative comparison of NIRS and fMRI across multiple cognitive tasks. [cited 2017 Nov 20]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3021967/pdf/nihms256402.pdf

90. Bailey DP, Locke CD. Breaking up prolonged sitting with light-intensity walking improves postprandial glycemia, but breaking up sitting with standing does not. J Sci Med Sport. 2015;18.

91. Ballard KD, Duguid RM, Berry CW, Dey P, Bruno RS, Marie Ward R, et al. Effects of prior aerobic exercise on sitting-induced vascular dysfunction in healthy men. Eur J Appl Physiol [Internet]. 123AD [cited 2017 Oct 23];1. Available from: https://link.springer.com/content/pdf/10.1007%2Fs00421-017-3738-2.pdf

92. Ciok J, Dolna A. [The role of glycemic index concept in carbohydrate metabolism]. Przegl Lek [Internet]. 2006 [cited 2017 Oct 4];63:287–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17036507

93. Little JP, Chilibeck PD, Ciona D, Forbes S, Rees H, Vandenberg A, et al. Effect of Low-and High-Glycemic-Index Meals on Metabolism and Performance During High-Intensity, Intermittent Exercise. Int J Sport Nutr Exerc Metab [Internet]. 2010 [cited 2018 May 3];20:447–56. Available from: https://pdfs.semanticscholar.org/cc0b/69fd5a9760713643523b69b42f376b5e510b.pdf

94. Jamurtas AZ, Tofas T, Fatouros I, Nikolaidis MG, Paschalis V, Yfanti C, et al. The effects of low and high glycemic index foods on exercise performance and beta-endorphin responses. J Int Soc Sports Nutr [Internet]. 2011 [cited 2018 May 3];8:15. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3219660/pdf/1550-2783-8-15.pdf

95. Kaplan RJ, Greenwood CE, Winocur G, Wolever TM. Cognitive performance is associated with glucose regulation in healthy elderly persons and can be enhanced with glucose and dietary carbohydrates. Am J Clin Nutr [Internet]. 2000;72:825–36. Available from: http://ajcn.nutrition.org/content/72/3/825.full

96. Nilsson A, Radeborg K, Björck I. Effects of differences in postprandial glycaemia on cognitive functions in healthy middle-aged participants. Eur J Clin Nutr [Internet]. 2009 [cited 2018 May 23];63:113–20. Available from: https://www.nature.com/articles/1602900.pdf

97. Moore LJS, Midgley AW, Thomas G, Thurlow S, Mcnaughton LR, Mcnaughton L. The Effects of Low and High Glycemic Index Meals on Time-Trial Performance. Int J Sports

Physiol Perform [Internet]. [cited 2018 May 3]; Available from:
http://www.humankinetics.com/acucustom/sitename/Documents/DocumentItem/17100.p
df

98. Kaviani M, Chilibeck P, Yee P, Zello G. The effect of consuming low- versus high-glycemic index meals after exercise on postprandial blood lipid response following a next-day high-fat meal. Nat Publ Gr [Internet]. 2016 [cited 2018 Apr 22]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4973139/pdf/nutd201626a.pdf

99. Ludwig DS, Majzoub JA, Al-Zahrani A, Dallal GE, Blanco I, Roberts SB. High Glycemic Index Foods, Overeating, and Obesity. [cited 2017 Oct 23]; Available from: http://pediatrics.aappublications.org/content/pediatrics/103/3/e26.full.pdf

100. Nilsson A, Stman E, Preston T, Björck I. Effects of GI vs content of cereal fibre of the evening meal on glucose tolerance at a subsequent standardized breakfast. Eur J Clin Nutr [Internet]. 2008 [cited 2017 Sep 19];62:712–20. Available from: https://www.nature.com/ejcn/journal/v62/n6/pdf/1602784a.pdf

101. Meng H, Matthan NR, Ausman LM, Lichtenstein AH, Mayer J. Effect of macronutrients and fiber on postprandial glycemic responses and meal glycemic index and glycemic load value determinations 1–3. [cited 2018 May 3]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5366046/pdf/ajcn144162.pdf

102. Clark C, Gardiner J, Mcburney M, Anderson S, Weatherspoon L, Henry D, et al. Effects of breakfast meal composition on second meal metabolic responses in adults with type 2 diabetes mellitus. Eur J Clin Nutr [Internet]. 2006 [cited 2018 May 3];60:1122–9. Available from: https://www.nature.com/articles/1602427.pdf

103. Vega-López S, Ausman LM, Matthan NR, Lichtenstein AH. Postprandial lipid responses to standard carbohydrates used to determine glycaemic index values. [cited 2018 May 3]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4429602/pdf/nihms683465.pdf

104. Bailey DP, Sardinha LB, Magalhães JP, Santos DA, Júdice PB. Sedentary Patterns, Physical Activity, and Cardiorespiratory Fitness in Association to Glycemic Control in Type 2 Diabetes Patients. Front Physiol Front Physiol [Internet]. 2017 [cited 2017 Sep 19];8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5409266/pdf/fphys-08-00262.pdf

105. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values.' Eur Heart J [Internet]. Oxford University Press; 2010 [cited 2018 Jun 11];31:2338–50. Available from: https://academic.oup.com/eurheartj/article/31/19/2338/441416

106. Wilkinson I, MacCallum H, Flint L, Cockcroft J, Newby D, Webb D. The influence of heart rate on augmentation index and central arterial pressure in humans. J Physiol [internet]. 2000. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2269933/pdf/tjp0525-0263.pdf

107. de Vegt, F., Dekker, J. M., Ruhe, H. G., Stehouwer, C. D., Nijpels, G., Bouter, L. M. & Heine, R. J. (1999) Hyperglycaemia is associated with all-cause and

cardiovascular mortality in the Hoorn population: the Hoorn Study. Diabetologia 42: 926– 931. Available from: https://link.springer.com/article/10.1007%2Fs001250051249

108. Credeur D, Miller S, Jones R, Stoner L, Dolbow D, Fryer S, Stone K, McCoy S. Impact of Prolonged Sitting on Peripheral and Central Vascular Health. American Journal of Cardiology [Internet]. 2019.

109. Baier D, Teren A, Wirkner K, Loeffler M, Scholz M. Parameters of pulse wave velocity: determinants and reference values assessed in the population-based study LIFE-Adult. Clinical Research in Cardiology. 2018. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6208658/pdf/392_2018_Article_1278.pdf

110. Yates T, Wilmot EG, Khunti K, Biddle S, Gorely T, Davies MJ. Stand up for your health: Is it time to rethink the physical activity paradigm? Diabetes Res Clin Pract [Internet]. 2011 [cited 2017 Oct 26];93:292–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21507499

111. *The Price of Inactivity, American Heart Association*, Heart.org, October 2015. http://www.heart.org/HEARTORG/HealthyLiving/PhysicalActivity/FitnessBasics/The-Price-of- Inactivity_UCM_307974_Article.jsp#.V-fDBPkrKM9

112. Rosenberg DE, Bellettiere J, Gardiner PA, Villarreal VN, Crist K, Kerr J. Independent Associations Between Sedentary Behaviors and Mental, Cognitive, Physical, and Functional Health Among Older Adults in Retirement Communities. J Gerontol A Biol Sci Med Sci [Internet]. 2016 [cited 2019 Apr 7];71:78–83. Available from: https://academic.oup.com/biomedgerontology/article-lookup/doi/10.1093/gerona/glv103

113. GENNUSO KP, GANGNON RE, MATTHEWS CE, THRAEN-BOROWSKI KM, COLBERT LH. Sedentary Behavior, Physical Activity, and Markers of Health in Older Adults. Med Sci Sport Exerc [Internet]. 2013 [cited 2019 Apr 7];45:1493–500. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23475142

114. Stamatakis E, Davis M, Stathi A, Hamer M. Associations between multiple indicators of objectively-measured and self-reported sedentary behaviour and cardiometabolic risk in older adults. Prev Med (Baltim) [Internet]. 2012 [cited 2019 Apr 7];54:82–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0091743511004270

115. Hamilton MT, Hamilton DG, Zderic TW. Sedentary behavior as a mediator of type 2 diabetes. [cited 2018 May 22]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4364419/pdf/nihms659264.pdf

116. Patterson, R., E. McNamara, M. Tainio, et al., *Sedentary behaviour and risk of all-cause, cardiovascular and cancer mortality, and incident type 2 diabetes: a systematic review and dose response meta-analysis.* Eur J Epidemiol, 2018.

117. Thosar, S.S., S.L. Bielko, K.J. Mather, J.D. Johnston, and J.P. Wallace, *Effect of prolonged sitting and breaks in sitting time on endothelial function.* Med Sci Sports Exerc, 2015. **47**(4): p. 843-9.

118. Morishima, T., R.M. Restaino, L.K. Walsh, J.A. Kanaley, and J. Padilla, *Prior exercise and standing as strategies to circumvent sitting-induced leg endothelial dysfunction.* Clin Sci (Lond), 2017. **131**(11): p. 1045-1053.

119. Kruse, N.T., W.E. Hughes, R.M. Benzo, L.J. Carr, and D.P. Casey, *Workplace Strategies to Prevent Sitting-induced Endothelial Dysfunction.* Med Sci Sports Exerc, 2018. **50**(4): p. 801-808.

120. Barone Gibbs, B., R.J. Kowalsky, S.J. Perdomo, et al., *Effect of alternating standing and sitting on blood pressure and pulse wave velocity during a simulated workday in adults with overweight/obesity.* J Hypertens, 2017. **35**(12): p. 2411-2418.

121. Evans, W., Stoner, L., Willey, Q., Kelsch, E., Credeur, D., Hanson, E. Local Exercise Does Not Prevent The Aortic Stiffening Response To Acute Prolonged Sitting: A Randomized Cross-Over Trial. Under review. 2019

122. Tremblay, M. S., Aubert, S., Barnes, J. D., Saunders, T. J., Carson, V., Latimer-Cheung, A. E., … Wondergem, R. (2017). Sedentary Behavior Research Network (SBRN) - Terminology Consensus Project process and outcome. *International Journal of Behavioral Nutrition and Physical Activity*, *14*(1). https://doi.org/10.1186/s12966-017-0525-8

123. Belz, GG. Elastic properties and Windkessel function of the human aorta. Cardiovascular Drugs and Therapy 9: 73-83, 1995.

124. Dunstan, D., Kingwell, B.A., Larsen, R., Healy, G., Cerin, E., Hamilton, M.T., Shaw, J.E., Bertovic, D.A., Zimmet, P.Z., Salmon, J., Owen, N. *Breaking Up Prolonged Sitting Reduces Postprandial Glucose and Insulin Responses*. Diabetes Care, 2012. **35**: p. 976-983.

125. Stone, K.J., Fryer, S.M., Ryan, T., Stoner, L. *The validity and reliability of continuous-wave near-infrared spectroscopy for the assessment of leg blood volume during an orthostatic challenge.* Atherosclerosis, 2016. **251**: p. 234-239.