INVESTIGATION OF THE EFFECTS OF SEDENTARY BEHAVIOUR AND MODERATE EXERCISE ON GLUCOSE TOLERANCE AND INSULIN SENSITIVITY.

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A thesis submitted in partial fulfillment of the requirements of the Honours Program in the Department of Applied Human Sciences

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ABSTRACT

Introduction: The primary objectives of this study is to demonstrate to what degree frequent light-intensity exercise effects glucose handling, as well as beginning to establish a light intensity exercise protocol that demonstrates the most significant benefit in glucose handling when breaking up frequent bouts of sedentary behaviour. Secondary objectives of the study involved looking at chronic disease risk factors, executive function following 3 hours of constant sedentary time and 3 hours of sedentary time broken up with a 3-minute bout of light intensity exercise every 30 minutes.

Methods: Participants completed two trails.

SIT Trial- Participants sat at a desk in an office for 3 hours with access to their own devices. Blood was drawn pre and post-trial to look for changes in metabolites. Blood glucose, heart rate, blood pressure was measured in half-hour increments following the start of the meal. Cognitive tests (Trail making test, Stroop task, N-Back test, and Flanker Task) were performed after the second last blood glucose test.

SIT-EX Trial- Participants performed the same tasks as the SIT trail except for a three-minute bout of light to moderate activity was completed following each of the blood glucose measurements. Cognitive tests (Trail making test, Stroop task, N-Back test, and Flanker Task) were performed after the last exercise bout.

Results: After analyzing all of the results from both trials, there was no significant difference between average SIT and SIT-EX blood glucose concentration (p=.73), heart rate ( p=.1519), systolic blood pressure (p=.4202), diastolic blood pressure ( p=.2985), Trail Making Test (Part A p=.72 Part B. p=.09 ) N-backtest (p=.76 ), Stroop test (p=.62), Flanker test (p=.60). Forty-three metabolites had a significant difference. Thirty-seven metabolites showed a significant difference between the fasted and postprandial blood draws. One metabolite (lysophosphatidylcholine) differed between the SIT and SIT-EX conditions and five metabolites effect due to an interaction between the two factors.

Conclusions: Further high-quality studies with more participants are needed to understand the effect of acute bouts of sedentary behaviour on cognitive function as well as changes in metabolites and the interaction frequent exercise bouts can have on those relationships.
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LITERATURE REVIEW

Prevalence of Sedentary Behaviour

Throughout an individual's life trajectory, they may experience significant life changes and adversity, which tend to result in a decline of their fitness level and physical activity as one matures into adulthood (Dwyer et al., 2008). The decrease in physical activity can be troublesome for the population's overall health. Research demonstrates that there is a weak to moderate inverse association between sedentary behaviour and physical activity (Mansoubi, Pearson, Biddle, & Clemes, 2014). Sedentary behaviour is defined as any activity done while sitting, lying, or reclining, while expending no more than 1.5 metabolic equivalents (Tremblay et al., 2017).

Compared to lifestyles in the past, a more extensive variety of individuals are engaged in sedentary behaviour today (Patterson et al., 2018). For example, the increase in sedentary time could be impacted by rapidly advancing technology, such as human transportation, methods used for communications, workplace efficiency, and home entertainment. Furthermore, there is a positive relationship between age and sedentary behaviours (the older the person, the more sedentary) (O’Donoghue et al., 2016). Children of the twentieth-century spend vast amounts of time throughout their day in sedentary behaviours. This is evident as children spend most of their time in school sedentary, coupled with the fact that they spend over half of their time after school in
sedentary behaviours. Arundell and colleagues state there is a prevalent concern for adolescents and youth as well (2016).

Canadians spend the majority of their time being sedentary; in fact, the average Canadian spends 8.4 and 9.6 hours per day engaged in sedentary behaviour, respectively (Roberts et al., 2017). Adults spend much of their day engaged in sedentary behaviours such as watching television, using a computer or driving in vehicles. Consequently, with an increase in sedentary time, there is also an increase in the adverse effects that are associated with it. Unfortunately, the increase in sedentary behaviour is not only a Canadian issue but rather a global concern as almost 60% of adults over the age of 60 reported sitting for more than four hours per day (Harvey, Sebastien, and Skeleton, 2013).

**Negative Effects of Sedentary Behaviour**

The adverse effects of sedentary behaviour affect multiple aspects of the body. Evidence suggests that sedentary behaviour has a direct influence on metabolism, bone mineral content, and vascular health through a person’s life span (Tremblay, Colley, Saunders, Healy & Owen, 2010). In 2012 and more recently in 2018, Saunders and colleagues systematically reviewed the available research examining changes in cardiometabolic indicators following exposure to ≤ 7 days of prolonged sedentary behaviour (2012) and the benefits of breaking up sedentary behaviour (2018). These reviews indicate that extended periods of sedentary behaviour consistently resulted in significant reductions in insulin sensitivity, glucose tolerance, and increased plasma triglyceride levels (Saunders, Larouche, Colley & Tremblay, 2012). Furthermore, the effect of five days of bed rest showed that reactive hyperemia was reduced by roughly
20% in the legs and 30% in the arms in participants (Hamburg et al. 2007). It has also been shown that approximately eight hours per day of sedentary behaviour correlates with adverse effects, and after this eight hours, there is an increase in non-linear relationships between the amount of sedentary behaviour and all-cause mortality, such as cardiovascular diseases (Patterson et al., 2018).

Sedentary behaviour has been suggested to be distinct from physical inactivity and an independent predictor of metabolic risk even if an individual meets current physical activity guidelines (Booth & Lees, 2007). In a clinical trial of non-obese adults, only one day of inactivity, long hours of sitting, and minimal walking or standing decreased insulin sensitivity even when energy intake was reduced to maintain energy balance (Stephens, Granados, Zderic, Hamilton & Braun, 2011). Further research has shown negative consequences for individuals who exercise in the morning but then have longer sedentary bouts throughout the day, and evidence supports that regular activity breaks were more effective than continuous physical activity at decreasing postprandial glycemia and insulinemia in healthy, normal-weight adults (Peddie et al., 2013).

**Cognitive Function and Sedentary behaviour**

Recent systematic reviews show a mixture of results when looking at the correlation between sedentary behaviour and cognitive functions in the workplace (Fanning et al., 2017). The results of cross-sectional and epidemiological studies have shown that physical exercise enhances cognitive functions in young and older adults (Fernandes, Arida & Gomez-Pinilla, 2017). Increasing sedentary behaviour in the workplace is a significant public health concern and a particularly relevant need for
further research as the effects of work-related sedentary time on cognition appear unclear in literature. Evidence from a recent systematic review supports the notion that over time, poor glycemic control can impair brain structure and function, and smaller effects were observed for tasks that measured processing compared to the task that measured memory (Geijselaers, Sep, Stehouwer & Biessels, 2015).

**Possible Mechanism Underlying Negative Effects of Sedentary Behaviour**

Recent reviews suggest that no single mechanism causes the adverse effects of sedentary behaviour, but instead there may be multiple mechanisms such as glucose transporter type 4 (GLUT4) activity (Tremblay et al., 2010). Suggested potential mechanisms for improvement in glucose regulation are likely to involve muscle contraction and localized increases in skeletal muscle glucose uptake, mediated by both the insulin and contraction-mediated (insulin-independent) glucose uptake pathways (Cartee, 2015). Numerous studies have analyzed participants and found between 7–19 days of bed-rest (Tabata et al. 1999; Op ‘t Eijnde et al., 2001; Biensø et al., 2012) has the potential to reduce muscle GLUT4 protein content when muscles have not been activated. These results coupled with studies that look at decreased activation due to denervation of a muscle, show a rapid decrease in both muscle GLUT-4 content and insulin-stimulated glucose uptake (Megeney et al. 1993). Although there are many adverse effects on a person, some of the most substantial statistical results represented in the literature are the increased risk of developing type 2 Diabetes (Biswas 2015).
Interventions to Decrease Sedentary Behaviour

There have been multiple methods of intervention studied to decrease a person’s sedentary behaviour. Well-controlled laboratory studies have suggested that reducing sedentary time may have benefits in terms of metabolic expenditure. Pooled analyses of outcomes for men and women indicated that energy expenditures while sitting, standing and during sit/stand transitions were significantly different from each other. A recent systematic review done by Dr. Saunders and colleagues showed there is evidence that suggests that a decrease in sedentary behaviour with intermittent breaks while standing, walking at a light intensity, walking a moderate intensity, arm ergometry, resistance training with bodyweight, resistance training with weights, alternating between sitting and standing, and walking upstairs intermittently (Saunders et al., 2018). The interventions varied in results with some trials showing negative correlations between sedentary behaviour and health risks while others were showing no effect. After reviewing all of the intervention methods stated above, it was suggested by the authors that acute periods of uninterrupted sitting results in significant increases in postprandial insulin and glucose levels, when compared to periods of sitting interrupted with light- or moderate-intensity physical activity (Saunders et al., 2018).

Metabolomics and Their Link To Insulin Resistance

Metabolomics refers to the systematic analysis of metabolites (sugars, amino acids, organic acids, nucleotides, and lipids) in a biological sample. Metabolites have been used as biomarkers for a variety of chronic diseases, or can be used to look for possible signs that an individual has a chronic disease or is at risk of developing one. The
evidence is beginning to surface regarding the utility of metabolomics in pre-diabetes, diabetes, and other chronic diseases. Recent reviews provide evidence that several blood amino acids may consistently be associated with the risk of developing type 2 diabetes (Guasch-Ferré et al., 2016). The evidence suggests there may be a positive correlations with Isoleucine, Leucine, Valine, Tyrosine, Phenylalanine, and Alanine, and a negative correlation with Glycine, Glutamine, and Histidine in regard to insulin resistances in people who’s blood was analyzed (Appendix A). The results from the previous review suggest that alterations in blood branch chain amino acids and aromatic amino acids identified using metabolomics techniques may be useful in identifying novel biomarkers of type 2 diabetes (Guasch-Ferré et al., 2016). Furthermore, understanding how a high-carb, high-Fat meal influences specific metabolites, as well as the effect, breaking up sedentary behaviour, can have on lowering the concentrations of these in healthy younger individuals and how we can combat signs of diseases earlier on in a person’s life would be beneficial for public health.

**Objectives of Study**

As a pilot study, many different objectives and outcomes are being analyzed to create a protocol that can be used for a larger research study in the future. The primary objectives of this study are to establish an exercise protocol that shows the most significant benefit in glucose handling after consuming a high carb, high-fat meal. Secondary objectives of the study involved looking at cardiometabolic risk factors and the participant's executive function between three hours of constant sedentary time and three hours of sedentary time broken up with a three-minute bout of exercise every 30
minutes. Finally, a target assay looking for possible changes in metabolites between conditions. I hypothesize that (1) by breaking up sedentary time with a three-minute light-resistance based activities; it will result in a decrease in blood glucose concentrations when comparing the exercise trial to the sedentary trial. (2) There will be changes in the participants’ metabolites when comparing the fasting to the postprandial blood samples. (3) There will be no significant changes in the participant’s blood pressure, heart rate, and cognitive function.
METHODS

Participant Recruitment

Healthy adults were recruited from the UPEI population and communities throughout Prince Edward Island. The individuals were recruited by posters in the community, social media advertisement, emails and through word of mouth. Potential participants contacted members of the research group by phone and/or email. Participants were aged 18-50 healthy, non-smoking, not diabetic or not pregnant. Exclusion criteria included participants who perform more than 150 minutes of moderate to physical activity a week, or had a food allergy to the meals that were provided to them during the trials. A Participant Questionnaire and PAR-Q was used to identify individuals who qualified for the study and were given to the participants at the introductory lab visit. Individuals with the five active diseases known to affect our outcome were also excluded (diabetes, kidney disease, liver disease, cardiovascular disease, or cancer).

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Figure 1. Participant Recruitment Flow Diagram
Experimental Protocol

Study design

The study was a randomized crossover trial conducted in Charlottetown, Prince Edward Island, between January-February 2018. The University of Prince Edward Ethics Committee approved the trial and written informed consent was obtained from all participants before the screening procedures.

Standardization of Diet and Exercise

Participants were asked to avoid physical activity and caffeine for 48 hours as well as alcohol for 24 hours before each session. Participants were sent a reminder email within 24 hours of the start time to remind them to fast at least 12 hours, and a food log was attached for participants to fill out when a what their last meal was the night before the trials. Participants were asked to eat similar meals before both trials. Water consumption was recorded during the first trial and participants were asked to consume a similar volume during the second trial.

First Visit

This first visit to the lab consisted of an informational meeting where the requirements of the study, the time commitment, and risk and benefits were described in detail, and the letter of information was distributed to interested participants. Before data collection, participants were provided with informed consent forms and provided an opportunity to ask questions about the study protocols. Interested participants were then
screened to determine their health status by assessing physiological variables. Baseline measurements recorded include height, weight, BP, waist circumference, % body fat. Eligible participants were then familiarized with the lab and the intervention procedures.

**Blood Collection**

Participants arrived at the clinic between 0845 and 0915 after an overnight fast of 12 h. The three-hour trial began when the first meal was given to participants. The first meal was after the collection of a fasting blood sample (Figure 2). A total of 2 blood (1 7.5mL SST red top, one 6mL K2EDTA purple top) samples were collected from each participant. One sample was collected 3 hours after the start of the first meal. Each blood sample was collected by syringe from veins located in the antecubital fossa. Both tubes were inverted as per BD vacutainer recommendations and stored at room temperature (20C) for 30-45 minutes. The purple top sample was used to collect peripheral blood mononuclear cell analysis at a later. (Appendix B) Red/Grey top samples were centrifuged (1300 g for 15 min at -4C) to separate serum from red blood cells. Serum was aliquoted 200µL into 1.5 ml cryovials. Within 2 hours of collection, serum was stored at -80C for later metabolomic analysis (TMIC Prime Metabolomics Profiling Assay) at The Metabolomic Innervation Center (TMIC).

**Test Meals**

During the first meal participants were given 15 minutes to consume 3 servings (466 grams) of melted Cows Creamery chocolate ice cream (Melted in fridge no later than 24hrs before participants were scheduled to complete a trial) (87g carbohydrate, 57g
fat, 15g protein, 960 calories) (Appendix C). This meal was offered at 0hrs of trial. Participants had 10 minutes to consume a second high carbohydrate meal (220 ml Minute maid orange juice and one Little Debbie Cosmic Brownie) (Appendix D). that was given 2 hrs after the start of the first meal Participants consumed water and libitum during the first intervention session, and the volume ingested was replicated in subsequent intervention sessions.

**Second and Third Visits**

Subjects experienced two conditions:

1) sitting for three consecutive hours (SIT) (Figure 2a)

2) sitting for three consecutive hours with three-minute activity breaks every 30 minutes (SIT+EX). (Figure 2b)

The SIT and SIT+EX conditions were performed in a random order (flip of a coin at the start of the first trial). Participants will not know which experiment they would be performing until entering the lab during the first trial. The SIT will be used to find baseline values of participant’s insulin resistance, glucose intolerance, cognitive functions, blood pressure, heart rate, and the effect a high carbohydrate, high-fat meal has on metabolites. The SIT+EX condition values will be used to determine the effect of physical exercises that improve glucose control, insulin sensitivity, cognitive functions, blood pressure, heart rate, and the impact a high carbohydrate, high-fat meal has on metabolites during periods of prolonged sitting in both the SIT and SIT-EX trials participants. Carryover effects were expected to be minimal because of the minimum 6-d washout period between consecutive interventions.
Heart rate, blood pressure was recorded (BpTRU, BPM-200) every 30 minutes for the duration of the 3-h testing session, which provided a total of 6 measurements. Blood glucose was measured every half hour (except for 2hrs) following the start of the meal with a hand-held glucometer (OneTouch Ultra®2 meters).

The exercise bouts were done in a designated area of the laboratory. While seated in their office chairs, participants were wheeled there following the blood glucose check. The exercise bouts consisted of three 30 second full body exercises with the 30s of marching in place after each exercise as demonstrated in Figure 3. The exercise bout completed every half hour following the meal (.5hr,1hr,1.5hr, 2hr,2.5hr) (Appendix E). A water 1L water bottle filled with tap water from a public campus water fountain (2.3lbs) was used for thrusters and kettlebell swings, while a red medium resistance Theraband was attached to the top of a door frame for the semi-squat shoulder extensions.

Participants were instructed on how to perform exercises during the first visit to the lab. No designated tempo was used; participants completed the exercises at their own pace. A researcher kept track of the number of reps throughout each bout but did not inform the participants of how many reps they had performed. Four cognitive tests were performed while at a designated desk between 2.5 and 3hrs of sedentary time.
Figure 2.

a. Timeline of the SIT Trial.

b. Timeline of the SIT-EX Trial

Figure 2 shows the Timelines for both trials. (a) is the SIT trial (Timeline of the SIT+EX trial. (EB= Exercise Bout, BP=Blood Pressure, FBD= Fasting Blood Draw, FP=Finger Prick (Blood Glucose), SDP= Second Blood Draw)
Figure 3. Breakdown of Exercise Bouts

<table>
<thead>
<tr>
<th>Thrusters</th>
<th>March in Place</th>
<th>Semi Squat Shoulder Extensions</th>
<th>March in Place</th>
<th>Kettlebell Swing</th>
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**Cognitive Test**

Four cognitive tests were performed during the last 30 minutes of sedentary time. The first cognitive test completed was the Trail Making Test (TMT) (Visual search, scanning, speed of processing and mental flexibility). A mirrored image of the initial TMT was used for the second trail completed by the trial. The other three cognitive tests were completed online (psytool kit) on a windows desktop located in the laboratory under supervision. The order of completion was the Stroop test (cognitive processing speed, attentional capacity, and executive function) (MacLeod, 1991) followed by the N-Back (working memory) (Jaeggi, Buschkuehl, Perrig, & Meier, 2010), and Flankers (Cognitive processing speed, attentional capacity, and executive function) (Eriksen, & Eriksen, 1974).

**Statistical Analyses**

Changes in the Blood Glucose were calculated by looking at the incremental net area under the curve (iAUC) (trapezoidal method) for blood glucose. With the use of the glucose, insulin, and triglyceride concentrations measured from each of the 16 time points across each intervention, iAUCs were computed by first calculating the total AUC with the trapezoid rule (which approximates the AUC by considering the areas between continuous time points to be trapezoid in shape; the areas of the individual trapezoids are
then summed to provide the total AUC) and then subtracting the area from the baseline concentration over the 3-h period. This provided a single value for each outcome per participant-intervention combination. Two-tailed t-tests were used to look for the differences between the SIT and SIT EX trials for all measured values (BP, HR, BG) except for the metabolomic data. Repeated measures two-way ANOVA tests (Prism 8, Graph Pad software) were completed on all metabolite values supplied by TMIC.
RESULTS

Six participants completed both the SIT and SIT-EX trials (Female=4, Male=2). Participants ranged from the community and UPEI students. Mean age 24.8 ± 8.1, mean weight 69.3 ± 10.0 kg, mean body mass index 25.4 ± 2.8 kg/m², mean waist circumference 81.9 ± 9.7 cm. Of the six participants who completed both trials, four had blood drawn on both trials and had serum samples sent analysis at TMIC.

Changes in the Concentration of Blood Glucose Between trials

The purpose of this study was to evaluate both the effect of high carb, high-fat meal on participants blood glucose concentrations as well looking for changes when comparing the SIT to SIT-EX trials. We did this by having participants perform two trials with a minimum of six days of washout period in between. Fasted blood glucose was taken, and then a standardized high carb high-fat meal was consumed after the participant's sedentary bout. The SIT trial was used to assess the effect of glucose metabolism once sedentary while a SIT-EX trial was used to look for a difference in metabolism with frequent bouts. Figure 4 demonstrates the average blood glucose for both the SIT and SIT-EX trials. When compared to prolonged sitting, regular activity breaks did not lower postprandial glucose. The net average IAUC for the SIT trial was 141.25 ± 97.98 mmol/L/min and 158.88 ± 55.83 mmol/L/min for the SIT-EX trail. (Figure 4) On average The Sit-Ex trial was 17.6 mmol/L/min more than the SIT, but there was no
statistical significance (p=.63, d=.22, 95% CI -0.915 - 1.356) Individual Blood Glucose IAUC comparisons can be seen in Appendix F.

**Figure 4.** Average blood glucose values throughout the SIT and SIT-EX Trails (Standard deviation bars)

**Figure 5.** Mean Area Under the Curve Comparison (Standard deviation bars)
**Breaking up Sedentary Behaviour Time and Cardiovasular Measurements**

Throughout the two conditions, blood pressure and heart rate were taken simultaneously using an electric blood pressure monitor (BpTRU model BPM200). Measurements were taken before fasting blood draw and every half hour following the beginning of the meal until the completion of the trial. Measurements were taken before blood glucose, and blood draws.

**Heart Rate**

Figure 6 shows the average heart rate for both the SIT and SIT-EX trials. When compared to prolonged sitting, regular activity breaks did not have a significant effect on heart rate. The average blood pressure for the SIT trial was 81.07 ± 2.29 bpm and which was on average 2.452 bpm higher then SIT-EX average heart rate 83.52 ± 5.7 bpm (p=.1519).

![Figure 6. SIT vs SIT-EX mean heart rate comparison (Standard deviation bars)](image-url)
**Blood Pressure**

Exercise is commonly recommended by health care professionals as it has been shown to decrease the risk of developing or combating hypertension. Exercise has been shown to improve BP although it returns to normal shortly finishing exercise. There is evidence to support that prolonged sitting can equal an increase in blood pressure for overweight and obese adults aged 45-65. However, there is less evidence looking at the effect of acute bouts of sedentary behaviour, and the correlation with blood pressure in younger adults (Larsen et al., 2014). The average ages of the participants (n=6) throughout this trial were 24, and the blood pressure was assessed every half hour through the trial.

**Systolic BP**

The average systolic BP for both the SIT and SIT-EX trials can be seen in figure 7. When compared to prolonged sitting, regular activity breaks did not have an effect on diastolic BP. The average systolic BP for the SIT trial was 112.6 ± 1.96 mmHg and which was on average 1.3±1.12 mmhg higher then SIT-EX average systolic BP 111.3 ± 3.27 mmHg (p=.4202). The mean SIT-EX BP bottomed out at 107mmHg and peaked at 113.7mmHG which was a range of 6.7mmHg in response to the high carb, high fat meal (0-120 minutes) and 5.7mmHg following the second meal (120-180min). During the same time frame, the mean SIT bp bottomed out at 110.4 mmHg and peaked at 113.2mmHG which was a range of 2.8 mmHg in response to the high carb, high fat meal (0-120 minutes) and 7.3mmHg following the second meal (120-180min ).
Diastolic BP

Figure 8 shows the average diastolic BP or both the SIT and SIT-EX trials. When compared to prolonged sitting, regular activity breaks did not affect diastolic BP. The average diastolic blood pressure for the SIT trial was 75.4 ± 1.69 mmHg which was on average 1.3±1.12 mmHg higher than SIT-EX average DBP 75.2 ± 3.06 mmHg (p=.2985). When looking at the difference between the ranges in DBP between the conditions, the SIT-EX had a range of 10.3 mmHG and the difference between the SIT low value and the high value of 5.5mmhg throughout the trials.
Sedentary Behaviour and Cognitive Functions

Sedentary behaviour has been an increasing area of research in the workforce. Employers are interested in decreasing the amount of money spent on health-related issues that correlate with sedentary behaviour. Employers are also interested in decreasing sedentary behaviour and the potential positive effect on productivity and cognitive function. During the last half hour of the trials, four cognitive tests were performed (2.5-3 hrs).

When comparing the SIT-EX to the SIT conditions, there was no significant difference in any of the cognitive tests. TMT (Part A p=.72 Part B. p=.09) (Figure 8a looked at scanning and processing speed, mental flexibility. The N back test (p=.76 ). Figure 8b) looked at working memory, Stroop test (p=.62) (Figure 8c). The Flanker test (p=.60). Figure 8d) looked at processing speed, attentional capacity, and executive function. SIT average TMT times for Part A was 23.97±6.01s and 46.26±16.38s for part
B while SIT-EX average TMT times for Part A which was 24.36±6.41s and 38.21±12.84s for Part B. The SIT-EX trail was on average .385s faster in Part A and 8.04s faster in Part B. The average percent of 3-back letters wrong for the SIT was 21.8 ± 14.31% while the SIT-EX trial was 25.7±18.74%. The average stroop effect was 64.33±143.71 ms for the SIT and 95.17±85.035 ms for the SIT-EX condition. The mean flanker effect was 24.83±78.96 ms for the SIT 52.83 ±18.74 ms for the SIT-EX condition. Individual participant comparison for the four cognitive tests can be seen in Appendix G, H, I, J, and K. There is a mixture of results and effects as trail B shows exercise has a positive effect on five of the six participants. The flanker effect shows adverse effects in five of the six participants. However, the stroop test that looks at similar cognitive functions showed positive effects in three participants and no difference for one participant.

Figure 9a  Figure 9b.
Figure 9c  Figure 9d

Figure 9. (a) TMT Part A and Part B Result Comparison Between SIT and SIT-EX. (b) N-Back Result Comparison Between SIT and SIT-EX. (c) Stroop Effect Comparison Between SIT and SIT-EX. (d) Flanker Effect Comparison Between SIT and SIT-EX. (Standard deviation bars)

**Difference Between SIT/SIT-EX as Well as Morning and Postprandial Blood_Draws**

Metabolites have been used for as biomarkers for a variety of chronic diseases. They can be used to look for possible signs that an individual has a chronic disease or is at risk. Understanding how a high-carb, high-Fat meal influences specific metabolites, as well as the effect, breaking up sedentary behaviour, can have on lowering the concentrations of these in healthy younger individuals and how we can combat signs of diseases earlier on in a person’s life would be beneficial for public health. Through both the SIT and SIT-EX trials, researchers collected both a fasted and postprandial blood sample. This sample was sent to TMIC located at the University of Alberta for analysis, and significant results are in Table 1. 43 metabolites had a significant difference. Thirty-seven metabolites showed a significant difference between the fasted and postprandial blood draws (Table 1c). One metabolite (LYSOC27:0) differed between the SIT and SIT-
EX conditions (Table 1a). and five metabolites affect due to an interaction between the two factors (Table 1b).

Table 1

(a) Metabolites with Significant Differences Between SIT and SITEX (p=<0.05)

<table>
<thead>
<tr>
<th>Name</th>
<th>Fasting BD</th>
<th>Postprandial BD</th>
<th>%Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>Avg</td>
<td>Avg</td>
</tr>
<tr>
<td>LYSOC17:0</td>
<td>0.0366</td>
<td>2.153</td>
<td>1.819</td>
</tr>
</tbody>
</table>

(b) Metabolites with Significant Differences (p=<0.05) Interaction Factor

<table>
<thead>
<tr>
<th>Name</th>
<th>SIT Fasting</th>
<th>Postprandial</th>
<th>SITEX Fasting</th>
<th>Postprandial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>Avg</td>
<td>SD</td>
<td>Avg</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.0108</td>
<td>172.25</td>
<td>39.05</td>
<td>116.45</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0.0355</td>
<td>2557.5</td>
<td>444.77</td>
<td>3990</td>
</tr>
<tr>
<td>Butyric acid C4</td>
<td>0.0277</td>
<td>1.043</td>
<td>0.199</td>
<td>1.372</td>
</tr>
<tr>
<td>Butyrylcarnitine C16:1</td>
<td>0.0182</td>
<td>0.273</td>
<td>0.094</td>
<td>0.240</td>
</tr>
<tr>
<td>Hexadecenoylcarnitine</td>
<td>0.0419</td>
<td>0.044</td>
<td>0.009</td>
<td>0.029</td>
</tr>
</tbody>
</table>
(c). Metabolites with Significant Differences Between Fasting and Postprandial (p=<05)

<table>
<thead>
<tr>
<th>Name</th>
<th>P</th>
<th>Fasting BD Avg</th>
<th>Postprandial BD Avg</th>
<th>%Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>0.0349</td>
<td>187.2</td>
<td>169.8</td>
<td>-9.294871795</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.0412</td>
<td>19.15</td>
<td>18</td>
<td>-6.005221932</td>
</tr>
<tr>
<td>Methionine-Sulfoxide</td>
<td>0.0241</td>
<td>0.5639</td>
<td>1.096</td>
<td>94.36070225</td>
</tr>
<tr>
<td>Acetyl-ornithine</td>
<td>0.0075</td>
<td>0.5471</td>
<td>0.6555</td>
<td>19.81356242</td>
</tr>
<tr>
<td>Citrulline</td>
<td>0.0019</td>
<td>19.86</td>
<td>14.44</td>
<td>-27.29103726</td>
</tr>
<tr>
<td>Total dimethylarginine</td>
<td>0.0349</td>
<td>0.8505</td>
<td>0.7711</td>
<td>-9.335684891</td>
</tr>
<tr>
<td>Creatine</td>
<td>0.0244</td>
<td>16.09</td>
<td>25.83</td>
<td>60.53449347</td>
</tr>
<tr>
<td>Betaine</td>
<td>0.0213</td>
<td>26.2</td>
<td>30.63</td>
<td>16.90839695</td>
</tr>
<tr>
<td>Methylhistidine</td>
<td>0.0161</td>
<td>5.959</td>
<td>4.906</td>
<td>-17.67075013</td>
</tr>
<tr>
<td>Alpha-Ketoglutaric acid</td>
<td>0.0372</td>
<td>11.2</td>
<td>16.41</td>
<td>46.51785714</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.0089</td>
<td>85.28</td>
<td>119.2</td>
<td>39.77485929</td>
</tr>
<tr>
<td>Pyruvic acid</td>
<td>0.0323</td>
<td>103.9</td>
<td>156.1</td>
<td>50.24061598</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.0102</td>
<td>5269</td>
<td>6962</td>
<td>32.13133422</td>
</tr>
<tr>
<td>LYSOC14:0</td>
<td>0.0224</td>
<td>5.007</td>
<td>6.076</td>
<td>21.35010985</td>
</tr>
<tr>
<td>LYSOC16:1</td>
<td>0.0287</td>
<td>3.591</td>
<td>4.293</td>
<td>19.54887218</td>
</tr>
<tr>
<td>LYSOC16:0</td>
<td>0.0333</td>
<td>123.3</td>
<td>140.6</td>
<td>14.03081914</td>
</tr>
<tr>
<td>LYSOC18:2</td>
<td>0.0108</td>
<td>19.33</td>
<td>26.79</td>
<td>38.59286084</td>
</tr>
<tr>
<td>LYSOC18:0</td>
<td>0.0308</td>
<td>33.77</td>
<td>37.45</td>
<td>10.89724608</td>
</tr>
<tr>
<td>LYSOC20:4</td>
<td>0.0184</td>
<td>3.203</td>
<td>3.639</td>
<td>13.61223853</td>
</tr>
<tr>
<td>LYSOC26:1</td>
<td>0.0265</td>
<td>0.1278</td>
<td>0.1564</td>
<td>22.37871674</td>
</tr>
<tr>
<td>20:25M</td>
<td>0.0375</td>
<td>0.8761</td>
<td>0.9713</td>
<td>10.86633946</td>
</tr>
<tr>
<td>PC36:6AA</td>
<td>0.0244</td>
<td>0.8719</td>
<td>1.009</td>
<td>15.72428031</td>
</tr>
<tr>
<td>PC36:0AA</td>
<td>0.0096</td>
<td>6.4</td>
<td>6.909</td>
<td>7.953125</td>
</tr>
<tr>
<td>PC38:6AA</td>
<td>0.0274</td>
<td>74.72</td>
<td>81.5</td>
<td>9.073875803</td>
</tr>
<tr>
<td>PC40:6AA</td>
<td>0.047</td>
<td>21.11</td>
<td>22.51</td>
<td>6.631927996</td>
</tr>
<tr>
<td>C3</td>
<td>0.0083</td>
<td>0.4221</td>
<td>0.4925</td>
<td>16.6785122</td>
</tr>
<tr>
<td>C10:1</td>
<td>0.0225</td>
<td>0.3582</td>
<td>0.2218</td>
<td>-38.07928532</td>
</tr>
<tr>
<td>C18:2</td>
<td>0.0232</td>
<td>0.08714</td>
<td>0.06788</td>
<td>-22.10236401</td>
</tr>
<tr>
<td>C18:1</td>
<td>0.0408</td>
<td>0.1123</td>
<td>0.08021</td>
<td>-28.57524488</td>
</tr>
<tr>
<td>C18</td>
<td>0.0008</td>
<td>0.04598</td>
<td>0.04088</td>
<td>-11.09177903</td>
</tr>
</tbody>
</table>
DISCUSSION

Changes in Blood Glucose Between Trials

When compared to prolonged sitting, regular activity breaks appeared not to lower the postprandial glucose concentration in the blood but actually increase it, although the findings were non-significant. The Sit-Ex IAUC was 17.7mmol/L/min larger than the SIT IAUC (d=.22, 95% CI -0.915 - 1.356, p=.63). This result does not match up with the findings in current literature as other studies looked at similar interventions to this study and showed a reduction in glucose concentrations when breaking up sedentary time frequently (Dunstan et al., 2012). There are a variety of possibilities why there was not a significant difference in these measurements when comparing the trials. For instance, one participant’s fasting blood glucose was 2.0 mmol/L high during the SIT condition in the fast SIT-EX measurements. When the lower fasting blood glucose was used for the for the IAUC calculations. There was a positive effect towards the exercise but was not significant (p=.72)

Postprandial glycemia is based on how quickly a carbohydrate is digested and absorbed and how quickly glucose is removed from the circulation (Falck, Davis & Liu-Ambrose, 2016). Carbohydrate is the primary determinant of blood glucose levels, with the amount of carbohydrate being the primary contributor. The type of carbohydrate and other characteristics of the food, such as its physical form, ripeness, type of starch, style of preparation (cooking method, temperature, time, and amount of moisture used), and
the degree of processing all influence the PPG response (Falck, Davis & Liu-Ambrose, 2016).

The shape of the curve was not as anticipated; it was expected that the blood glucose would spike initially as it did following the first meal, but would remain low and not spike again until after the second meal was consumed. This second spike in blood glucose before two hours was not anticipated initially, but upon further review of the literature, the glucose spike response was similar to another study that looked at the glucose response to a meal with similar macronutrient values. (Akins et al., 2019) (Appendix L). There was a rise of plasma blood glucose following the meal 2 hours after the initial high carb, high-fat meal.

Stress may have caused a factor in participants blood glucose as research has shown that stress or anxiety can release glucagon and epinephrine (adrenaline) levels rise and increase glucose being released from the liver (Marcovecchio & Chiarelli, 2012). At the same time, growth hormone and cortisol levels rise, this causes body tissues (muscle and fat) to be less sensitive to insulin. Other potential stress factors could have included participants being anxious around researchers and all but two students going through midterm exams and studying during their sedentary time. There was no direct measurement of HR during or after exercise bout although average heart rate differences were non-significant between trials. The age and fitness levels of the participants may have affected the results as well. Research speculated that increasing age is associated with attenuated catecholamine responses and lower carbohydrate oxidation during activity (Yardley, Brockman & Bracken, 2018). Because the participants in this study
were younger (mean age 24.8) and had functional mobility in their arms throughout the trial, and small movements could have been sufficient enough to increase glucose uptake.

**Breaking up Sedentary Behaviour Time and Cardiovascular Measurements**

**Heart Rate**

There was no significant change when comparing the SIT-EX trial to the SIT trial. (p>.05). There was on average 2.45 bpm more during the SIT-EX trail compared to the SIT trail. Unfortunately, no subjective measurements were used to measure the heart rate following the exercise bouts. Increased heart rate could have been from anticipating exercise as well.

**Blood Pressure**

After analyzing, the recorded measurements from the trials suggested there were no significant differences between both the SBP (p=.4202) and DBP (p=.2985) when comparing the two conditions. These results contrast results from a study completed in 2014. The results from that study showed a significant difference when comparing both light and moderate physical activity (walking for 2 minutes every 20 minutes) to uninterrupted sedentary time. Interrupting sitting time with either light and moderate physical activity bouts of walking significantly lowered SBP by 2-3 mmHg and DBP by two mmHg, relative to continuous sitting (Larsen et al., 2014). One of the reasons there was a difference in results could be due to the nature that the participants had to lift their legs when we had to move our participants for finger pricks every half hour and although instructed not to move around, were able to move their legs to get comfortable. This
movement could affect blood pressure as an earlier study suggested three hours of sitting resulted in impaired superficial femoral artery flow-mediated dilation but not biracial artery flow-mediated dilation (Thosar, Bielko, Wiggins & Wallace, 2014). Although 3 hours of sitting did not reduce brachial artery flow-mediated dilation; it impaired shear patterns in the BA (Thosar, Bielko, Wiggins & Wallace, 2014). During this study, participants did not move their legs throughout the trial while they could move their legs in this study. The ability to engage in small leg movements by participants has the potential to change results. Furthermore, interpretation of a single time-of-day BP must be in a behavioural context that considers additional factors, such as dietary and fluid intake, physical activity, emotions, stress, and drugs (including caffeine and nicotine) (Dempsey, Larsen, Dunstan, Owen & Kingwell, 2018). Although dietary and fluid intake was controlled and participants reported not being on any prescription drugs through the trial, the level of emotion or stress may have been a more significant effect then on the sedentary time.

**Cognitive Test Suggests No Effect in Response to Exercise Bouts**

The trail making test is a hard copy test that was designed to be given to participants once but to decrease on test-retest bias, another model of the trail making test was created. It was a mirrored image of the original TMT that was performed during the first trials which have been shown for sequential testing of executive functioning (Wagner, Helmreich, Dahmen, Lieb & Tadic, 2011)

There were no significant differences between the TMT part A (p=.72), and part B (p=.090), N-back test (p=.76), Stroop test (p=.62), and Flanker test (p=.60) between the SIT and SIT-EX conditions. The duration of time spent sedentary may have been too
short to look for a difference in cognitive function. The possible benefits of associated with the light-intensity activity for the brain does not manifest in the acute setting, but rather over a more extended period following protection from repeated glucose excursions (Wheeler et al., 2017).

A study from 2017 concluded that compared to uninterrupted sitting, short bouts of standing or light-intensity cycling and walking might improve acute cognitive performance (Mullane, Buman, Zeigler, Crespo & Gaesser, 2017). These short bouts of activity where at the same intensity although the duration of sedentary breaks was longer. Furthermore, a review in 2018 suggested there was no short-term and medium-term interventions that reported a significant improvement in cognitive functioning when measures are taken to decrease sedentary behaviour in the workplace (Magnon, Vallet & Auxiette, 2018). Furthermore, the state of arousal may have affected the cognitive scores, and the time interval between exercise onset and cognitive test performance revealed that participants' performance declined during the initial 10 min of exercise and subsequent 10 min interval (Lambourne & Tomporowski, 2010).

The Difference Between SIT/SIT-EX as Well as Morning/Postprandial Blood Draws

The TMIC Prime Metabolomics Profiling Assay identifies 139 separate metabolites in a serum sample. Of the 16 samples tested (one SIT fasted, SIT postprandial, SIT-EX fasted, and SIT-EX postprandial sample per participant). Forty-three metabolites had a significant difference (p=<.05). Thirty-seven metabolites showed a significant difference between the fasted and postprandial blood draws. One metabolite was significantly different between the SIT and SIT-EX conditions and five metabolites
were significantly different because of interaction between the two factors of the previous systematic reviews published looking at metabolites as possible biomarkers for insulin resistance and Diabetes. Valine was the only significantly changed throughout the trial that was among the metabolites linked to diabetes and insulin resistance in previous reviews, and the effect was not due to the exercise but to the meal itself. This leads to the conclusion that breaking up sedentary behaviour during an acute bout of sedentary behaviour does not have a significant effect on metabolites. When comparing the fasted to postprandial samples, thirty-seven significantly affected metabolites may lead to the conclusion that there is a difference between viewing metabolites fasted and during a fed state. No distinction can be concluded that the trials themselves affected as there were no baseline values to compare too.
SUMMARY

A recent review (Saunders et al., 2018) has shown there is a positive effect on glucose response when breaking up sedentary behaviour with short bouts of activity (Appendix M). This is beneficial as from a public health viewpoint, breaking up sitting time with light intensity bouts can be incorporated into a variety of different everyday tasks, including the workplace and domestic environments. Moreover, the exercise bouts during the SIT-EX were designed to be done with little coaching and in a home or work environment. Furthermore, although our findings suggested an insignificant change in blood glucose, regular light activity breaks have a variety of health benefits. Throughout the analysis of the results, there is key information that can be taken away from this study, although there were no significant findings these results allow future researchers to assess the current protocol used for this study and make changes to further develop a protocol to accurately test glucose, cognitive, heart rate, blood pressure and metabolites.
LIMITATIONS

This study had several limitations. Only 6 participants completed both trials and 4 participants were able to have blood samples analyzed. BP and HR were measured every half hour as a single measurement (rather than serial measurements). Participants pre-trial meals the night before were not regulated and were self-reported. When performing the second trial, participants were asked to do similar tasks during their first trial, but these were self-reported from participants as well. Exercise intensity was not measured objectively. The amount and/or quality of sleep was not assessed. Participants also did not have a quiet period before starting the sedentary time. Although they were completed in the final half hour of the trial cognitive tests did not begin at an exact time following the 2.5hr measurements. Finally, scripts were not used for cognitive tests and exercise intensity was not regulated throughout SIT-EX conditions.
FUTURE RESEARCH

Future studies should involve a larger number of participants, as well as having quiet time before starting the trial were participants are sedentary. Participants should come in and sit down for an extended period before first blood draws and meal as previously done in studies (Dempsey et al., 2016). Cognitive tests should be done to standardized instructions and practice of each cognitive test should be performed before the first trial. When looking at the blood glucose curves, a high fat high-carb meal has a less researched glucose response curve when compared to an OGTT. A high carb, low-fat meal may show greater effects in response to the exercise bouts. Currently, there is another pilot study at UPEI with the same exercise bouts, but with a high carb low-fat protein meal. Although there has been a small number of participants, the glucose curve moved in a more predictable pattern, and there appears to be a difference when comparing the exercise and sedentary trials (Appendix N).
References


APPENDICES

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List of Metabolites Relative Risk for Type 2 Diabetes (Guasch-Ferré et al., 2016)
## APPENDIX B
Red/Grey Top Serum Preparation Protocol

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Label Tube</td>
<td></td>
</tr>
<tr>
<td>Invert 5-6 time</td>
<td></td>
</tr>
<tr>
<td>Leave Upright 30-45 minutes (no longer than 60 min) (RT)</td>
<td></td>
</tr>
<tr>
<td>Centrifuge for 15 minutes at 1300g at -4*</td>
<td></td>
</tr>
<tr>
<td>Aspirate serum at RT into falcon tube. (15 ml)</td>
<td></td>
</tr>
<tr>
<td>(new Pipette tip each time.)</td>
<td></td>
</tr>
<tr>
<td>Centrifuge for 15 minutes at 1300g at -4*</td>
<td></td>
</tr>
<tr>
<td>Inspect serum for turbidity.</td>
<td></td>
</tr>
<tr>
<td>Aliquot into 1.5 ml cryovials</td>
<td></td>
</tr>
<tr>
<td>Store in -80 within 1-2 hours</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX C

Cows ice-cream nutritional value (Meal 1)

<table>
<thead>
<tr>
<th>Nutrition Facts</th>
<th>% Daily Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories 320</td>
<td>24 %</td>
</tr>
<tr>
<td>Fat 18 g</td>
<td>24 %</td>
</tr>
<tr>
<td>Saturated 11 g</td>
<td>55 %</td>
</tr>
<tr>
<td>+ Trans 0.5 g</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate 29 g</td>
<td></td>
</tr>
<tr>
<td>Fiber 3 g</td>
<td>11 %</td>
</tr>
<tr>
<td>Sugars 26 g</td>
<td>26 %</td>
</tr>
<tr>
<td>Protein 5 g</td>
<td></td>
</tr>
<tr>
<td>Cholesterol 80 mg</td>
<td>27 %</td>
</tr>
<tr>
<td>Sodium 70 mg</td>
<td>3 %</td>
</tr>
<tr>
<td>Potassium 0 mg</td>
<td>0 %</td>
</tr>
<tr>
<td>Calcium 150 mg</td>
<td>12 %</td>
</tr>
<tr>
<td>Iron 1.75 mg</td>
<td>10 %</td>
</tr>
</tbody>
</table>

*5% or less is a little 15% or more is a lot

**Ingredients:** Milk ingredients, sugar, eggs, cocoa, chocolate liquor, salt, pure vanilla (water, alcohol, sugar, and vanilla bean extractives)

**Contains:** Milk, Egg
APPENDIX D
Nutritional Information for Cosmic Brownie and Orange Juice (Meal 2)
3 Minute SIT-EX Exercise Bout

Thrusters 30s

March In Place 30s

Semi Squat
Shoulder Extension 30s

March In Pace 30s

Kettle Bell Swing 30s

March In Pace 30s
APPENDIX F

Individual IAUC, Individual Comparison

Positive Values = Improvement with Exercise Bout
Negative Values = Decrease with Exercise Bouts

SIT Minus SITEX
APPENDIX G
Trail Making Test, Part A, Individual Comparison

Positive Values = Improvement with Exercise Bout
Negative Values=Decrease with Exercise Bouts
APPENDIX H
Trail Making Test, Part B, Individual Comparison

Positive Values = Improvement with Exercise Bout
Negative Values = Decrease with Exercise Bouts
APPENDIX I

Stroop Test Results, Individual Comparison

Positive Values = Improvement with Exercise Bout
Negative Values = Decrease with Exercise Bouts
APPENDIX J

Flanker Test Results, Individual Comparison

Positive Values = Improvement with Exercise Bout
Negative Values = Decrease with Exercise Bouts
APPENDIX K

N-BackTest Results, Individual Comparison

Positive Values = Improvement with Exercise Bout
Negative Values = Decrease with Exercise Bouts
APPENDIX L

Glucose Curve From Recent Study.
(Akins et al., 2019)
APPENDIX M

Review Looking At Effects Of Short Exercise Bouts On Post Prandial Glucose Response (Saunder et al., 2018)

![Figure 3](image.png)

Effect (Cohen’s d) of regular activity breaks (<10 min in duration) compared to prolonged sitting on postprandial glucose responses. Studies are sorted by carbohydrate content of the test meal. The diamond indicates the summary estimate with associated 95% confidence interval.
APPENDIX N
Glucose Response to Exercise Bouts following a High Carb, Low Fat Meal
PERMISSION TO USE HONOURS PAPER

Title of paper: INVESTIGATION OF THE EFFECTS OF SEDENTARY BEHAVIOUR AND MODERATE EXERCISE ON GLUCOSE TOLERANCE AND INSULIN SENSITIVITY.
Name of Author: Marcellus Campbell
Department: Human Applied Sciences
Degree: Kinesiology
Year: 2019
Name of Supervisor(s): Dr.Adam Johnston & Dr.Travis Saunders

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