PROLONGED SITTING WITH OR WITHOUT HIGH GLYCEMIC INDEX MEAL AND THE ACUTE EFFECTS ON CEREBROVASCULAR FUNCTION IN HEALTHY ADULTS

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A thesis submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Masters of Arts in the Department of Exercise and Sports Science (Exercise Physiology).

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ABSTRACT

Kathryn Burnet: Prolonged Sitting With or Without High Glycemic Index Meal and the Acute Effects on Cerebrovascular Function in Healthy Adults
(Under the direction of Lee Stoner)

The study purpose was to determine if prolonged (3-hr) sitting (a) resulted in a decreased total brain blood flow (Q_{BF}) and executive function, and (b) whether this decrease is exacerbated by a high glycemic index meal (HGI). Subjects (n=18) participated in a HGI and low glycemic index (LGI) condition. Doppler Ultrasound was used to measure Q_{BF} using the equation: [(ICA blood flow + VA blood flow) x 2 (ml min^{-1})]. Executive function was assessed using the Stroop Task and Trail Making using the Trail Making Test – Part B. Q_{BF} decreased during sitting, as shown through the LGI condition, but increased in HGI, with a condition effect (P=0.04). No differences were observed in the Stroop and Trail Making tests. Future studies are needed that measure total brain blood flow, cerebral blood flow velocity, perfusion and autoregulation simultaneously to help further understand the effects of prolonged sitting on cerebrovascular function.

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AUC	Area Under the Curve
BF	Blood Flow
CBF	Cerebral Blood Flow
CCA	Common Carotid Artery
CGM	Continuous Glucose Monitor(ing)
CONSORT	Consolidated Standards of Reporting Trials
CVD	Cardiovascular Disease
DeoxyHb	Deoxygenated hemoglobin
FMD	Flow Mediated Dilation
GI	Glycemic Index
Hb	Hemoglobin
HGI	High Glycemic Index
ICA	Internal Carotid Artery
LGI	Low Glycemic Index
MVPA	Moderate to Vigorous Physical Activity
NIRS	Near-infrared spectroscopy
PWV	Pulse Wave Velocity
Q_{BF}	Total Brain Blood Flow
RPE	Borg Rate of Perceived Exertion
T2DM	Type II Diabetes Mellitus
TCD	Transcranial Doppler
tHb	Total hemoglobin
VA	Vertebral Artery

CHAPTER 1: INTRODUCTION

High amounts of sedentary behavior (SB) is associated with poor cerebrovascular health [1,2], including reductions in brain volume [1] and a decline in cognitive function [3,4]. However, less is known regarding the mechanism(s) linking repeated exposure to acute sedentary behavior on chronic cerebrovascular complications. A potential pathway, and one which has been suggested to link repeated prolonged sitting exposure to peripheral and central cardiovascular complications, is brain blood flow (BF). Studies exploring this potential pathway have found that within acute bouts of prolonged sitting BF to the brain, total BF, and middle cerebral artery BF declines [Credeur – unpublished data, [5]]. Interestingly, cerebral perfusion is maintained throughout three hours of sitting [Stoner – unpublished]. However, it is important to consider the conditions at which an individual sits, such as during eating. Poor dietary choices such as foods high in refined sugar and subsequently with a high glycemic index (HGI), are associated with chronic effects such as cognitive dysfunction [6–10]. Acutely, less is known about the effects of HGI foods on cerebrovascular function. Together, HGI foods and prolonged sitting may have detrimental acute effects on cerebrovascular function. Thus, there is a critical need to examine the effects of prolonged sitting and HGI meals on measures of cerebrovascular function.

The long-term goal is to recommend dietary changes during sedentary behavior to optimize cerebrovascular function. While both sedentary behavior and repeated consumption of HGI foods are associated with metabolic, vascular, and cognition dysfunction, this study focused on cerebrovascular and cognitive function. No known study has investigated the combined effect of an HGI meal and prolonged sitting on the cerebrovascular system and cognitive function. To achieve this, our study compared a HGI meal to a low glycemic index (LGI) meal during a

session of 3 hours of sitting and explored the acute changes from pre to post sitting on BF to the brain in young, healthy adults. This population was selected for this preliminary study to minimize confounding variables due to aging or disease. This study also looked at perfusion within the prefrontal cortex, cognitive function (specifically, executive function and memory), and brain fog. We hypothesized that (1) prolonged sitting will (a) result in decreased total brain blood flow (Q_{BF}), and (b) this decrease in total brain BF will be exacerbated by HGI. Our secondary hypothesis includes: compared to a LGI meal, a HGI meal will exacerbate the effects of prolonged sitting on cognitive function and prefrontal cortex perfusion. These findings may inform public health recommendations regarding sedentary behavior and dietary habits for the prevention of poor cerebrovascular function.

CHAPTER 2: LITERATURE REVIEW

Introduction: Risks of Sedentary Behavior

In today's American society, adults typically spend 50-70% of their waking hours sitting [11]. This can be attributed to the way westernized societies interact with the world around them. Communication, transportation, and entertainment technologies, as well as work place and educational environments favor sedentary behavior. This increase in sedentary behavior has been shown to have detrimental cardiometabolic effects, and these effects may impact cerebrovascular and cognitive function. Beginning with the London Bus Study in 1966, Morris' data demonstrated that active conductors were less prone to having a greater incidence of fatal myocardial infarction as compared to the more sedentary drivers [12]. More recently, this has been verified with objective data, and Borgundvaag et al. found that moderate to vigorous physical activity (MVPA) may be associated with a substantially lower mortality risk [13]. However, as previously stated, adults typically spend majority of waking hours sitting, and recent evidence supports that prolonged sitting time is associated with CVD risk, independent of leisure-time physical activity [14]. Many studies continually support this finding, in fact, there is 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 levels [15]. More specifically, these deleterious health outcomes include impaired physical function [16], CVD [17,18], T2DM [19], reductions in brain volume [1,20], and cognitive decline [3,4,16,21].

Epidemiological evidence supports a link between sedentary behavior and a risk for vascular, metabolic, and cognitive decline. However, as sedentary behavior becomes more

prevalent in society, it is important to examine the acute effects of sedentary behavior to further understand how its repeated exposure leads to chronic complications. Examining the effects of prolonged sitting has become a recent area of research interest. The following will outline what is known about the acute vascular, metabolic, and cerebrovascular effects of sedentary behavior.

Consideration 1: Acute Responses to Prolonged Sitting

Vascular

Repeated exposure to prolonged sitting, defined as an acute bout of sedentary behavior (>30 minutes), may directly compromise whole body CV health. In 2015, Thosar et al. found that 3 hours of sitting was associated with a significant impairment in shear rate and superficial femoral artery flow mediated dilation (FMD) [22]. FMD is widely believed to reflect nitric oxide-mediated endothelium-dependent vascular function [23]. Blunted FMD is suggestive of impaired macrovascular endothelial function, but could also be the result of an attenuated reactive hyperemia impaired microvascular function [24]. Similarly, Restaino et al. sought to examine the impact of 6 hours of uninterrupted sitting on micro- and macrovascular dilator function of the lower and upper limbs, assessed by reactive hyperemia and associated FMD response (19). They found that prolonged sitting did reduce lower leg micro- and macrovascular function. Restaino et al. also established that sitting-induced leg endothelial dysfunction is mediated by a reduction in shear stress [25], and several preventative measures were soon found to prevent this reduction in endothelial function caused by prolonged sitting [26]. Shear stress is the stress imposed on the vascular wall by red blood cells travelling close to the vessel wall, i.e. shear stress is dependent on BF [27]. The BF induced shear stress acts on the vascular endothelium, the inner-most lining of blood vessels, and initiates release of nitric oxide [28]. The absence of shear stress leads to increased resistance from constricted vessels, decreasing BF and impairing vascular function.

Further support that sitting-induced leg endothelial dysfunction is mediated by a reduction in shear stress was observed in Hitos et al.; they observed reduced BF reduced by 40% in the legs after just 1.5 hours of sitting [29]. A proposed mechanism for this large decrease in BF is a decrease in muscle “pump” activity [30]. If the muscles in the legs are inactive while in a seated position for a long period of time, blood will pool in the lower extremities. When blood pools in the lower limbs, there is less venous return, which will impair cardiac output. Subsequently, the vessels will constrict to maintain blood pressure. When the vessels constrict, there is an increased resistance to local BF. The local resistance is increased by clotting factors [29] accumulating in these regions due to slow moving blood. Increased resistance will reduce BF and thus BF decreases in the lower extremities during prolonged sitting.

Metabolic

Prolonged uninterrupted sitting is also adversely related to postprandial hyperglycemia [31], due to a lack of muscular contraction stimulated glucose uptake. Postprandial hyperglycemia refers to an exaggerated elevation in blood glucose following consumption of a meal [32]. This postprandial hyperglycemia increases cardiometabolic risk, and has been shown to contribute to pancreatic beta cell failure and progression to late-stage diabetes [19]. Furthermore, postprandial glucose elevation is a key predictor of acute microvascular and macrovascular complications [31]. Additionally, recent studies suggest prolonged sedentary time impairs glucose metabolism [33], due to a lack of skeletal muscle contraction-stimulated glucose uptake.

Cerebrovascular

Normal brain function relies on the regulation of cerebral blood flow (CBF) and glucose disposal, both of which may become impaired with prolonged sitting. Regulation of CBF is dependent upon sufficient venous return to ensure maintenance of total brain BF, cerebral

autoregulation to maintain perfusion pressures [34], and microcirculatory recruitment to maintain perfusion [34,35]. As previously mentioned, excessive sitting time, especially with a lack of skeletal muscle activity, can lead to venous pooling and decreased total brain BF and impaired glycemic control [36,37]. Glycemic control is dependent on adequate delivery and uptake, especially by skeletal muscle. This is accomplished by pairing a glucose transporter (GLUT4), which can be stimulated by insulin, or skeletal muscle contractions [38,39]. Within the brain, insulin-sensitive glucose transporters (GLUT1 and GLUT3) are required to transport glucose across the blood brain barrier [40]. However, exposure to hyperglycemia, which may occur post-prandially and/or with prolonged sitting, results in decreased brain glucose transport [41]. The down-regulation of brain glucose transport, which may initially serve to protect the brain, may exaggerate the effects of subsequent hypoglycemia and ultimately damage the brain [42]. Finally, prolonged exposure to hyperglycemia and repeated exposure to hypoglycemia can damage the microvasculature, impair brain structure and function, and impair cognitive function [43,44].

Summary

Prolonged sitting has been linked to vascular, metabolic, cerebrovascular, and cognitive dysfunction. While the literature on the effects of prolonged sitting on acute cerebrovascular functioning are nascent, a large body of evidence exists for the association between physical activity and improved cerebral perfusion and cognitive function [45]. It is important to explore this literature in order to better understand the effects on prolonged sitting. This evidence will be demonstrated in the next section.

Consideration 2: Effects of Acute Bouts of Physical Activity on Cognitive Function

There is been a strong association between improved cognitive function and acute bouts of physical activity shown in literature [46,47]. Low to moderate intensity physical activity has been shown to increase cognitive function and cerebral BF [21,48–50]. An aspect of cognitive

function, executive function, is an umbrella term for the neurologically-based skills involving processes that all have to do with managing oneself and one's resources in order to achieve a goal [51]. The prefrontal cortex region in the brain correlates to executive functioning, as shown by perfusion changes via MRI scanning during a test of executive function [52].

Although this has yet to be fully explored, breaking up prolonged sitting may lead to cognitive improvements due to improved cerebral blood flow. This is speculated due to the following evidence. The effects of breaking up sitting with standing and/or walking on postprandial hyperglycemia have been observed [32,53–55]. Bailey et al. found that interrupting prolonged sitting with brief light-intensity walking, but not standing, has a beneficial effect on postprandial response [53]. Benatti et al. found breaking up sitting with standing across a 9-hour period acutely reduced the postprandial glycemic response [36].

Collectively, in light of the evidence discussed above, it can be speculated that reduced cerebral blood flow and impaired glucose metabolism may be associated with acute cognitive dysfunction. However, it is important to remember that humans are typically engaged in many behavioral choices while sitting. There may be a concomitant effect of behavioral choices on the effects of prolonged sitting. The impact of acute behavioral choices (i.e. dietary intake, such as consumption of high GI meals) within each lifestyle paradigm could augment or attenuate the previously observed outcomes of sedentary behavior. These effects will be explored in the following section.

Consideration 3: Interactions between Acute Sedentary Behavior and Lifestyle Choices

There may be interactions between acute sedentary behavior and other lifestyle choices. These lifestyle choices include, but are not limited to, mental stress, alcohol use, and dietary intake. In this review of literature, dietary intake will be of primary focus, specifically a high glycemic index meal. Humans consume a variety of meals each day, consisting of varying macronutrient content. However, it is important to consider how these nutrients, in particular

simple carbohydrates, are affecting our bodies, specifically cardiovascular and cerebrovascular outcomes.

Glycemic index (GI) and glycemic response

It is not uncommon to consume a meal high in refined sugar, or high glycemic index, while sitting at a desk. The health effects of dietary carbohydrate are of great interest to health professionals, the general public, and policy makers [56]. Carbohydrate rich foods differ in their effect of increasing blood glucose. This property of carbohydrate-rich foods is called “glycemic index” (GI), a relative ranking of carbohydrate in foods according to how they affect blood glucose levels [57]. The postprandial period is the time after consumption of a meal. Postprandial hyperglycemia refers to an exaggerated elevation in blood glucose following consumption of a meal [32]. However, the response differs between a low GI meal and a high GI meal. Consumption of a low GI meal leads to a more constant postprandial blood glucose concentration, while a high GI meal leads to a rapid increase in plasma glucose concentration followed by a concomitant high insulin secretion by the pancreas [6]. This high insulin response results in a rapid blood glucose disposal, which may cause the blood glucose concentration to decrease below the fasting concentration in the later postprandial period [58]. This unstable blood glucose profile is associated with CVD, T2DM, and impaired cognitive functioning. These associations will be discussed in the following sections.

Glycemic Index and Vascular Function

As mentioned previously, a high GI meal contributes to an unstable postprandial, or after meal, blood glucose concentration. Specifically, a high GI meal results in postprandial hyperglycemia. A systematic review by Loader et al. indicates that whole endothelial function is impaired by acute induction of hyperglycemia [59]. This vascular effect has been shown to predict future CVD mortality in both diabetic and normoglycemic individuals [60]. Although the mechanisms by which postprandial hyperglycemia induces vascular dysfunction are not fully

understood, the key event associated with this dysfunction is oxidative stress-mediated disruptions in nitric oxide homeostasis (44). In addition to CVD, dietary GI alters risk for T2DM [6]. This risk and the mechanisms leading to it will be discussed in the next section.

Glycemic Index and Metabolic Function

As outlined in section 2.2, the literature supports strong and consistent evidence linking prolonged sitting to hyperglycemia (17). Insulin resistance and impaired beta cell function may occur with a high GI meal through the direct effects of hyperglycemia (45). Hyperglycemia is known to cause beta cell dysfunction, or glucotoxicity [61]. GI has been demonstrated to lead to chronic events such as CVD and T2DM and acute effects such as oxidative stress, insulin resistance, and impaired beta cell function. Next, the effect of GI on cognitive function will be discussed.

Glycemic Index and Cognitive Function

The brain is entirely dependent on glucose as its main source of energy [58]. It is undoubtedly true that a failure of blood glucose supply to the brain produces a significant loss of brain function [62]. A high GI meal would result is an acute rise in blood glucose levels, followed by a decrease in blood glucose levels following the insulin response. This unstable blood glucose profile is associated with decreased cognitive functioning [8]. In healthy participants, ingestion of glucose has been shown to reduce regional cerebral blood flow (CBF) [63]. This may indicate that CBF is acutely sensitive to glucose levels. In fact, the changes in blood glucose concentration rather than the absolute concentration are critical for modulation of cognitive function [10]. Both prolonged exposure to hyperglycemia and repeated exposure to hypoglycemia can induce microvascular damage and impair endothelial function leading to cerebral hypoperfusion [42–44]. Therefore, cerebral blood flow decreases during acute hyperglycemia [64,65]. In conclusion, the brain may lack both cerebral blood flow and blood glucose following a high GI meal.

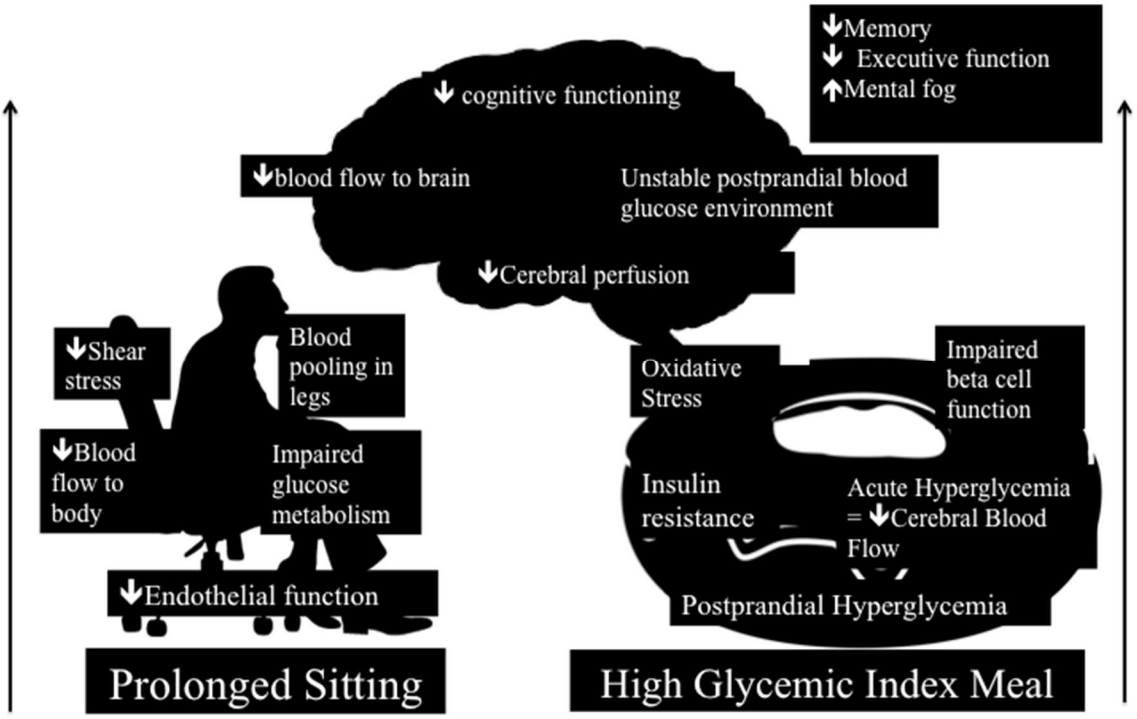
Additionally, as mentioned in the previous section, insulin resistance may occur with consumption of a high GI meal. Insulin resistance is a state of decreased responsiveness of target tissues to normal circulating levels of insulin [66]. The brain contains insulin receptors with important roles in cognitive function that are affected by insulin resistance [66]. In fact, insulin resistance is closely related to occurrence and development of cognitive dysfunction [67]. Additionally, impaired insulin signaling is relevant to the pathophysiologic mechanisms of cognitive impairment (61).

Through GI' effect on both blood glucose levels and insulin sensitivity and blood flow, it is suggested a high GI may have acute effects on cognition. However, a question that remains is what may be the combined effect of prolonged sitting and a high GI meal on cerebrovascular and cognitive function. The next section will outline what may occur, and the potential mechanisms related to the outcome.

Consideration 4: Acute Effects of Prolonged Sitting and a High Glycemic Index Meal

Both prolonged sitting and a high GI meal have been independently associated with negative effects on cognitive functioning. To our knowledge, no study has looked at the combined effect of prolonged sitting and a high GI on acute effects of cognitive functioning. **Figure 1** represents the potential or a combined effect of the two stimuli.

Figure 1. Acute Effect of Prolonged Sitting and a High Glycemic Index Meal on Cognitive Function



The figure represents the effects from both stimuli, and the combined effect in the brain. We know that prolonged sitting is associated with impaired vasculature. More specifically, the mechanism behind this impaired vascular function is decreased endothelial function. This decrease in endothelial function is explained by decreased shear stress. A lack of muscle pump activity leads to blood pooling in the legs and impaired glucose metabolism. This in turn decreases blood flow to the rest of the body. On the other side, a high GI meal is predicted to exacerbate these mechanisms. The postprandial hyperglycemia associated with a high GI meal will have detrimental effects on cerebral blood flow. Additionally, a high GI meal has been linked to oxidative stress, insulin resistance, and impaired beta cell function. Together, these combined mechanisms may overall decrease blood flow to the brain and cause an unstable postprandial blood glucose environment. Both of these mechanisms are linked to deleterious effects on cognitive functioning, and more specifically executive function.

Implications/Why is this study needed?

Very few studies exist exploring the effects of acute periods of prolonged sitting on cerebrovascular function, yet, chronic sedentary behavior has been associated with long term cerebrovascular impairments [42]. Several experimental studies have demonstrated that prolonged sitting results in unfavorable vascular effects such as increases in diastolic blood pressure [68,69], impairments in vascular function [24], and impairments in endothelial function [22]. It is not currently known if these detrimental vascular effects of prolonged sitting also occur in the cerebrovasculature. Related to this, the brain relies on blood flow to deliver oxygen and glucose, so decreases in total brain blood flow could affect it's functioning. Thus, it is imperative to explore the effects of prolonged sitting on total brain blood flow. Additionally, it is important to consider how lifestyle behaviors, in this case – consumption of a high GI meal, may affect total brain blood flow.

Methodological Considerations

In order to test these combined stimuli on cerebrovascular and cognitive functioning, several methodological considerations were examined. Two major considerations were external and internal validity. External validity represents the generalizability of the study, and appropriate subjects and study design will be discussed considering this. Internal validity refers to whether the effects observed in the study were due to manipulation of the independent variable, and instrumentation will be discussed considering this. These considerations, among others, will be outlined in the following sections.

Study Design Considerations

The appropriate choice in study design is essential for the successful execution of research. There are many designs to choose from, and each design has its own strengths and weaknesses, and the need to understand these limitations is necessary to arrive at correct study conclusions [70]. First, we considered an observational study. Observational studies,

which include cohort studies and case-control study designs, have several advantages such as an ability to look at multiple risk factors and multiple outcomes, greater timeliness, and a broader range of patients, however, we would not be able to manipulate anything [71]. Next study design considered was a randomized controlled trial, the gold standard for clinical research. However, this would require participants to be divided into two groups and may require more participants, and thus would not be feasible for a Master's Thesis project. The study design selected was a double-blind randomized cross over design with two experimental group conditions (low glycemic index [LGI] and high glycemic index meal [HGI]) preceded by a familiarization session. The study design was double blinded since the subjects did not know which condition they were receiving and the researchers were blinded as an undergraduate lab assistant randomly assigned the conditions.

Following selection of the study design, it was important to define the bout of prolonged sitting. Three hours of prolonged sitting has been shown to cause a reduction in shear rate, popliteal FMD, reduced hyperemic blood flow, and increased calf circumference [25]. A three-hour bout of sitting was also previously used in unpublished studies from our laboratory with designs similar to our protocol.

Measurement Considerations

Blood Flow to the Brain

There are a number of imagining techniques to examine blood flow to the brain. First, magnetic resonance imaging (MRI) can be used to noninvasively image cerebral blood flow, but this is an extremely expensive option. Transcranial Doppler (TCD) ultrasound is another option providing fast, noninvasive, real-time measures of blood velocity. Ideally, either of these options would have been used, but due to equipment availability, Doppler Ultrasound was chosen for this study. Blood flow was measured in three cerebral arteries: common carotid artery (CCA), internal carotid artery (ICA), and vertebral artery (VA). Global CBF or blood flow to the brain was

calculated as the sum of the blood flow in the ICA and VA [(ICA blood flow + VA blood flow) × 2 (ml min^{-1})] to be able to compare to previous literature [(Credeur – unpublished data), [72]]. Additionally, the use of ICA+VA*2 accounted for blood flow solely the brain, and not scalp and face. The ICA and VA both directly perfuse the Circle of Willis.

Cognitive Tests

In determining the best cognitive test to utilize, it is important to examine past literature and the measures of cognitive function used. In a systematic review by Falck et al [73], thirteen different measures of cognitive function were used to assess the association between sedentary behavior and cognitive function. The following domains were examined: memory, executive function, processing speed, and perceptual organizing and planning. This thesis will focus on executive function, since the NIRS probe will be placed over the prefrontal cortex. Previous unpublished research in our lab used the Stroop Test to assess executive function. However, Falck et al. [74] recommended use of the Trail Making Test - Part B for executive function to allow for comparisons across future studies. Additionally, this thesis aimed to use quick tests to avoid interference with vascular measurements (i.e. avoid increasing blood pressure). An additional consideration is the timing of the administration of cognitive tests. In order to not affect vascular measurements throughout the protocol, tests were given pre and post the 3-hour sitting protocol. The Stroop Test and Trail Making Test, Part B, are both simply and quick to administer, thus, this study used both the Stroop Test and the Trail Making Test, Part B, to assess executive function.

In addition to assessing executive function, we were also interested in memory, as many subjectively experience decreased memory and increased brain fog with sitting. As we already administered two tests, we wanted the memory test to be quick and simple. The Hopkins Verbal Learning Task (HVLT) is widely recognized as a brief test of memory, with an administration

time of 10 minutes [75]. Therefore, we created a modified version - incorporated word lists from the HVLT and administered a 12 word recall pre- and post- test.

Brain Fog

We were interested in measuring brain fog as subjective feelings of brain fog have been experienced with sitting. There is no known reliable and valid objective measure of brain fog, so we subjectively measured brain fog using a modified Borg Rate of Perceived Exertion (RPE) scale.

Cerebral Perfusion

Cerebral perfusion could have been measured using MRI but this would have been difficult and costly. Therefore, cerebral perfusion was measured using a continuous-wave near-infrared spectroscopy (NIRS) device (Portalite, Artinis, Netherlands). The NIRS probe measured perfusion and relative changes in total hemoglobin [76]. To do this, the probe emits an infrared light, which passes through skin, adipose tissue, muscle, and bone. A receiver registers the absorbance of light waves passed through the adjacent tissues continuously in real-time. The probe is programmed to determine the light wavelength absorbance spectra for oxygenated hemoglobin (Hb), deoxygenated hemoglobin (deoxyHb), and total hemoglobin (tHb). For the experimental protocol, the NIRS probe was positioned on the forehead, approximately 3 cm to the right of center, directly over the eyebrow. The rationale for this placement was based off the international Electroencephalography 10-20 system for brain mapping and placement of electrodes for measuring the pre-frontal cortex [76]. Importantly, perfusion changes within this region have been shown to correlate to executive functioning, as denoted by perfusion changes via MRI scanning during tests of executive function [52].

Glucose Monitoring

There are several different ways to measure blood glucose. These include glucometers, whole blood, plasma, or serum blood tests, and continuous glucose monitors. Continuous

glucose monitors were used since they are reasonably unobtrusive, small, comfortable, and provide information unattainable by intermittent capillary blood glucose including “24/7” coverage and the ability to characterize glycemic variability [77].

Meal Intervention

In order to induce the greatest response, we selected a solution-based meal. We provided a glucose solution for the HGI meal and a fructose solution for the LGI meal. The literature was searched extensively to determine the most appropriate high and low meal choices. Most studies matched for macronutrient content and the average low GI used was 36, and the average high GI used was 80. With use of a meal solution, the experiment was double-blinded as both solutions looked the same. The meals were matched g/kg of the participant’s weight. A standardized dinner was given the night before testing.

Population/Sampling

Young healthy adults (18-35 years) were recruited for the study to ensure complete brain development and avoid the potential for age-related cognitive decline. Using young subjects avoided potential confounding variables, since younger people are typically healthier. Then, it was easier to draw direct conclusions on the effects of prolonged sitting. Additionally, the subjects were active (>90 minutes a week of moderate exercise), to induce a greater effect from prolonged sitting since the subjects were adapted to physical activity. This was considered due to a previous study from our laboratory (unpublished), and lack of major findings from sedentary subjects. Although a more generalizable sample was preferred, we recruited a homogenous group at this early stage to reduce confounding by participant characteristics.

Statistical Considerations

For this study, we were interested in repeated measures analysis. First, we considered a multivariate test, but with many time points for assessing brain blood flow, it was risky to run a multivariate approach since it requires a complete case analysis. For example, if a subject is

missing one time point, they would be dropped from the entire analysis through listwise deletion. Therefore, we used 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 by doing a mixed linear model we are allowing subjects to vary depending on the baselines of the outcomes of interest. The independent variables for the study are time (10, 90, 170) and condition (HGI, LGI).

Other Considerations

Sex as a Biological Factor

We cannot rule out that sex differences exist in cerebrovascular function. However, this study is not adequately powered to determine whether there are sex differences in cerebrovascular impairment and risk interactions. However, it is known that men and women have different trajectories for acquisition of CVD risk factors over the course of a lifetime.

Ethnicity/Race

While this study attempted to recruit a racially diverse sample, this study was not adequately powered to determine whether there are race/ethnic differences in cerebrovascular impairment and risk interactions.

Generalizability

Our results may only be generalizable to young, healthy, and active individuals. Therefore, the generalizability of our results is low due to our selection of a homogenous study population in order to reduce the potential for confounding.

Pre-assessment control

Our pre-assessment controls included refraining from moderate to vigorous physical activity and alcohol 24 hours prior to the experimental days. Accelerometers were used to confirm participants abstained from moderate-to-vigorous physical activity (MVPA) the day before. We provided a standardized dinner which was repeated the night before each testing

day, at least 12 hours before the start of testing, and texts were sent to ensure participants ate the meal at the same time before each experimental day. Finally, we required an overnight fast. Continuous glucose monitors were inserted at least 12 hours prior to the experimental day to ensure stable glucose levels in our participants. These pre-assessment controls helped to minimize confounding variables. Additionally, we reported on pre-assessment physical activity levels, sleep, glucose levels, and mode of transport to ensure a relatively homogenous sample. In our results, if we were to experience any outliers, we would be able to see if any of these variables may have affected the participant's results.

Summary

The primary study question was to determine whether prolonged sitting decreases total brain BF and the role of an HGI meal on this association. We hypothesized that relative to the LGI meal, an HGI meal would increase the effects of prolonged sitting on reduced BF to the brain, decreased cerebral perfusion, and decreased cognitive function. This research is highly relevant to society as the documented acute effects of prolonged sitting on cognitive function are scarce, and the effect of a diet on these associations has yet to be observed. The outcomes of this study may make a public health statement and encourage people to sit down less and eat differently in their workplace or school setting.

CHAPTER 3: METHODOLOGY

This study is reported in accordance with CONSORT (Consolidated Standards of Reporting Trials) guidelines[78]. Ethical approval was obtained from the University of North Carolina at Chapel Hill institutional review board, and all participants were provided written informed consent prior to participating in the study.

Participants

Twenty males and females, ages 18-35 were recruited for the study from the University of North Carolina Chapel Hill campus and surrounded Chapel Hill area. Participants were recruited between November 2018 to February 2019, and all experimental visits occurred between November 2018 and March 2019. All participants were block randomly assigned to condition A or B (HGI or LGI) using a random number generator (randomizer.org).

Exclusion criteria included: less than 90 minutes of self-reported structured exercise per week, any known cardio-metabolic disorders, pregnant women, smokers, and currently taking any medication known to affect cardiovascular function. Because this is the first study of its kind and because elderly and diseased populations have different vascular sensitivity, a young, healthy, homogenous population was recruited to reduce the potential for confounding variables. In women, because fluctuations in estrogen can affect cardiovascular measures, the first testing session was performed within the first 1-7 d of the onset of their menstrual cycle, and the second condition was performed within 7 d of the previous visit. Women reporting contraceptive use were tested during the placebo week.

Experimental Design

A double-blind randomized crossover design with two experimental conditions (consumption of low glycemic index meal [LGI] and consumption of high glycemic index meal [HGI]), preceded

by a familiarization session. Both conditions were matched with a three hour sitting period. Three hours was chosen based on prior studies [26,32,79]. Additionally, the peak postprandial glucose concentration time is estimated to be two hours post-meal consumption [80]. Testing began between 6:00-10:00AM in the Cardiometabolic Lab in the Applied Physiology Laboratory within Fetzer Hall at the University of North Carolina – Chapel Hill (APL).

Prior to participation, subjects took part in an overnight fast while abstaining from alcohol and exercise 24 hours prior to experiment. Accelerometers were used to confirm participants abstained from moderate-to-vigorous physical activity (MVPA) the day before. Additionally, the night before, a standardized dinner was given based on the subjects' individual food preferences and food allergies. This standardized dinner was repeated the night before each testing day, at least 12 hours before the start of testing, and texts were sent to ensure participants ate the meal at the same time before each experimental day.

Pre-assessment

Prior to subject participation, ethical approval was obtained through the IRB and the Office of Human Research Ethics at the University of North Carolina – Chapel Hill (#17-2532). All subjects filled out a medical history questionnaire (Appendix A) and informed consent (Appendix B). If female, a pregnancy test was provided as well as confirmation subject is in day 1-7 of cycle for experimental visits. Then, using online randomization software (www.randomizer.org) subjects were randomized into either LGI or HGI intervention for first visit.

Familiarization

Subjects reported to the APL to review documentation and informed consent. At this time, females were given a urine pregnancy test. After completing the medical health history and consent forms, subjects height was taken using a stadiometer (Perspective Enterprises, Portage, Michigan), and weight was taken via calibrated scale (Health o meter)(weighed (in kg)

and height was taken (in cm)) .and then positioned on a on a three-section table (Armedica AM353 Hi-Lo, Tiger Medical, Irvington, NJ). In the supine position, the continuous glucose monitor (CGM)(iPro2, Medtronic, Northridge, CA) was inserted into the subject's abdomen, approximately 5 cm lateral to the umbilicus. The CGM was inserted at least 12 hours before the start of the experimental testing visit for each visit. Following insertion of the CGM, subjects were instructed how to use a glucometer (Contour Next One). A finger prick was needed at least 1 hour after insertion of the CGM (per Medtronic instructions) and before bed. If a subject wore the CGM for multiple days, they were instructed (and reminded through text message) to prick every 12 hours. The glucose levels measured through the glucometer was recorded with the time of prick. An accelerometer (ActiSleep +; ActiGraph LLC, Fort Walton Beach, FL) was also placed on the ankle to covary for spontaneous movement as well as to ensure subjects refrained from exercise 24 hours prior to the experimental day. A 24-hour food log was given to each subject to record their foods the day before each experimental day. The day before experimental day 2, subjects were asked to eat similar meals as the day before experimental day 1. Finally, subjects were familiarized with the cognitive assessments using the standardized directions (Appendix C). Upon leaving the lab, subjects were given a reminders sheet (Appendix D) and their standardized dinner.

Experimental Visits

Subjects arrived to the APL (22.36 ±2°C, 26.09 ± 8.94% humidity) between 6:00 and 10:00 AM fasted (for Visit 2: 2-8 days following the first experimental visit). Subjects were fasted and refrained from caffeine intake for at least 12 hours, and alcohol and MVPA for at least 24 hours prior to arrival. Upon arrival, fasting blood glucose levels were measured using a glucometer and participants filled out a sleep diary (Appendix E). Following 20 minutes of quiet rest, the subjects were read the standardized cognitive assessment instructions (Appendix C) and asked to assess their brain fog, then performed three 20 second Stroop test measurements, followed by three trials of the Trail Making B test. After this, subjects were read

aloud memory words from the Hopkins Verbal Learning Task (at the rate of 1 word every 2 seconds). Following the cognitive assessments, three 10 second ultrasound measurements at the Common Carotid artery and a video was recorded. Subjects were asked to hold their breath for each 10 second measurements, and 1 minute was given between each 10 second measurement. Then, a 30 second video was taken at both the Internal Carotid and Vertebral arteries. After the ultrasound measurements, pulse wave velocity and pulse wave analysis measurements were taken.

Subjects were then brought to an upright, seated position using an Armedica AM353 Hi-lo Treatment Table (Tiger Medical, TIGER#TM83695) with their feet flat on the ground with approximately 90 degrees of knee flexion. If their feet could not touch the ground, a platform was used. Angle of chair recline was measured, as well as the distance from the bottom of the chair to the ground to ensure equivalent chair placement for the second visit. The HGI (glucose; GI: 100) or low GI (fructose; GI: 19) beverage (solution in g/kg of subject weight mixed with 300 mL of water, lemon juice given as option to add to make solution palatable) was then administered and subjects were given 10 minutes to drink it. The 3 hour sitting protocol began following consumption of the beverage. During the 3 hours, subjects were instructed to not fidget, and subjects watched their selected low-stimulatory documentary/show. The purpose of the subjects watching these shows was to prevent large fluctuations in cognitive stimulation that homework, cell-phones, or games may have caused. This option provided a minimal stimulus to maintain wakefulness and prevent subjects from falling asleep. While sitting, ultrasound videos, cardiovascular measurements, and Brain Fog were collected at 10, 90, and 170 minutes. NIRS and continuous glucose monitoring (CGM) were collected continuously. Immediately prior to and following the sitting protocol, NIRS signals were marked to acquire pre-to-post change scores. After 180 minutes, subjects were transferred back to the supine position to conclude with the cognitive assessments, ultrasound measurements, and cardiovascular measurements.

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 can 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, subjects 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 subjects getting up to use the restroom at any point during the study.

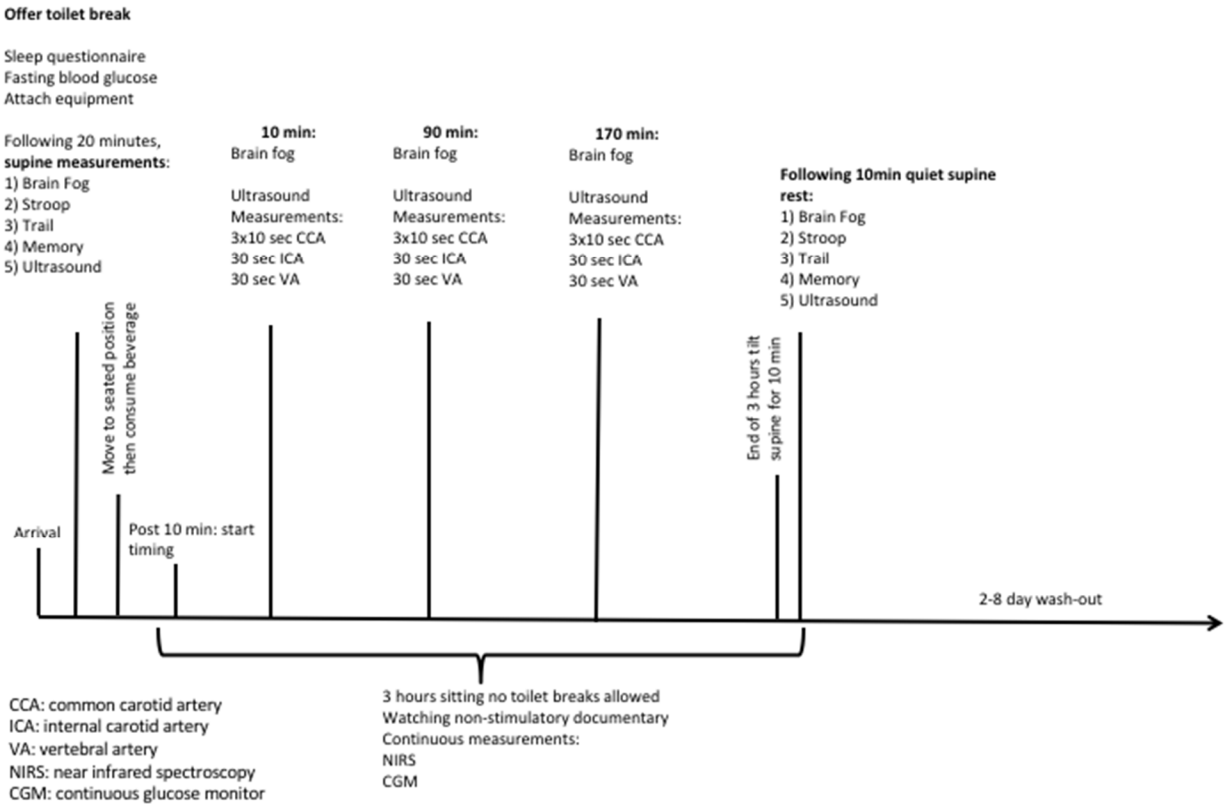


Figure 2. Experimental Day Timeline for Cerebrovascular/Cognitive Measurements

Experimental Measures

Primary Outcome: Total Brain Blood Flow

Total brain blood flow was assessed using Doppler Ultrasound at three cerebral arteries: common carotid (CCA), internal carotid (ICA), and vertebral (VA) during time points 0, 10, 90,

170, and 180 to explore the effects of sitting and glycemic index on cerebrovascular function. At time points 0 and 180, the subject was in a supine position, and while at time points 10, 90, and 170, the subject was seated. At the common carotid, three 10 second videos were taken to determine cerebrovascular hemodynamics. During these 10 seconds, subjects were asked to hold their breath to avoid noise from respiration on the pulsed wave mode. At the internal and vertebral arteries, 30 second videos were recorded and further broken into 10 second videos during analysis. Total blood flow to the brain was calculated as the sum of the blood flow in the ICA and VA [(ICA blood flow + VA blood flow) x 2 (ml min^{-1})]. This equation is used to account for blood flow sorely to the brain, not face and scalp. The ICA and VA both directly perfuse the Circle of Willis.

Secondary Outcome: Cognition

Stroop

To assess if executive function was affected by prolonged sitting and glycemic index and perhaps declines in cerebral perfusion, the Stroop Word-Color Task (Stroop) was administered on an iPad [81]. The Stroop interference test involves 4 words (blue, yellow, green, and red) being randomly presented, consecutively. The color that each word is presented in is either congruent (Word) or incongruent (Color) with the relevant semantic information. For this task, 20 seconds was allotted for each trial, and subjects needed to complete as many iterations as quickly and correctly as possible. Response time, total number of iterations, and percentage correct were recorded. Stroop has been done previously within our lab group [Stoner – unpublished, Fryer – unpublished] which allows for comparison of results. Acceptable reliability has been reported for both Word (ICC: 0.71) and Color (IC: 0.79) Tests in young adults [82].

Trail-Making Test – Part B

The Trail-Making Test – Part B was also used to assess executive function. This test was recommended by prior studies to use as an assessment of executive function in young,

healthy populations [Stoner – unpublished, [73]], thus may be more appropriate than the Stroop Test. The Trail-Making Test – Part B presents numbers and letters placed in a semi-random fixed order, in such a manner to avoid overlapping lines being drawn. The subject connected 25 encircled numbers and letters in numerical and alphabetical order, alternating between the numbers and letters. For example, the first number ‘‘1’’ is followed by the first letter ‘‘A,’’ followed by the second number ‘‘2’’ then second letter ‘‘B’’ and so on [83]. Time to completion was recorded, and the test was repeated 3 times in a row.

Memory

To explore the effects of prolonged sitting and glycemic index on memory, a simple memory test was given. We created our own memory test to fit our time needs. Participants were read word lists from the Hopkins Verbal Learning Test at the rate of 1 word every 2 seconds during the pre-test, supine position cognitive testing period. The Hopkins Verbal Learning test word lists contain 12 words, 4 words from 3 semantic categories. After 3 hours of sitting, subjects were asked if they can recall any of the words during the post-test. It is important to clarify we did not use the Hopkins Verbal Learning Test, we only used word lists from it. While this is not a validated and reliable measurement, it was a simple and quick way to explore the effects of sitting and glycemic index on memory.

Secondary Outcome: Brain Fog

As an exploratory measurement to assess brain fog, a rate of perceived exertion scale was used and adapted to fit brain fog. This measurement was used in unpublished studies (Piepmeier). This is not a standard assessment, nor is it reliable or valid, but its purpose was exploratory. This scale can be found in Appendix F.

Mechanistic Outcome: Cerebral Perfusion

Cerebral perfusion was measured using a continuous-wave near infrared spectroscopy (NIRS) device (Portalite, Artinis, Netherlands) throughout the testing session. The NIRS probe

measures perfusion and relative changes in total hemoglobin [84]. For the experimental protocol, the NIRS probe was positioned on the forehead, approximately 3 cm to the right of center, directly over the eyebrow. The rationale for this placement was based off the international Electroencephalography 10-20 system for brain mapping and placement of electrodes for measuring the pre-frontal cortex [76]. Most importantly, perfusion within the prefrontal cortex has been shown to correlate to executive functioning [52]. The NIRS device was fixed to the skin with bio adhesive tape and the location was marked (and a picture was taken) to ensure identical placement on the second day. The optode was covered with a dark opaque cloth to prevent signal contamination by ambient light, as per the manufacturer recommendations. For each time point, data were averaged over 30 seconds.

Continuous Blood Glucose Monitoring

A continuous glucose monitor (iPro2, Medtronic, Northridge, CA) was used throughout the experimental procedure. It was placed on the participant 24 hours prior to Day 1 and taken out upon completion of Day 1 or Day 2, depending on the number of days between each testing session. Continuous blood glucose monitors give “24/7” coverage and the ability to characterize glycemic variability [77].

Randomization

The randomization was performed by chance, where 2 sets of 10 unique numbers were generated from a number range of 1-20 (www.randomizer.org). The randomization procedure was performed by a research assistant.

This study was a double-blinded study. Solutions were assigned as “A” and “B”. An undergraduate research assistant who was not assessing outcomes covered each solution with construction paper and black tape. Both participants and those assessing outcomes were blinded to interventions. Solutions were unmasked following statistical analyses.

Sample Size

Calculations to determine minimum sample size was based on Elizabeth Kelsch's 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 [85], 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, which is approximately 17 participants. 20 participants were recruited to account for drop-outs.

Quality Control

For brain blood flow, all ultrasound measurements were conducted by EK and all Cardiovascular Suite analyses (a relatively subjective analysis) were conducted by KB. Brain blood flow analysis took at least 10 hours per subject, so KB and two research assistants worked together on analysis in Microsoft Excel. At the conclusion of the study, a random selection of 10% of the data sets (e.g. from 3 participants) were re-scored by an independent observer.

Data Management and Statistical Analysis

All statistical analyses were performed using jamovi (2018, Version 0.9). The α level was set *a priori* for all statistical procedures at α=0.05. Supine (Pre vs. Post) and sitting (min 10, 70 and 170) date were analyzed separately. For the sitting data, 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 subject. The NIRS data was relative to baseline, and therefore the baseline was 0, and the intercepts were fixed. 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. Raw data are

presented as mean [standard deviation] and mixed model data are presented as mean [95% confidence interval].

CHAPTER 4: RESULTS

Participants

Twenty participants (22.6 [3.1] y, 33% F, 24.3 [3.7] kg/m^2) were recruited. Participants self-identified as non-Hispanic White (n=14), African American (n=2), and Middle-eastern (n=4). Subject demographics are shown in Table 1. All female participants were on some type of birth control method.

Age	X	22.6
	SD	3.10
BMI	X	24.3
	SD	3.70
Height (cm)	X	172.6
	SD	8.70
Weight (kg)	X	72.8
	SD	15.0
HR (bpm)	X	58.5
	SD	11.1
SBP (mmHg)	X	121.9
	SD	11.1
DBP (mmHg)	X	58.2
	SD	5.50
Sex (% Female)		33%
Total PA (min/week)	X	280
	SD	175

Table 1. Participant characteristics.

18 participants successfully completed both experimental trials (Figure 1). 2 participants dropped out due to illness (n=1) and time (n=1). Total brain blood flow could not be calculated for 3 participants for the HGI condition and 7 participants for the LGI condition, due to poor quality videos of the ICA and VA (Figure 2).

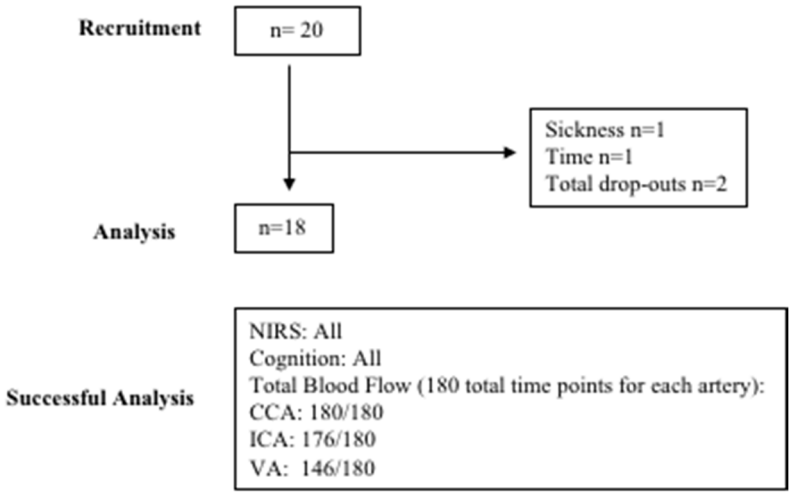


Figure 3. CONSORT diagram. *CCA Common Carotid Artery, ICA Internal Carotid Artery, VA Vertebral Artery.*

Control Data

For the night before data collection, the total minutes in bed averaged 304.7 ± 25.1 minutes for the HGI condition and 433.3 ± 53.5 for the LGI condition. There was no significant difference between total MVPA (moderate-vigorous physical activity) during the 12-24 hours prior to experimental visits (HGI: 42.2 ± 33.5, LGI: 52.4 ± 40.8 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, $p<0.05$).

Baseline data

Baseline data is reported in Tables 2, 3, and 4. No significant differences were observed between conditions for CCA Bfmean (P=0.510), ICA Bfmean (P=0.260), VA Bfmean (P=0.434), or for any of the NIRS or cognitive variables.

			CCA Bfmean *m/s*	ICA Bfmean *m/s*	VA Bfmean *m/s*
Baseline	HGI	X	4527	3603	1636
		SD	1157	1510	1162
Baseline	LGI	X	4801	2968	1545
		SD	1367	1682	1074
		P	0.510	0.260	0.434
		ES	0.22	-0.40	-0.08

Table 2. Total brain blood flow baseline data. *HGI High glycemic index, LGI Low glycemic index, Bfmean Blood Flow Mean, CCA Common Carotid Artery, ICA Internal Carotid Artery, VA Vertebral Artery.*

			NIRS			
			SRS HbO2	SRS HHb	SRS tHb	TSI *%*
Baseline	HGI	X	37.454	27.949	65.403	57.179
		SD	12.162	8.942	17.955	7.568
Baseline	LGI	X	42.777	26.701	69.478	60.937
		SD	14.356	7.283	19.690	7.262
		P	0.211	0.610	0.474	0.117
		ES	0.40	-0.15	0.22	0.51

Table 3. Prefrontal cortex cerebral perfusion baseline data. *HGI High glycemic index, LGI Low glycemic index, SRS HbO2 Oxy hemoglobin, SRS HHb Deoxy hemoglobin, SRS tHb Total hemoglobin, TSI Total Saturation Index.*

			Stroop			Trail	Fog
			RT *ms*	Correct #	Accuracy *%*	Time *ms*	Score *%*
Baseline	HGI	X	0.9	16.7	99.7	25.9	2.4
		SD	0.2	4.3	1.0	8.6	1.5
Baseline	LGI	X	0.9	18.2	99.7	23.4	2.2
		SD	0.0	3.7	1.1	7.7	1.2
		P	0.170	0.107	0.860	0.263	0.466
		ES	0.32	0.37	0.06	-0.30	-0.16

Table 4. Stroop, Trail, and Fog baseline data. *HGI High glycemic index, LGI Low glycemic index, RT Reaction Time.*

Continuous Blood Glucose Monitoring

Average glucose levels (mg/dL) across 180 minutes are reported in Figure 3. 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 significant, large effect (P=0.00, ES:-1.2). Additionally, for AUC, there was a significant large effect (P=0.00, ES:-1.15).

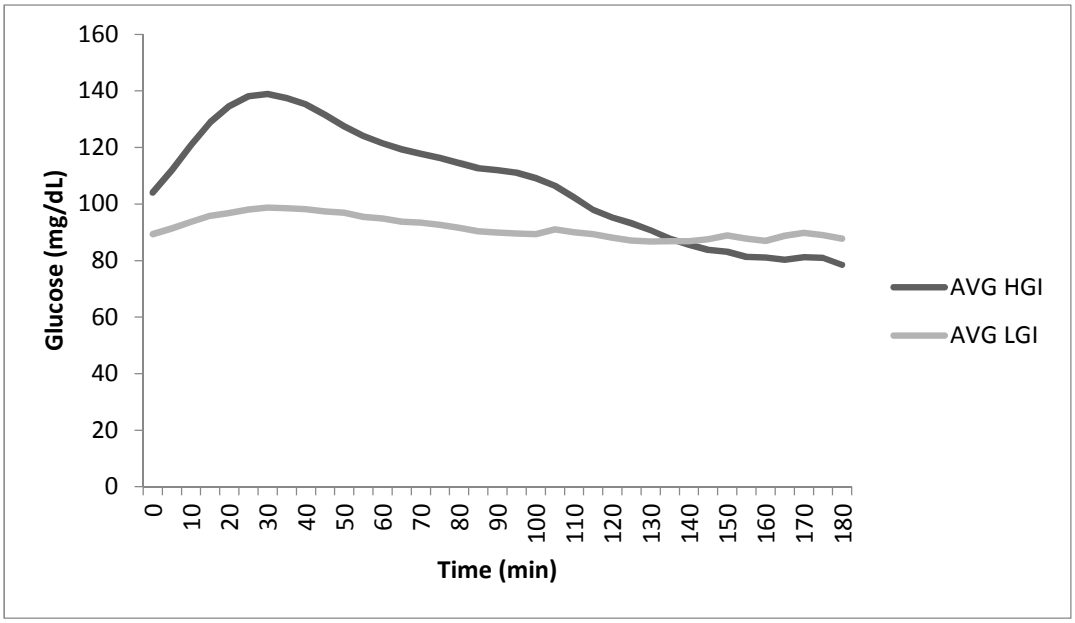


Figure 4. Average Glucose Across 3 hours of sitting. *HGI High glycemic index, LGI Low glycemic index.*

Primary Outcome: Total Brain Blood Flow

Our primary outcome was Q_{BF}. There was no interaction effect and no effect of time, however, there was a significant, small condition effect (P=0.04, ES: -0.06) (Table 5). For LGI, Q_{BF} decreased by 2203.2 ml/min (95% CI: -5136 to 2719), and for HGI, Q_{BF} increased by 73.8 ml/min (95% CI: -2719 to 73)(Figure 4).

Table 5 also reports BF values for each artery supplying the head. Q_{BF} is comprised of BF from VA_{BF} and ICA_{BF}. Changes were not significant for VA_{BF}, but for the ICA_{BF}, the changes mirror Q_{BF}, in that there was a significant, small condition effect (P=0.043, ES: -0.11). For CCA_{BF} there was a time effect across conditions (P=0.002, ES: -0.38). Across condition, BF decreased. Brain blood flow in the supine position is shown in Table 5, while blood flow to the brain data in the seated position is in Appendix G.

			CCA Bfmean *(ml/min)*	ICA Bfmean *(ml/min)*	VA Bfmean *(ml/min)*	Q(mean) Bfmean *(ml/min)*
Pre	HGI	X	4527	3603	1636	6889
		SD	1157	1510	1162	2906
	LGI	X	4801	2968	1545	6237
		SD	1367	1510	1162	2906
Post	HGI	X	3778	3110	1944	6949
		SD	975	2065	1972	5808
	LGI	X	4162	2259	1141	4490
		SD	1022	1178	786	2320
Interaction		P	0.798	0.709	0.165	0.189
Time		P	0.002	0.102	0.786	0.340
		ES	-0.38	-0.20	-0.04	-0.13
Condition		P	0.129	0.043	0.076	0.040
		ES	0.11	-0.11	-0.09	-0.06

Table 5. Supine: Brain Blood Flow. *HGI High glycemic index, LGI Low glycemic index, Bfmean Blood Flow Mean, CCA Common Carotid Artery, ICA Internal Carotid Artery, VA Vertebral Artery.*

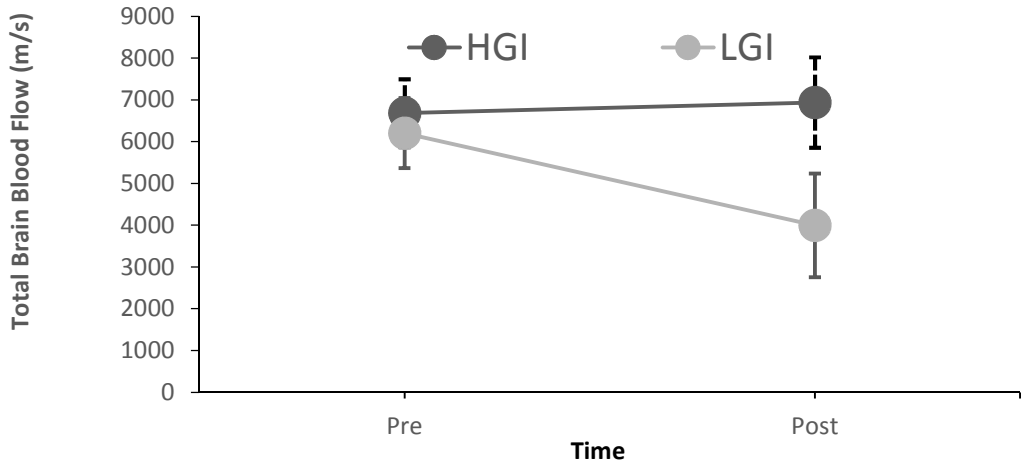


Figure 5. Total Brain Blood Flow; Pre to Post

Secondary Outcome: Cognition

Table 6 shows supine results from the Stroop Test, Trail Making Test, and Memory Test. There were no interaction effects for these assessments. There was a significant time effect

(P=0.023, ES: -0.39), as well as significant condition effect (P=0.046, ES: 0.046) for the Trail Making Test.

			Stroop			Trail	Memory
			RT ms	Correct #	Accuracy %	Time ms	Correct #
Pre	HGI	X	0.88	16.69	99.67	25.86	
		SD	0.20	4.30	1.00	8.62	
	LGI	X	0.90	18.20	99.70	23.41	
		SD	0.20	3.70	1.10	7.66	
Post	HGI	X	0.90	18.40	100.00	23.00	0.50
		SD	0.20	4.10	0.00	6.91	0.20
	LGI	X	1.00	18.80	99.80	21.00	0.50
		SD	0.20	3.20	1.00	5.57	0.18
Interaction		P	0.439	0.273	0.443	0.836	0.375
Time		P	0.125	0.032	0.401	0.023	
		ES	0.26	0.37	0.14	-0.39	
Condition		P	1.368	0.273	0.665	0.046	
		ES	0.28	0.40	-0.10	-0.48	

Table 6. Supine: Stroop, Trail, Memory.

Figure 5 shows seated results from the Brain Fog scale. There was no interaction effect and no significant differences between conditions HGI and LGI (P=0.612), but there was a significant, moderate (P=0.025, ES: 0.37) time effect, suggesting in both conditions subjects became foggier.

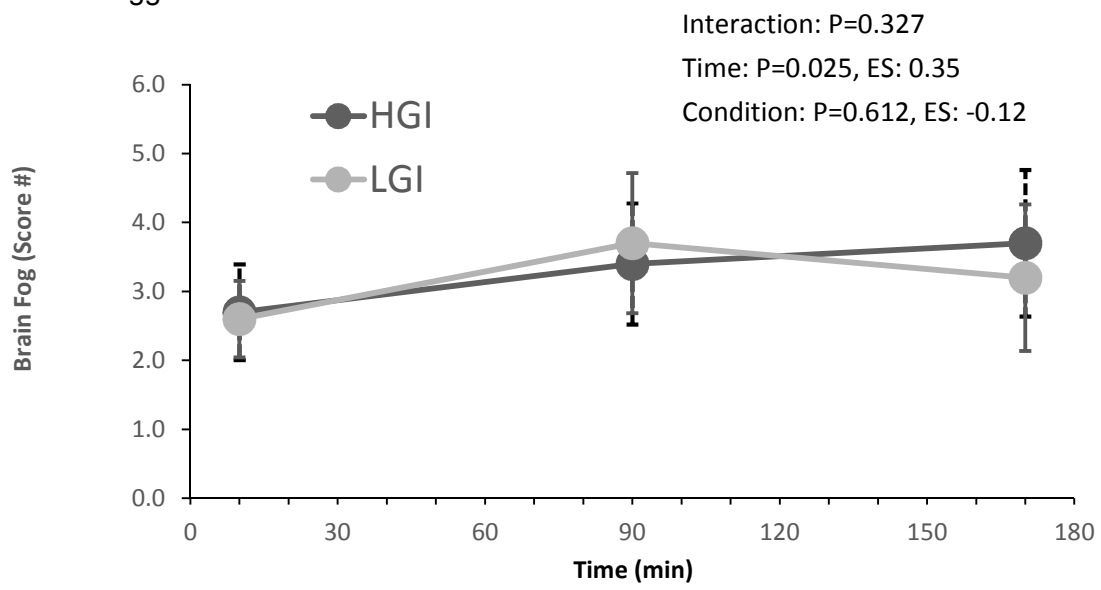


Figure 6. Brain Fog; Seated.

Mechanistic Outcomes: Oscillatory Blood Flow and Arterial Stiffness

Table 7 shows oscillatory blood flow and arterial stiffness in the supine position. There were no interaction effects and no significant differences between conditions HGI and LGI.

			CCA			ICA			VA		
			Stiff	PWV (β)	Osc.	Stiff	PWV (β)	Osc.	Stiff	PWV (β)	Osc.
			AU	*m/s*	*ratio*	*AU*	*m/s*	*ratio*	*AU*	*m/s*	*ratio*
Pre	HGI	X	9.93	5.22	0.53	12.00	5.64	1.77	12.79	6.25	3.85
		SD	2.90	0.91	1.01	4.11	1.01	3.57	11.01	1.66	4.92
	LGI	X	9.80	5.13	0.43	12.19	5.62	2.22	15.03	6.22	4.95
		SD	2.77	0.85	0.47	4.11	1.01	3.57	11.01	1.66	4.92
Post	HGI	X	9.25	4.97	1.16	12.55	5.76	3.13	16.53	6.52	8.28
		SD	3.28	0.86	1.91	5.58	1.44	4.24	13.91	2.23	18.09
	LGI	X	10.00	5.16	0.37	11.33	5.42	1.46	13.47	5.90	12.93
		SD	3.72	0.88	0.37	6.47	1.47	1.95	6.85	1.43	23.32
Interaction		P	0.359	0.197	0.143	0.493	0.510	0.244	0.255	0.478	0.844
Time		P	0.631	0.359	0.288	0.876	0.880	0.756	0.723	0.966	0.302
		ES	-0.06	-0.11	0.13	-0.02	-0.02	0.04	0.05	0.01	0.14
Condition		P	0.512	0.561	0.051	0.610	0.372	0.488	0.818	0.547	0.498
		ES	0.95	1.78	-3.91	-0.50	-1.84	-0.73	-0.14	-0.91	0.32

Table 7. Supine: Oscillatory Blood Flow and Arterial Stiffness. *HGI High glycemic index, LGI Low glycemic index, Bfmean Blood Flow Mean, CCA Common Carotid Artery, ICA Internal Carotid Artery, VA Vertebral Artery, Stiff Stiffness, PWV Pulse Wave Velocity, Osc. Oscillatory.*

Mechanistic Outcome: Cerebral Perfusion

Table 8 shows prefrontal cortex cerebral perfusion in the supine position. There were no interaction effects and no significant differences between conditions HGI and LGI.

			SRS HbO2	SRS HHb	SRS tHb	TSI(%)
Pre	HGI	X	37.45	27.95	65.40	57.18
		SD	12.16	8.94	17.96	7.57
	LGI	X	42.78	26.70	69.48	60.94
		SD	12.16	8.94	17.96	7.57
Post	HGI	X	41.11	27.62	68.73	59.74
		SD	15.24	10.58	24.74	5.88
	LGI	X	41.16	25.57	66.73	60.91
		SD	14.35	6.65	19.01	7.76
Interaction		P	0.209	0.738	0.302	0.272
Time		P	0.584	0.642	0.840	0.282
		ES	0.10	-0.08	0.04	0.19
Condition		P	0.324	0.226	0.854	0.064
		ES	0.25	-0.31	0.05	0.47

Table 8. Supine: Probe 1 - Prefrontal Cortex Cerebral Perfusion.

CHAPTER 5: DISCUSSION

The aim of the current study was to evaluate the combined effects of prolonged sitting and glycemic index (HGI vs. LGI) on total brain blood flow and cognitive function (executive function, memory, and brain fog). It was hypothesized that (1) prolonged sitting will (a) result in decreased total brain blood flow, and (b) this decrease will be exacerbated by HGI. The major findings of this study are that total brain blood flow decreased with prolonged sitting in the LGI condition, total brain blood flow increased in the HGI condition, and that sitting resulted in an increase in brain fog irrespective of condition. Prefrontal cortex cerebral perfusion and Stroop test scores did not change with condition or time, and contrary to expected, executive function improved across time in both conditions.

Limitations and Strengths

To better contextualize the discussion, the limitations and strengths of the current study must be stated. First, the generalizability of our study is low 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 cardiometabolic diseases. Second, while we did measure total brain blood flow and perfusion, future studies should consider continuously monitoring blood flow within the brain. Third, brain fog was a subjective measurement, which may present some bias. However, there is no comparable measure used in other literature, no other study has specifically measured brain fog while sitting. This is an important measurement as many individuals report feeling “foggy” when sitting for long periods of time. In fact, our study found sitting resulted in an increase in perceived brain fog. Finally, we were not adequately powered to examine sex differences in our hypotheses; however, 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 first look into how consumption of a HGI meal affects total brain blood flow during prolonged sitting, which helps to expand knowledge on the effects of prolonged sitting. Third, trial conditions were standardized for potential confounders such as diet, physical activity, and baseline values. Finally, this study is an adequately powered double blind crossover design, which limits most bias in our results.

Comparison to Literature: Brain Blood Flow

Our first hypothesis was that sitting results in decreased $Q_{BF,}$ and this was shown through our control condition, LGI. The LGI impact on Q_{BF} is consistent with previous literature in that prolonged sitting reduces cerebral blood flow [86]. Contrary to our second hypothesis that a HGI meal would further decrease Q_{BF}, a HGI meal resulted in an increase in Q_{BF}. The hypothesis was predicted as prolonged exposure to hyperglycemia and repeated exposure to hypoglycemia can induce microvascular damage and impair endothelial function leading to cerebral hypoperfusion [42–44], and thus cerebral blood flow may decrease during acute hyperglycemia [64,65]. Our study, however, looked at young healthy adults and an acute serving of a high glycemic index meal, and thus our subjects may have been able to protect themselves against damage.

Previous studies have found that middle cerebral artery blood velocity [86] and cerebral blood flow velocity [88] decreases with an acute bout of prolonged sitting. Interestingly, this study and our previous study (unpublished) demonstrate that prefrontal cortex cerebral perfusion does not decrease with prolonged sitting. This indicates that the prefrontal cortex microvasculature may auto-regulate to ensure adequate oxygen delivery [34], at least in young, healthy populations. However, with respect to our measure of cerebral perfusion, it is important to consider placement of the NIRS probes on the prefrontal cortex. In this study, we positioned the probe on the forehead, approximately 3 cm to the right of center, directly over the eyebrow.

This placement is also known as FP2 and the rationale was based off the international Electroencephalography 10-20 system for brain mapping and placement of electrodes for measuring the pre-frontal cortex [76]. A limitation of using this placement (the 10-20 system) is assumption of the placement over the prefrontal cortex. Other placements have been used, such as AF4 (unpublished data – Faulkner). However, we pursued our location based upon previous studies completed in our laboratory (unpublished), but comparing positioning should be considered in future studies.

Comparison to Literature: Cognition

Executive function (assessed by both the Stroop and Trail Making Test, Part B), did not get worse with prolonged sitting and the HGI condition. In fact, the Trail Making Test, Part B, results improved over time across both conditions. Although both sitting and HGI meals tend to independently decrease cognitive function chronically [42,73], these findings align with a previous study in our group [Stoner – unpublished] that found executive function (Stroop) did not get worse with prolonged sitting. We selected to administer both the Stroop and Trail as they are widely recognized assessments of executive function and are relatively short tests, which will minimize mental stimulation and interference with vascular measurements. Our subjects' age, level of education, and the lack of time between the tests (3 hours) may have prevented changes in executive function. In fact, these tests may not have been challenging enough for our subjects, and there may been a learning effect. We did not allow for practice sessions and instead allowed our participants to take the test three times in a row. We also read aloud standardized instructions prior to administering the test.

Although executive function did not worsen with sitting, brain fog did. Brain fog did not differ by condition, but there was a significant effect by time. These findings are similar to a recent study by Perdomo et al [88], who looked at the effects of alternating standing and sitting compared to prolonged sitting on cerebrovascular hemodynamics across an entire workday.

Perdomo et al. assessed measures of sleepiness, mental effort, and fatigue and found these measures did not significantly differ by condition, but did significantly differ by time. It is clear that with prolonged sitting, there is an increase in perceived brain fog, sleepiness, mental effort, and fatigue; and these subjective measurements may be a better estimate of the effects on the brain. Therefore, future studies looking at prolonged sitting should continue to use these measures and see if they differ by any conditions.

Implications

While previous studies have associated chronic sedentary behavior with reductions in brain volume[1] and a decline in cognitive function [3,4], less is known about the mechanism(s) linking repeated acute sedentary behavior exposure to chronic cerebrovascular complications and how lifestyle factors, such as poor dietary choices like HGI meals, may impact these complications. A potential pathway, and one which has been suggested to link repeated prolonged sitting exposure to peripheral and central cardiovascular complications, is blood flow. Two recent studies reported that prolonged sitting decreases middle cerebral artery blood flow [5] and cerebral blood flow velocity [5,88]. In this study, we found that total brain blood flow did decrease across 3 hours, but an HGI meal increases total brain blood flow. However, it is important to state that this study was conducted on young, healthy, active participants, and the findings may not be generalizable. Next, future studies are needed that measure total brain blood flow, cerebral blood flow velocity, perfusion and autoregulation simultaneously to help further understand the effects of prolonged sitting on cerebrovascular function. It is also recommended that future studies utilize executive function tests which are appropriate for the population of interest and minimize prolonged mental stimulation. Finally, this study should also be repeated in older populations and those with chronic disease, so findings are more generalizable.

Conclusions

The purpose of this study was to determine, in young healthy active adults, the effects of prolonged sitting (3 hours), with or without a HGI meal, on total brain blood flow, cognition (executive function, memory, and brain fog), and prefrontal cortex cerebral perfusion. We found that prolonged sitting does decrease total brain blood flow, but contrary to expected, a HGI meal results in an increase in total brain blood flow. While we found that total brain blood flow decreases with sitting, oxygen delivery to the brain is maintained. However, our next question is if we will see similar results in an older population, which may further contribute to the puzzle of why chronic sedentary behavior is associated with cognitive decline.

APPENDIX A: MEDICAL HISTORY FORM

Department of Exercise and Sport Science
Medical History Questionnaire Screening

Subject:_____
Cell Phone #:_____

Patient History

		YES	NO
1.	Do you have any health problems at the present time?	_____	_____
2.	If yes, please describe:_____		
3.	Have you ever been told you have heart trouble?	_____	_____
4.	If yes, please describe:_____		
5.	Do you ever get pain in your chest?	_____	_____
6.	Do you ever feel light-headed or have you ever fainted?	_____	_____
7.	If yes, please describe:_____		
8.	Have you ever been told that you have high blood pressure?	_____	_____
9.	If yes, please describe:_____		
10.	Have you ever had difficulty breathing at rest or with exertion?	_____	_____
11.	If yes, please describe:_____		
12.	Have you ever been treated for infectious mononucleosis, hepatitis, pneumonia, or another infectious disease during the past year?	_____	_____
13.	If yes, name the disease:_____		
14.	Have you ever been treated for or told you might have diabetes?	_____	_____
15.	Have you ever been treated for low blood sugar?	_____	_____
16.	Have you ever experienced heat stroke or heat exhaustion?	_____	_____
17.	If yes, when?_____		
18.	Are you now taking any pills, medications, or supplements?	_____	_____
19.	If yes, please list:_____		
20.	Have you had any recent (within 1 year) difficulties with your:		
	a. Feet	_____	_____
	b. Legs	_____	_____
	c. Back	_____	_____

Menstrual Cycle

21. What was the start date of your most recent menstrual cycle? _____

Family History

22. Has anyone in your family (grandparent, father, mother, and/or sibling) experienced any of the following?
    a. Sudden death _____ _____
    b. Cardiac disease _____ _____
    c. Marfan's syndrome _____ _____

Activity History

23. During your early childhood (to age 12) would you say you were:
    Very active ____ Quite active____ Moderately active____ Seldom active____
24. During your adolescent years (age 13-18) would you say you were:
    Very active ____ Quite active____ Moderately active____ Seldom active____
25. Did you participate in:
    a. Intramural high school sports? _____ _____
    b. Community sponsored sports? _____ _____
    c. Varsity high school sports? _____ _____
    d. Active family recreation? _____ _____
26. Since leaving high school, how active have you been?
    Very active ____ Quite active____ Active____ Inactive____
27. Have you previous participated in strength training _____ _____
28. Do you participate in any moderate to vigorous activity at present? _____ _____
29. If yes, please list:

Activity	Frequency	Duration	Intensity

30. Whom shall we notify in case of emergency?
    Name:_____
    Phone: (Home)_____ (Work)_____

Signature:_____ Date:_____

APPENDIX B: CONSENT FORM

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. During this exercise visit, the participants will receive a standardized frozen meal to consume 12 hours prior to the 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

APPENDIX C: COGNITIVE INSTRUCTIONS

Stroop Test:

This test is called the Stroop Test. It will show you a color word and ask you to respond to the color of the word, not the color that the letters spell. Ex: if the word “blue” shows up in yellow colored font – you will select yellow. The timer will be set for 20 seconds, and once I press “start”, the first word will pop up. Tap the correct color with your pointer or middle finger. The test will stop after 20 seconds.

Trail Making B:

This test is called the Trail Making B Test. It requires you to move sequentially through a series of 25 target dots as quickly as possible. The targets are a combination of numbers and letter so you are required to tap them in an alternating fashion (ex: 1-A, 2-B, 3-C, etc.) You will begin with finding the number “1”. You are required to tap these numbers or letters and they will connect.

Word Recall:

I will read you a list of words at the rate of 1 word every 2 seconds. I will ask you to recall these words post-test.

Foggy Scale:

Brain fog is a general term for dysfunction in focus and memory. I am about to show you a scale of 0-10, 0 being not foggy at all, and 10 is maximal fogginess and an inability to focus. Please point to how foggy you feel.

APPENDIX D: REMINDERS SHEET

Your patient ID: SGI_____

Your CGM was inserted at: _____

CGM things to know:

- You can decide if you would like to take out your CGM after Day 1 or after Day 2. If you take it out after Day 1, we will need to schedule you to come in the night before Day 2.
- You can shower and bathe normally with the CGM
- If you have any issues with CGM, please text Katie 919-924-4201 and Liz 516-449-2102
- DO NOT THROW IT AWAY
- Please use glucometer and prick at the following times:
    - At least 1 hour after leaving lab. This time is: _____
    - Right before you go to bed

    → text glucose level to Katie and Liz (919-924-4201, 516-449-2102)

Day before testing visit:

- Please fill out 24 hour food log.
- Please refrain from vigorous physical activity
- Eat dinner 12 hours before visit. Please remember the dinner is the meal we provided you. **Please try not to eat anything else with this**.
- Please eat meal by: _____.
    - Do not consume coffee or alcohol after this time.
- If female, do pregnancy test and text results to Katie & Liz.

Morning of testing visit:

- Please pack *shorts* and a *t-shirt* for visit. A blanket will be provided. (You're welcome to bring your own.
- Please pack laptop and headphones for visit.
- Please fill out *24 hour food log* and bring to lab in morning.

Quick check list:

___ Prick at least one hour after insertion

___ Prick before bed

___ TO BRING MORNING OF VISIT: glucometer, 24 hour food log, shorts/t-shirt, accelerometer (do not remove), laptop, charger, headphones, and parking pass (if needed)

APPENDIX E: SLEEP DIARY

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?_____

Appendix F: Brain Fog Scale

How “foggy” do you feel?

Borg’s CR-10 Scale

0 Not foggy – very focused

1

2 A little foggy

3

4

5 Moderately foggy

6

7

8 Very foggy

9

O Maximal fogginess – cannot focus

APPENDIX G: EXTRA DATA

Seated: Blood Flow to Brain

			CCA Bfmean *m/s*	ICA Bfmean *m/s*	VA Bfmean *m/s*	Q (mean) Bfmean *m/s*
10 Min	HGI	X	3928	3256	1239	5761
		SD	*1164*	*1012*	*728*	*2813*
	LGI	X	3847	2545	1410	5251
		SD	*906*	*1078*	*607*	*2116*
90 Min	HGI	X	3506	2327	1153	4621
		SD	*576*	*996*	*675*	*2060*
	LGI	X	3814	3071	1301	5532
		SD	*897*	*1362*	*588*	*1775*
170 Min	HGI	X	3464	2632	1769	6195
		SD	*590*	*993*	*1185*	*2843*
	LGI	X	3851	2650	1497	5826
		SD	*957*	*1649*	*826*	*2299*
Interaction		*P*	0.383	0.035	0.487	0.384
Time		*P*	*0.346*	*0.605*	*0.191*	*0.27*
		ES	-0.12	-0.07	0.06	0.15
Condition		*P*	*0.167*	*0.928*	*0.994*	*0.93*
		ES	0.10	0.01	0.0166982	0.00

Supine: All NIRS

			tHb_1 *µM*	tHb_2 *µM*	tHb_3 *µM*	HbO2_1 *µM*	HbO2_2 *µM*	HbO2_3 *µM*	HHb_1 *µM*	HHb_2 *µM*	HHb_3 *µM*	SRS HbO2	SRS HHb	SRS tHb	TSI *%*
Pre	HGI	X	28.66129	34.86946	39.41264	18.22047	21.5136	23.38136	10.44081	13.355844	16.03127	37.45409	27.94867	65.4028	57.1792
		SD	*48.25*	*58.44*	*65.66*	*29.86*	*35.21*	*37.72*	*18.49*	*23.37*	*28.01*	*12.16*	*8.94*	*17.96*	*7.57*
	LGI	X	28.09309	35.88069	41.44698	16.29946	20.9004889	24.32849	11.79364	14.980228	17.11847	42.77723	26.70065	69.4779	60.936767
		SD	*48.25*	*58.44*	*65.66*	*29.86*	*35.21*	*37.72*	*18.49*	*23.37*	*28.01*	*12.16*	*8.94*	*17.96*	*7.57*
Post	HGI	X	31.42	38.14	44.19	19.76	23.10	26.64	11.66	15.04	17.55	41.11	27.62	68.73	59.74
		SD	*48.21*	*60.23*	*69.67*	*29.91*	*36.96*	*40.93*	*18.46*	*23.49*	*28.83*	*15.24*	*10.58*	*24.74*	*5.88*
	LGI	X	28.73887	35.28186	41.08591	16.48483	20.2207778	23.95045	12.25403	15.061094	17.13542	41.16084	25.5654	66.7262	60.9125
		SD	*48.65*	*59.42*	*67.50*	*27.82*	*34.05*	*38.37*	*22.20*	*26.52*	*29.81*	*14.35*	*6.65*	*19.01*	*7.76*
Interaction		*P*	0.922	0.832	0.795	0.861	0.797	0.671	0.973	0.874	0.928	0.209	0.738	0.302	0.272
Time		*P*	*0.78*	*0.94*	*0.85*	*0.77*	*0.96*	*0.77*	*0.81*	*0.85*	*0.92*	*0.58*	*0.64*	*0.84*	*0.28*
		ES	0.05	0.01	0.03	0.05	-0.01	0.05	0.04	0.03	0.02	0.10	-0.08	0.04	0.19
Condition		*P*	*0.80*	*0.99*	*0.92*	*0.18*	*0.59*	*0.93*	*0.57*	*0.63*	*0.78*	*0.32*	*0.23*	*0.85*	*0.06*
		ES	-0.06	0.00	0.02	-0.34	-0.13	-0.02	0.14	0.12	0.07	0.25	-0.31	0.05	0.47

Seated: All NIRS

			tHb_1 *µM*	tHb_2 *µM*	tHb_3 *µM*	HbO2_1 *µM*	HbO2_2 *µM*	HbO2_3 *µM*	HHb_1 *µM*	HHb_2 *µM*	HHb_3 *µM*	SRS HbO2	SRS HHb	SRS tHb	TSI *%*
10 Min	HGI	X	29.94166	35.50844	38.25592	17.45226	20.11245	21.43928	12.48939	15.396006	16.81661	33.14394	25.93592	59.0799	55.865394
		SD	*48.53*	*61.56*	*67.87*	*30.21*	*37.65*	*39.15*	*18.43*	*24.10*	*28.79*	*13.10*	*9.58*	*20.08*	*8.91*
	LGI	X	30.22737	34.37938	38.26327	17.82789	19.7758222	21.85443	12.39949	14.60355	16.40884	33.12041	24.75589	57.8763	57.049839
		SD	*48.09*	*57.94*	*63.82*	*26.84*	*32.53*	*35.53*	*22.45*	*26.44*	*28.94*	*11.97*	*8.78*	*19.02*	*9.13*
90 Min	HGI	X	31.77	37.11	41.72	18.90	21.39	24.07	12.86	15.72	17.64	34.66	25.21	59.88	58.09
		SD	*49.26*	*61.71*	*68.29*	*30.29*	*37.39*	*39.49*	*19.06*	*24.44*	*28.86*	*11.65*	*8.66*	*18.41*	*8.34*
	LGI	X	30.88558	37.65359	40.77703	16.12292	20.1632056	22.18272	14.76267	17.490417	18.59429	36.63239	25.23265	61.865	59.306244
		SD	*50.40*	*60.10*	*66.91*	*28.52*	*34.21*	*37.59*	*23.11*	*27.03*	*29.99*	*14.42*	*9.18*	*21.50*	*12.19*
170 Min	HGI	X	30.53582	36.25997	38.41808	16.53237	19.52875	21.06809	14.00346	16.731239	17.34997	32.42384	23.17847	55.6023	57.158628
		SD	*48.98*	*61.26*	*68.96*	*30.12*	*36.99*	*39.78*	*18.95*	*24.39*	*29.22*	*11.64*	*9.39*	*18.81*	*8.31*
	LGI	X	28.69172	35.20622	38.01072	15.28612	18.8031444	21.03122	13.40559	16.403117	16.97948	34.55444	23.32147	57.8759	60.008739
		SD	*49.97*	*60.01*	*66.77*	*28.60*	*34.51*	*37.79*	*22.83*	*26.85*	*29.77*	*12.56*	*9.05*	*20.23*	*7.68*
Interaction		*P*	0.932	0.971	0.937	0.874	0.946	0.901	0.966	0.989	0.967	0.539	0.906	0.681	0.526
Time		*P*	*0.97*	*0.95*	*0.98*	*0.96*	*0.97*	*0.98*	*0.95*	*0.95*	*0.95*	*0.53*	*0.56*	*0.55*	*0.39*
		ES	-0.04	-0.05	-0.02	0.00	-0.04	0.01	-0.06	-0.05	-0.05	0.00	-0.14	-0.06	0.18
Condition		*P*	*0.95*	*0.74*	*0.94*	*0.20*	*0.78*	*0.79*	*0.37*	*0.40*	*0.69*	*0.69*	*0.26*	*0.80*	*0.14*
		ES	-0.02	0.08	0.02	-0.32	-0.07	-0.07	0.22	0.21	0.10	0.10	-0.29	-0.07	0.37

Seated: Stiffness

			CCA			ICA			VA		
			Stiff	PWV (β)	Osc.	Stiff	PWV (β)	Osc.	Stiff	PWV (β)	Osc.
			AU	*m/s*	*ratio*	*AU*	*m/s*	*ratio*	*AU*	*m/s*	*ratio*
10 Min	A	X	10.18837	5.203462	0.908654	10.11599	5.20862291	2.590505	14.4958	6.3590608	15.26264
		SD	*3.30*	*0.87*	*1.58*	*3.53*	*0.76*	*4.85*	*9.03*	*1.44*	*22.42*
	B	X	9.541892	5.222923	1.040656	11.2045	5.45322541	3.00246	16.57842	6.7599764	5.512493
		SD	*3.49*	*1.01*	*1.48*	*6.54*	*1.39*	*6.10*	*10.41*	*1.74*	*9.48*
90 Min	A	X	9.86	5.27	0.74	10.56	5.32	2.64	17.42	6.97	12.68
		SD	*2.93*	*0.89*	*1.38*	*5.04*	*1.19*	*3.69*	*15.30*	*2.38*	*19.04*
	B	X	12.26072	5.934516	0.687117	13.72861	6.2163476	2.8372	14.85638	6.8935996	11.13958
		SD	*4.02*	*1.09*	*0.69*	*6.67*	*1.40*	*5.23*	*7.11*	*1.16*	*18.80*
170 Min	A	X	7.899335	5.274986	0.802031	11.64678	5.68738043	1.684019	16.17518	6.9342226	8.627386
		SD	*7.28*	*0.83*	*1.01*	*5.55*	*1.35*	*1.99*	*7.80*	*1.58*	*12.01*
	B	X	11.45808	5.763836	0.87961	10.36547	5.61571132	7.279111	16.47213	6.862656	10.02898
		SD	*2.81*	*0.91*	*1.00*	*4.94*	*0.92*	*13.66*	*7.60*	*1.39*	*15.23*
Interaction		*P*	0.045	0.063	0.931	0.123	0.072	0.11	0.541	0.678	0.645
Time		*P*	*0.241*	*0.042*	*0.716*	*0.464*	*0.409*	*0.562*	*0.895*	*0.534*	*0.767*
		ES	-0.02	0.21	-0.05	-0.03	0.05	0.10	-66.65	40.15	-5.07
Condition		*P*	*0.014*	*0.001*	*0.792*	*0.238*	*0.033*	*0.076*	*0.955*	*0.919*	*0.599*
		ES	2.62	8.81	0.49	1.36	5.95	1.67	0.221637	-0.0158355	0.176857

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