CAN SEDENTARY TIME ATTENUATE THE ACUTE EFFECT OF EXERCISE ON INSULIN ACTION?

By

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A thesis submitted to the School of Kinesiology and Health Studies

In conformity with the requirements for

the degree of Master of Science

Queen’s University

Kingston, Ontario, Canada

(August 19, 2014)

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ABSTRACT

Impaired insulin action predicts the development of type 2 diabetes. Evidence is growing that sedentary time (SED) is a risk factor for morbidity and mortality, independent of moderate to vigorous physical activity (MVPA) levels. While it is clear from large-scale epidemiological studies that SED is associated with impaired insulin action, higher levels of evidence from intervention studies are sorely lacking. The objective of the present investigation was to determine whether a pragmatic 1 hour increase in daily SED for 4 days could mask the acute effect of exercise on insulin action.

The study enrolled 15 sedentary, abdominally obese men and women. Participants completed 3 conditions, each of which was 4 days in length. A counterbalanced randomized crossover design was used, in which participants served as their own controls. In the baseline condition participants were instructed to maintain habitual levels of PA. In the exercise condition participants were asked to perform 1 hour of supervised moderate-to-vigorous exercise per day. In the exercise + sedentary condition participants completed 1 hour of moderate-to-vigorous exercise per day and were also asked to increase daily SED by 1 hour from baseline. PA and SED were assessed using accelerometry. Measurements of insulin action were obtained from an oral glucose tolerance test (OGTT) performed in the fasting state the morning after the 4 days of each condition.

Unexpectedly, SED in the exercise condition decreased from baseline (9:28±0:45 hr:min vs. 8:14±1:07 hr:min, p<0.001), whereas SED was maintained at baseline levels in the exercise + sedentary condition. The exercise and exercise + sedentary conditions were therefore renamed as ‘exercise – sedentary’ and ‘exercise’, respectively. Insulin action was improved following the exercise – sedentary condition in comparison to baseline (1601±657 pmol·L⁻¹·2h vs. 1181±419 pmol·L⁻¹·2h, p<0.01). An improvement in insulin action was also observed in the exercise condition by comparison to baseline (1601±657 pmol·L⁻¹·2h vs. 1362±456 pmol·L⁻¹·2h, p<0.05). The improvement in insulin action
following the exercise – sedentary condition in comparison to the exercise condition approached statistical significance (1362±456 pmol·L⁻¹·2h vs. 1181±419 pmol·L⁻¹·2h, p=0.058).

These findings provide preliminary evidence in support of the notion that SED, in addition to the adoption of PA, may be an important behaviour to target for management of insulin action.
CO-AUTHORSHIP

I was responsible for collecting and analyzing all accelerometry data. I collected the majority of the cardiorespiratory fitness and anthropometry data. I performed all statistical analyses on the data and wrote the manuscript. Dr. Robert Ross reviewed, edited, and co-wrote the manuscript.
THESIS CONTRIBUTIONS

The data obtained for my thesis is from an investigation conducted by myself and my supervisor, Dr. Robert Ross. Data was collected by myself and other graduate students. The study comprised of 15 participants.

Louise De Lannoy recruited the majority of participants, and met and communicated with interested participants. I provided all instructions to subjects for completing the study. These instructions include but are not limited to: how/when to wear the accelerometer, when to wear the pedometer and how to track daily steps, how to change daily sedentary time depending on the study condition, how to record diet the day before each OGTT. I collected and analyzed all accelerometry data and collected the majority of anthropometric and cardiorespiratory fitness data. The remainder of these tasks was completed by the other graduate students in the lab. These students are: John Clarke, Andrea Brennan, Sara Giovannetti, Alex Ricketts, Billy Bostad, Angela Fernandez, and Theresa Cowan. Myself and each of these students contributed to the exercise supervision in the study.

Tammy Scott-Zelt, with the help of Nicole Florent, was responsible for conducting all fasting blood draws and oral glucose tolerance tests.
ACKNOWLEDGMENTS

I would first like to thank my supervisor Bob for the considerable amount of time and energy you have invested in me over the last two years. You are an unbelievable writer and there is no doubt that my writing ability has improved after publishing a paper with you in the fall of 2013 and then having worked together on my thesis manuscript. My study presented many challenges, and it would not have been without your guidance that is was completed. You have provided me with a great deal of honest constructive criticism over the past couple years. You have also provided me with sincere and enthusiastic compliments for the successes that I have achieved during my time as a master’s student. I appreciate both of these equally. Moving forward in life, I hope to develop the same passion for my work that you have for yours.

I would then like to thank all the participants for their hard work. I would not have a manuscript if it wasn’t for each and everyone one of you. You have all been a pleasure to work with and I feel blessed to have worked with such friendly, enthusiastic people.

Over the last 2 years I have had the opportunity to work with many graduate students so I will thank them here in the order that I have met them. Trevor, I know we were never officially members of the Ross lab at the same time but I wanted to thank you nonetheless. You were an amazing TA in my 4th year and I enjoyed talking to you about the Sens. I remember when I came into your office in the summer of 2012 and was asking you about being a master’s student in Bob’s lab. We then went down to Bob’s office and I asked him if he would take me as a grad student, and that’s how this whole journey got started. Crazy that we will now be studying in Ireland together. Einat and Gifferd, I could always count on you two for a smile or a laugh. I hope you are both doing well in your current endeavours. Andrea and Sara, you are both some of the funniest, happiest, most generous people I have ever met. I have shared too many memorable moments with each of you over the last 2 years. I
probably could have finished my degree a lot sooner if you guys hadn’t been so much fun to be around. John, you have provided me with more assistance and insight than I could ever repay. You were always happy to help me when I needed it. I’m not sure our lab could have functioned without you over the last 2 years. Thank you for all your work. Angela, Theresa, Billy, and Alex, I couldn’t have asked for a better group of first years to finish my master’s with. You each helped me immensely with my thesis project, and made me excited to come into the lab each day. The four of you are all very talented and I expect big things to come from the Ross lab in the next year.

Tammy, I can’t thank you enough for the work you have done for my thesis project. My project simply could not have been completed without someone as dependable and reliable as you. I have learned a lot from you regarding the interpersonal skills required to conduct tests such as fasting blood draws and OGTTs on participants/patients. The work and the passion that you put into the past 2 Christmas parties were just as inspiring. I am lucky to have worked with you over the last 2 years. Thank you Nicole for your help in performing the blood tests in my study as well. It was great working with you this past year.

Louise, thank you so much for all your hard work in recruiting participants to my study. It wasn’t easy recruiting for this study but just about every time a potential participant came in to meet with you they always signed up! Participants have often commented on how friendly and easy to talk to you are. Thank you as well for your assistance in writing and editing the CDA grant submission. Your help has made the past year run as smoothly as I could have hoped.

Melinda, thank you for all your hard work over the last two years. I learned a lot from you about recruiting subjects (the good and the bad), and how to properly manage my study. Your support and assistance allowed my study to run smoothly. You have always been happy to help me when I
needed it and have more good stories to tell than a 2 year master’s can handle. Paula, you are an absolute computer genius. You helped me retrieve data for me for my independent study and taught me a lot about data management. Even when you’re having a bad day you always seem to be smiling and laughing harder than any of us. The lab is lucky to have you on their team.

To my Mom and Dad, thank you very much for your support and encouragement during my time as a master’s student. I am grateful for the genuine interest you have had in my thesis project.
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<th>Full Form</th>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BP</td>
<td>Blood pressure</td>
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<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
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<td>FFA</td>
<td>Free fatty acids</td>
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<td>GLUT-4</td>
<td>Glucose transporter 4</td>
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<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
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<tr>
<td>HOMA-IR</td>
<td>Homeostatic model assessment of insulin resistance</td>
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<tr>
<td>IMTG</td>
<td>Intramyocellular triglycerides</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>LPA</td>
<td>Light physical activity</td>
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<tr>
<td>MVPA</td>
<td>Moderate to vigorous physical activity</td>
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<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>PA</td>
<td>Physical activity</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>------------------------------------</td>
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<tr>
<td>SED</td>
<td>Sedentary time</td>
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<tr>
<td>SAT</td>
<td>Subcutaneous adipose tissue</td>
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<tr>
<td>TG</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>VAT</td>
<td>Visceral adipose tissue</td>
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<tr>
<td>WC</td>
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Chapter 1: General Introduction

Impaired insulin action, as measured by elevated insulin area under the curve (AUC) during an oral glucose tolerance test (OGTT), is associated with increased all-cause and cardiovascular mortality [1] and also predicts the risk of coronary heart disease (CHD) and stroke [2]. Sedentary time (SED) is emerging as a novel risk factor for morbidity and mortality [3-6], where it is associated with derangements in insulin action [3]. Indeed, in a large epidemiological study of 173 men and women Healy et al. demonstrated that SED predicted 2-hour glucose beyond exercise that meets consensus physical activity (PA) guidelines [3]. This suggests that, independent of moderate-vigorous physical activity (MVPA) levels, SED is unfavorably associated with blood glucose and that reducing SED should be a treatment target in the prevention of diabetes and cardiovascular disease. Subsequent studies have repeated this observation in a variety of populations [5, 7, 8]. These cross-sectional observations are insightful and have stimulated much research into the independent effects of SED time on health risk. In fact, this research has contributed to the development of preliminary public health guidelines for SED in Canada [9].

Strikingly, absent from the literature are intervention trials wherein SED is manipulated in order to determine its effect on insulin action. A singular exception to this is a short-term intervention conducted by Duvivier et al. (2013) which used a randomized cross-over design to examine the effects of manipulating daily SED and PA on insulin sensitivity and lipid profiles [10]. Here, the authors concluded that a daily bout of MVPA cannot compensate for the negative metabolic effects of excessive SED. This observation has profound clinical implications, suggesting that adopting exercise consistent with consensus guidelines is not associated with improvement in insulin sensitivity if SED is increased. Other studies have confirmed the
observation that MVPA does not predict metabolic health risk beyond SED [11, 12]. On the other hand, several large cross-sectional studies refute this observation, suggesting that MVPA explains risk independent of SED [13, 14]. These contradictory findings strongly suggest that further investigation is required to fully elucidate the unique effects of PA and SED on metabolic health.

The conclusion reached by Duvivier et al. (2013), while insightful, is weakened by significant limitations in study design. Most importantly, participants sat for 13-14 hours daily which is an unrealistic amount of SED to incur and thus significantly limits the external validity of the study. Furthermore, the subjects in the study were healthy, university students and thus do not represent a demographic with an elevated risk for chronic disease. Lastly, reference measures for both metabolic variables and PA and SED were not obtained at baseline and therefore the magnitude of the change that occurred in each of these variables could not be assessed.

To our knowledge there are no carefully designed intervention strategies that have rigorously manipulated SED and PA on insulin action in men and women with the high-risk form of obesity. Thus, questions with important practical and clinical implications remain unanswered: “Does an increase in SED negate the beneficial effects of exercise on insulin action?” and “Is the adoption of PA consistent with consensus guidelines associated with improvements in insulin action regardless of a pragmatic (~1 hour) increase in SED?” Answers to these questions are important and would lend empirical support to cross-sectional evidence linking SED with elevated health risk in adults.
Thus we performed a counterbalanced, randomized crossover design study to investigate the independent and combined effects of SED and exercise time on insulin action in abdominally obese men and women. We chose to study abdominally obese men and women because they are at substantially increased risk for insulin resistance and because it is estimated that 37% of Canadians between the ages of 30 and 65 are abdominally obese [15].
Chapter 2: Literature Review

2.1 Introduction

This review will address the current literature regarding sedentary time and its influence on insulin action. The review is divided into three distinct sections. First, section 2.2 will provide a review of our primary outcome, insulin action. In this chapter, we will describe and define insulin action, examine the ectopic fat model as a mechanism of insulin resistance, and summarize the effects of exercise on insulin action along with potential mechanisms. Second, section 2.3 will review literature relevant to the behaviour of interest, SED, and its influence on insulin action. This section will first briefly describe the prevalence and health implications of SED. Following this, we will discuss how SED and PA can be measured objectively using accelerometry. The remaining portion of the section will discuss the evidence, from both epidemiological and intervention studies, linking SED with impaired insulin action, and will conclude with a review of potential mechanisms. Third, section 2.4 will summarize the literature review.

2.2 Insulin resistance and insulin action

Insulin resistance refers to the inability of insulin to stimulate the disposal of glucose into skeletal muscle. Insulin resistance is both an antecedent [16] and a characteristic of type 2 diabetes [17], and precedes the development of cardiovascular disease (CVD) as well [18, 19]. Insulin sensitivity, the antithesis of insulin resistance, refers to the ability of insulin to stimulate glucose uptake into skeletal muscle. A criterion measure of insulin resistance can be obtained using the hyperinsulinemic-euglycemic clamp technique [20]. Conversely, more pragmatic measures of insulin resistance/sensitivity, such as the homeostatic model assessment of insulin
resistance (HOMA-IR) [21] and the Matsuda index [22], can be obtained from the oral glucose tolerance test (OGTT).

Insulin action, like insulin resistance/sensitivity, also refers to the ability of insulin to stimulate disposal of glucose into skeletal muscle. While all measures of insulin resistance would be considered measures of insulin action, the term insulin action also encompasses insulin-related variables which are not considered to be the traditional measures of insulin resistance, some of which are described above. Insulin area under the curve during a 2-hour OGTT, for example, is a measure of insulin function that is not considered a traditional measure of insulin resistance and is therefore considered to be a measure of insulin action. Elevated insulin area under the curve is associated with increased all-cause and cardiovascular mortality [1] and also predicts the risk of coronary heart disease (CHD) and stroke [2].

2.2.1 The ectopic fat model: a mechanism of insulin resistance

Much research has been conducted to determine the mechanism by which glucose disposal is impaired in individuals with insulin resistance. Research suggests that the impairment in insulin-stimulated glucose disposal results from an impairment in glucose transporter 4 (GLUT-4) translocation. What remains unclear, however, is how exactly GLUT-4 translocation is impaired. While the pathophysiology of skeletal muscle insulin resistance is not fully understood, the ectopic fat model is currently accepted by many as the principal mechanism in the development of insulin resistance. In this model, “overflow” of lipid from adipose tissues results in the accumulation of triglycerides (TG) at ectopic sites such as cardiac and skeletal muscle, the liver, and visceral adipose tissue (VAT). Deposition of fat at these ectopic sites is believed to be associated with the development of insulin resistance. While other models of insulin resistance exist such as that of chronic inflammation [23], we have chosen to focus on the
ectopic model in this review given its prominence in the literature and because we believe that our behaviour of interest, SED, may exert its effect on insulin action through this pathway.

Several studies have demonstrated that elevated levels of plasma free fatty acids (FFA), resulting from insulin-resistant adipose tissue and ectopic lipid accumulation, are implicated in the development of skeletal muscle insulin resistance. Insulin inhibits hormone sensitive lipase (HSL) in adipose tissue [24]. However, adipose tissue can become insulin-resistant [25], resulting in increased lipolysis and an increase in circulating lipid into the plasma. Subcutaneous adipose tissue (SAT) insulin resistance is believed to be associated with the development of skeletal muscle insulin resistance. Lipolysis of lipid from SAT may result in the accumulation of lipid in less desirable locations such as skeletal muscle, cardiac muscle, the liver, and VAT [26, 27]. Lipid located at these ectopic tissues are hypertrophied, resistant to the anti-lipolytic effects of insulin [28], and highly sensitive to the lipolytic effect of catecholamines[29]. Therefore in addition to the increase in circulating lipids from insulin resistant SAT, ectopically located lipids also contribute to an elevation in plasma FFA by increasing FFA secretion into the blood. VAT, in particular, is highly lipolytic [30, 31]. Visceral adipocytes that acquire excess fat become dysfunctional and have a reduced capacity to store lipid, resulting in an elevated secretion of FFA which are then transported to the liver via the portal vein and stored as TG [32]. There is a strong association between excess liver fat and metabolic risk factors that characterize the metabolic syndrome [33, 34]
2.2.2 Intramyocellular triglycerides, lipotoxic metabolites, and insulin resistance

The elevation in plasma FFA is believed to play a causal role in the development of insulin resistance. There is much evidence to suggest that the increases in plasma FFA lead to the accumulation of intramuscular triglycerides (IMTG) [35-37]. This increase in IMTG stores as a result of increased plasma FFA is accompanied by a reduction in peripheral insulin sensitivity [35-37]. This observation has prompted some to argue that the IMTG are causally related to the development of insulin resistance. However, given that IMTG are metabolically innocuous, and that endurance athletes are both highly insulin sensitive and have elevated IMTG, the causal relationship between IMTG and the development of insulin resistance has been challenged [37].

The elevated IMTG in endurance athletes is a favorable adaptation resulting from repeated bouts of IMTG depletion and repletion during and following exercise, respectively. Furthermore the accumulation of IMTG in endurance athletes is matched by increased fat oxidation. Conversely, in sedentary insulin resistant individuals, the accumulation of IMTG is a result of a metabolically inflexible myocyte that preferentially stores lipid, and oxidizes carbohydrate. Importantly, the reduced lipolysis of IMTG to FFA in the muscle is proposed to result in the formation of lipotoxic metabolites such as diacylglycerol (DAG) and ceramides.

One hypothesis is that these lipotoxic metabolites impair insulin signalling, and thus impair GLUT-4 translocation. In fact, emerging evidence reveals that it is the ceramides in particular, and not the DAG, that play a central role in the development of insulin resistance. Ceramides synthesis is initiated by the reaction of FFA with the amino acid serine (Figure 1). Ceramides are believed to impair insulin signalling by inhibiting Akt/PKB phosphorylation and activation [38, 39]. Elevated total myocellular DAG content concomitant with increased insulin sensitivity has been observed in normal weight endurance trained athletes vs obese sedentary
Figure 1. Enzymatic reactions controlling the synthesis and degradation of ceramides (taken from Summers [40]) (for permission see Appendix G)
individuals, suggesting that myocellular DAG content may not be associated with insulin resistance [41]. Conversely, ceramide content was higher in obese individuals [41]. Inhibition of ceramide synthesis has been shown to negate the antagonistic effect of saturated FFA on insulin signal transduction and furthermore, ceramide accumulation is associated with an augmentation in the inhibitory effects of saturated FFA on insulin signal transduction [42]. DAG, by contrast, did not have these same FFA effects on insulin signalling [42].

Ceramides can be glucosylated, phosphorylated, or deacylated to produce an array of other metabolites referred to as sphingolipids (Figure 1). Emerging evidence reveals that these sphingolipids may be responsible for impairments in insulin action that are independent of ceramide content. Glucosylceramides are a specific group of sphingolipids produced by the glucosylation of ceramides via the enzyme glucosylceramide synthase (GCS). Evidence in support of the role of glucosylceramides in impaired insulin action emanate from improvements in insulin action observed following GCS inhibition. In this manner, improvements in insulin action can be attributed to reductions in glucosylceramides and not ceramides. GCS can be selectively inhibited at the adipocyte by AMP-DNM, and thus unlike the improvements in insulin action which occur following inhibition of ceramide synthesis at the myocyte, improvements in insulin action following GCS inhibition would demonstrate that glucosylceramides exert their positive effects on insulin action at the adipocyte. As expected, GCS inhibition by AMP-DNM at the adipocyte in mice results in an array of benefits related to insulin action: improved insulin sensitivity in obese mice[43, 44], resolution of hepatic steatosis (i.e. fatty liver)[45, 46], and improved adipocyte morphology[47].
2.2.3 Effects of exercise on insulin action

There is irrefutable evidence that exercise, both acute and chronic, improves insulin-mediated glucose uptake independent of age, race, or gender [48-50]. It has been previously demonstrated that exercise improves insulin action by increasing GLUT-4 expression in skeletal muscle [51-53]. The exercise-induced increase in GLUT-4 expression may be mediated through increases in AMP-activated protein kinase (AMPK), calcium (Ca\(^{2+}\)), and phosphatidylinositol-3-kinase (PI3K) [54-57]. Increased GLUT-4 protein content reduces blood glucose and the risk for insulin resistance and diabetes [48].

Another mechanism by which exercise may influence insulin action is through the reduction of lipotoxic metabolites. As previously described, the ectopic fat model is regarded by many as a primary mechanism of insulin resistance and has prompted some to determine whether the lipotoxic intermediates residing in the myocyte can be reduced with exercise. Dube et al. have demonstrated that following 16 weeks of moderate intensity exercise, regardless of IMTG content, obese men and women have reduced ceramide content [58]. Bruce et al. observed similar results after 8 weeks of endurance training [59]. There are two pathways through which exercise may decrease the accumulation of lipotoxic metabolites. First, exercise training results in more frequent bouts of increased energy expenditure and thus increased IMTG turnover. Second, exercise training increases mitochondrial function [58] and thus improves the oxidative capacity of the muscle, enabling increased IMTG turnover.

2.3 Sedentary behaviour

As noted in the introduction in Chapter 1, SED is emerging as a behaviour that independently influences insulin action. Currently only 15% of adult Canadians regularly perform the minimum amount of PA to substantially reduce the risk of chronic diseases and
illness [60-62]. Conversely, many Canadian adults habitually accumulate large volumes of SED throughout the day; approximately 69% of a typical day is spent in SED [62]. Similar observations have been made in other countries around the globe, thus these trends are not unique to Canada [3, 63, 64]. Unfortunately, increased time spent in SED is associated with negative health outcomes such as increased risk of mortality and the metabolic risk factors that predict T2D and CVD [3, 5-7, 65, 66].

2.3.1 Measuring sedentary behaviour and physical activity objectively: Accelerometry

The accelerometer measures the acceleration of body movements, providing assessments of intensity, movement, and duration. Most accelerometers contain piezoelectric sensors to estimate the acceleration of movements. Moreover, advanced accelerometers measure acceleration in three dimensions [67]. The accelerometer translates the acceleration of movements into a voltage signal which is then converted from analog to digital numbers, producing the accelerometer’s output of counts [67]. Counts are normally averaged over an epoch of 1 minute, a reasonable duration given the limitations of short epochs (very low energy expenditure) and long epochs (misclassification of high or low intensities due to averaging).

Threshold values have been developed by Freedson et al. [68] to characterize the intensity of PA, and have enabled researchers to better understand the relationship between PA and health risks. These cut points have been used previously by our group [69] and others [5]. The cut points are as follows: sedentary behavior (SED, <100 counts per min (cpm)); total physical activity (TPA, >100 cpm), which can be further subdivided into light physical activity (LPA, 100-1951cpm), moderate physical activity (MPA, 1952-5724 cpm), and vigorous physical activity (VPA, >5725 cpm).
Although accelerometers provide valuable information, they also have limitations. Firstly, not all types of accelerometers measure PA using the same technique which may cause different evaluations of PA [70]. Secondly, some activities (such as cycling) cannot be accurately evaluated by accelerometer due to less movement in the hip, where the accelerometer is worn [70]. Thirdly, accelerometers are not highly sensitive for upper body movements [71]. Lastly, uniaxial accelerometers are sensitive for evaluating speed, but not elevation [72], however triaxial accelerometers may provide a solution for this problem, enabling a more accurate inclusion of activities such as running on an incline into measures of daily PA.

2.3.2 Negative effects of sedentary time on insulin action: evidence from cross-sectional/epidemiological research

Ekelund and colleagues [73] and Healy and associates [3, 5] were the first to assess the relationship between objectively measured (by accelerometry) SED and health outcomes, most namely insulin action. Whereas Ekelund et al. [73] reported that SED was not associated with HOMA-IR, Healy et al. [3, 5] observed a significant association of SED with 2-hour glucose. Importantly, in a large sample of 173 men and women, SED predicted 2-hour glucose after statistical control for MVPA [3], suggesting that reducing SED should be a treatment target in the management and prevention of type 2 diabetes. Subsequent to these initial studies, some have reported observations equivalent to Healy et al. [5, 7, 8], whereas others report findings in agreeance with Ekelund and colleagues [63, 74] (Table 1). Thus, there is inconsistency within the literature and at present, the relationship between SED and insulin action requires clarification.
Table 1. Independent associations between objectively measured sedentary behaviour and insulin action

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample</th>
<th>Measure of Insulin Action</th>
<th>Statistical Test and Result</th>
</tr>
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<tbody>
<tr>
<td>Ekelund et al. 2007</td>
<td>103 men, 155 women</td>
<td>Clustered metabolic risk (includes fasting insulin)</td>
<td>Linear regression, $\beta=0.07$, 95% CI $-0.01$ – $0.14$, p=0.1</td>
</tr>
<tr>
<td>Healy et al. 2007</td>
<td>60 men, 107 women</td>
<td>2-hour glucose</td>
<td>Linear regression, $\beta=0.29$, 95% CI $0.11$ – $0.48$, p=0.002</td>
</tr>
<tr>
<td>Balkau et al. 2008</td>
<td>346 men, 455 women</td>
<td>Glucose infusion rate during hyperinsulinemic-euglycemic clamp</td>
<td>P for trend, p&gt;0.05</td>
</tr>
<tr>
<td>Ekelund et al. 2009</td>
<td>81 men, 111 women</td>
<td>Fasting insulin, HOMA-IR</td>
<td>Linear regression, Fasting insulin: $\beta=-0.001$, 95% CI $0.0013$ – $0.00005$, p=0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HOMA-IR: $\beta=-0.001$, 95% CI $0.002$ – $0.0004$, p=0.21</td>
</tr>
<tr>
<td>Helmerhorst et al. 2009</td>
<td>166 men, 210 women</td>
<td>Log fasting insulin</td>
<td>Linear regression, $\beta=0.003$, 95% CI $0.0006$ – $0.006$, p=0.015</td>
</tr>
<tr>
<td>Healy et al. 2011</td>
<td>2378 men, 2379 women</td>
<td>Fasting insulin, HOMA-%B, HOMA-%S*</td>
<td>P for trend, p&lt;0.05</td>
</tr>
</tbody>
</table>

*HOMA-%B: Homeostatic model assessment – Beta cell; HOMA-%S: Homeostatic model assessment – Insulin Sensitivity
Although disparate findings exist regarding the effect of SED on insulin action, taken as a whole, the epidemiological studies indicate that SED, without accounting for MVPA, is deleteriously associated with insulin action. Similarly, there is unequivocal evidence that MVPA, without adjustment for SED, is favourably associated with insulin action. However, the large cross-sectional studies which have examined the independent effects of SED and MVPA on insulin action, after adjustment for one another, have produced inconsistent findings. Stated otherwise, whether or not insulin action and other markers of health risk are influenced by an interaction between SED and MVPA is currently not well understood.

As mentioned above, Healy et al. [3] demonstrated that SED predicts health risk, independent of MVPA. Similarly, others have demonstrated that SED is associated with metabolic risk after adjustment for MVPA [5, 11]. Conversely, Peterson et al. [75] recently demonstrated in a large representative sample of 5268 men and women that adults with the highest MVPA across tertiles of SED did not differ significantly with respect to cardiometabolic risk factors, or obesity and adiposity. Additionally, SED was not a predictor of a metabolically abnormal phenotype (elevated blood pressure, TG, fasting glucose, HOMA-IR, and low high density lipoprotein (HDL) cholesterol) after adjustment for MVPA. These results collectively suggest that SED, when adjusted for MPVA, is a poor predictor of health risk and that time spent in MVPA is a strong predictor of cardiometabolic health. Similarly, others have demonstrated in children and adolescents that MVPA predicts cardiometabolic health independent of SED, while SED, after adjustment for MVPA, is not associated with elevated health risk [13, 14]. Some studies have even suggested that MVPA, when adjusted for SED, is not associated with elevated health risk. For instance, Cooper et al. observed that MVPA is not associated with metabolic risk after accounting for SED [11], while Gennuso et al. demonstrated that meeting the PA
guidelines did not influence the relationship between SED and cardiometabolic risk [12]. Taken together, the results from each of these studies clearly illustrate that there is conflicting evidence regarding the interactive effect of SED and MVPA on insulin action and other measures of metabolic health, and that further research is required to determine how each variable affects health risk after controlling for the other.

2.3.3 Sedentary time and impaired insulin action: evidence from interventions

While it is clear that prolonged periods of physical inactivity (i.e. bed rest) produce marked impairments in insulin action [76-78], these situations are extreme and do not represent realistic amounts of SED that are typical of free-living humans. To overcome this limitation a recent study investigated the effects of a 2-week reduction in ambulatory activity on metabolic parameters in healthy young males[79, 80]. Participants were free-living and were instructed to reduce their daily steps to 1500 per day for 2 weeks, thereby providing a much more pragmatic model of sedentary behaviour than bed rest. At the end of the study period, there was an increase in plasma insulin and TG, and intra-abdominal fat mass along with a reduction of glucose uptake, cardiorespiratory fitness, and leg lean mass. Equivalent findings were reported by Stephen et al. [81] who observed a 39% reduction in insulin action after only one day of sitting (mean steps = 264) versus a minimal sitting condition (mean steps = 9914). These studies provide evidence of the negative effects of a reduction in daily activity (or increased SED) on insulin action that more closely resembles the lifestyle of free living humans and is not as extreme as bed rest. However, reducing daily steps to ≤1500 is still an extreme and unrealistic amount of SED given that Canadian men and women take on average 9500 and 8400 steps per day, respectively [62]. Furthermore, although these studies manipulated SED indirectly by reducing daily steps, the aforementioned studies did not measure SED objectively using
accelerometry. Therefore, these studies were unable to determine how a change in SED, the particular exposure which is associated with negative health outcomes, influenced insulin action.

To our knowledge, only one intervention trial has been conducted which has manipulated SED to determine its effect on insulin action. Recently, Duvivier et al. [10] utilized a randomized cross-over design trial to determine whether replacing 1 hour of SED with 1 hour of exercise could compensate for the deleterious effects of 14 hours of daily sitting time on insulin action. Seventeen (14 female, 3 male) healthy, university students completed 3 conditions, each 4 days in length. In the sitting regime, subjects were instructed to sit 14 hr/day, walk 1hr/day, stand 1hr/day, and sleep 8 hr/day. In the exercise regime, 1 hour of sitting was replaced with 1 hour of supervised, vigorous bicycling per day, and the rest of the day was spent similarly as the sitting condition. Additionally, there was a minimal intensity PA condition where subjects replaced 6 hours of sitting with 4 hours of light walking and 2 hours of standing. It was demonstrated that, despite substituting 1 hour of sitting with 1 hour of MVPA, there was no difference in insulin action as measured by insulin AUC between the sitting and exercise conditions (Figure 2). Given that exercise typically has a profound acute effect on measures of insulin action, this observation is both powerful and perplexing. These results suggest that a substantially elevated amount of SED is able to mask the benefits of exercise on insulin action, and that SED effects insulin action, independent of MVPA.
Figure 2. Average insulin AUC for the three conditions (sitting, exercise, minimal intensity PA) in the Duvivier study (taken from Duvivier et al. [10]) (for permission see Appendix H)
Although the study by Duvivier et al. provides great insight into the independent effect of SED on insulin action, there were two major limitations which limit the generalizability of its findings. Most importantly, participants sat for 13-14 hours daily which is an unrealistic amount of SED to incur given that the average daily SED of Canadian adults is approximately 10 hours [62], and thus significantly limits the external validity of the study. Furthermore, the subjects in the study were healthy, university students and thus do not represent a demographic with an elevated risk for chronic disease. In addition to the limitations related to poor generalizability, another limitation was that reference measures of metabolic variables or SED and PA were not obtained at baseline. Therefore whether an increase in daily sitting time is associated with deleterious metabolic effects, and the magnitude of the increase in SED that occurred in the sitting condition could not be assessed.

2.3.4 Sedentary behaviour and impaired insulin action: potential mechanisms

A small body of literature has examined the mechanisms by which SED may influence metabolic health. Available evidence suggests that the deleterious effects of SED on metabolic health may be mediated by changes in the activity of the enzyme lipoprotein lipase (LPL). Evidence from animal studies have demonstrated that LPL facilitates the entry of FFA into the myocyte and the adipocyte, and that low levels of LPL are associated with elevated levels of plasma TG and reduced levels of HDL cholesterol [82]. Bey and Hamilton [83] utilized the hind-limb unloading technique; whereby rodents were suspended by their tail to prevent ambulation of their hind limbs. After 12 hours of hind-limb unloading LPL activity decreased by 63% compared to control animals (animals allowed to ambulate freely). Along with the decrease in LPL activity, decreases in triglyceride-derived fatty acid uptake and HDL cholesterol concentration were observed. In healthy men and women, an 18% decrease in LPL activity
along with a decrease in HDL and an increase in both triglyceride and fasting insulin levels was documented following 20 days of bed rest without change in body weight [84]. Similarly, Hamburg et al. [76] observed a significant increase in total cholesterol, plasma TG, glucose, and a large (67%) reduction in insulin action after 5 days of complete bed rest (i.e. 23.5 hours per day) in healthy adults. Although it is clear from these extreme cases of sedentary behaviour that LPL activity is reduced following prolonged periods of SED, whether a pragmatic increase in SED (e.g. 1 hour) results in appreciable changes in LPL activity remains unknown. Therefore any changes in insulin action or metabolic health in general following a pragmatic increase in SED may not necessarily be due to the marked reductions in LPL activity following bed rest or hind-limb unloading.

While the link between reduced LPL activity and increased plasma TG and decreased HDL is evident it is unclear how a reduction in LPL activity would result in an impairment in insulin action specifically. Based on the notion that impaired insulin signalling in sedentary individuals results from an inflexible myocyte (one that oxidizes carbohydrate and stores lipid) it would be reasonable to conclude that the reduction in LPL activity and thus reduced rate of FFA entry into the myocyte would result in the preferential sparing of IMTG and oxidation of carbohydrate. The reduced oxidation of IMTG may result in the accumulation of lipotoxic metabolites such as ceramides or glucosylceramides which could then impair insulin signalling.

2.4 Summary

Impaired insulin action, as measured by elevated insulin AUC during an OGTT, is associated with increased all-cause and cardiovascular mortality [1] and also predicts the risk of CHD and stroke [2]. SED is emerging as a novel risk factor for morbidity and mortality [3-6], where it is associated with derangements in insulin action [3]. In fact, using objectively measured
PA and SED, Healy et al. demonstrated that SED predicted 2-hour glucose independent of time spent in MVPA [3]. Similar observations have been repeated in a variety of populations. Though these observations are insightful, higher levels of evidence emanating from intervention trials are required to confirm the deleterious impact of SED behaviour on insulin action. To our knowledge only one study has manipulated SED to determine its effect on insulin action. Here, Duvivier et al. employed a randomized crossover design to determine whether replacing 1 hour of SED with 1 hour of exercise can compensate for the negative metabolic effects of excessive sitting time [10]. The primary finding from this study was that substituting 1 hour of SED with 1 hour of exercise does not improve insulin action when SED is elevated (13-14 hours daily). This observation has significant clinical implications, suggesting that performing exercise consistent with the Canadian Physical Activity Guidelines does not improve insulin resistance if SED is elevated. While the conclusion reached by Duvivier et al. is insightful, there were significant limitations in study design which limit the clinical relevance of the study’s findings. Most importantly, participants sat for 13-14 hours per day which is not pragmatic and does not represent typical levels of SED observed in Canadians. Additionally, participants were healthy, university students and thus do not represent a demographic at risk for the development of type 2 diabetes and cardiovascular disease.
Chapter 3 – Can sedentary time attenuate the acute effect of exercise on insulin action?
3.1 Abstract

OBJECTIVES: To determine whether a 1 hour increase in daily sedentary time (SED) can negate the acute effects of exercise on insulin action.

RESEARCH DESIGN AND METHODS: We studied 15 abdominally obese men and women (49.7±10.7 years, waist circumference: 106.7±10.6 cm). Participants completed 3 conditions, each of which was 4 days in duration. A counterbalanced randomized crossover design was used, in which participants served as their own controls. In the baseline condition participants were instructed to maintain habitual levels of physical activity (PA). In the exercise condition participants were asked to perform 1 hour of supervised moderate-to-vigorous exercise at 65% of VO₂ peak each day. In the exercise + sedentary condition participants completed 1 hour of moderate-to-vigorous exercise per day and were also asked to increase daily SED by 1 hour from baseline. PA and SED were assessed using accelerometry. Measurements of insulin action were obtained from an oral glucose tolerance test (OGTT) performed in the fasting state the morning immediately after the 4 days of each condition.

RESULTS: Unexpectedly, SED in the exercise condition decreased from baseline (9:28±0:45hr:min vs. 8:14±1:07hr:min, p<0.001), whereas SED was maintained at baseline levels in the exercise + sedentary condition. The exercise and exercise + sedentary conditions were therefore renamed as ‘exercise – sedentary’ and ‘exercise’, respectively. Insulin action was improved following the exercise – sedentary condition in comparison to baseline (1601±657pmol·L⁻¹·2h vs. 1181±419pmol·L⁻¹·2h, p<0.01). An improvement in insulin action was also observed in the exercise condition by comparison to baseline (1601±657pmol·L⁻¹·2h vs. 1362±456pmol·L⁻¹·2h, p<0.05). The improvement in insulin action following the exercise –
sedentary condition in comparison to the exercise condition approached statistical significance but was not statistically significant (1362±456 pmol·L⁻¹·2h vs. 1181±419 pmol·L⁻¹·2h, p=0.058).

**CONCLUSION:** A 1 hour decrease in SED in combination with 1 hour of moderate-to-vigorous exercise may be associated with improvement in insulin action by comparison to 1 hour of exercise alone. These results extend the notion that in addition to performing PA consistent with the PA guidelines, reducing SED warrants consideration as a behavioral strategy designed to improve insulin action and hence, decrease risk of T2D and CVD.

**Key words:** sedentary, insulin action, physical activity, exercise
3.2 Introduction

Impaired insulin action, as measured by elevated insulin area under the curve (AUC) during an oral glucose tolerance test (OGTT), is associated with increased all-cause and cardiovascular mortality [1] and also predicts the risk of coronary heart disease (CHD) and stroke [2]. Sedentary time (SED) is emerging as a novel risk factor for morbidity and mortality [3-6], where it is associated with derangements in insulin action [3]. Indeed, in a large epidemiological study of 173 men and women Healy et al. demonstrated that SED predicted 2-hour glucose beyond exercise that meets consensus physical activity (PA) guidelines [3]. This suggests that, independent of moderate-vigorous physical activity (MVPA) levels, SED is unfavorably associated with blood glucose and that reducing SED should be a treatment target in the prevention of diabetes and cardiovascular disease. Subsequent studies have repeated this observation in a variety of populations [5, 7, 8]. These cross-sectional observations are insightful and have stimulated much research into the independent effects of SED on health risk. In fact, this research has contributed to the development of preliminary public health guidelines for SED in Canada [9].

Strikingly, absent from the literature are intervention trials wherein SED is manipulated in order to determine its effect on insulin action. A singular exception to this is a short-term intervention conducted by Duvivier et al. (2013) which used a randomized cross-over design to examine the effects of manipulating daily SED and PA on insulin sensitivity and lipid profiles [10]. Here, the authors concluded that a daily bout of MVPA cannot compensate for the negative metabolic effects of excessive SED. This observation has profound clinical implications, suggesting that adopting exercise consistent with consensus guidelines is not associated with improvement in insulin sensitivity if SED is increased. Other studies have confirmed the
observation that MVPA does not predict metabolic health risk beyond SED [11, 12]. On the other hand, several large cross-sectional studies refute this observation, suggesting that MVPA explains risk independent of SED [13, 14]. These contradictory findings strongly suggest that further investigation is required to fully elucidate the unique effects of PA and SED on metabolic health.

The conclusion reached by Duvivier et al. (2013), while insightful, is weakened by significant limitations in study design. Most importantly, participants sat for 13-14 hours daily which is an unrealistic amount of SED to incur and thus significantly limits the external validity of the study. Furthermore, the subjects in the study were healthy, university students and thus do not represent a demographic with an elevated risk for chronic disease. Lastly, reference measures for both metabolic variables and PA and SED were not obtained at baseline and therefore the magnitude of the change that occurred in each of these variables could not be assessed.

To our knowledge there are no carefully designed intervention strategies that have rigorously manipulated SED and PA time on insulin action in men and women with the high-risk form of obesity. Thus, questions with important practical and clinical implications remain unanswered: “Does an increase in SED negate the beneficial effects of exercise on insulin action?” and “Is the adoption of PA consistent with consensus guidelines associated with improvements in insulin action regardless of a pragmatic (~1 hour) increase in SED?” Answers to these questions are important and would lend empirical support to cross-sectional evidence linking SED with elevated health risk in adults.
Thus we performed a counterbalanced, randomized crossover design study to investigate the independent and combined effects of sedentary and exercise time on insulin action in abdominally obese men and women. We chose to study abdominally obese men and women because they are at substantially increased risk for insulin resistance and because it is estimated that 37% of Canadians between the ages of 30 and 65 are abdominally obese [15].

3.3 Methods

3.3.1 Participants

Participants were men and women aged 30 to 65 years who were inactive (less than 1 hour of self-reported planned PA per week), did not smoke, and had an elevated waist circumference (WC) (102cm in men and 88cm in women). The exclusion criteria included any physical impairment that would make PA difficult or unsafe according to the participant’s physician (history of myocardial infarction, stroke, angioplasty or bypass surgery in the past 6 months; unstable angina, ischemia and peripheral artery disease); if they had diabetes or were taking glucose-lowering medication, working night shifts, and an average daily SED greater than 12 hours. The study was approved by the Queen’s University Research Ethics Board. All participants gave written informed consent and successfully completed the Physical Activity Readiness Questionnaire-Plus (PAR-Q+) before participation in the study.

3.3.2 Study design

A counterbalanced, randomized crossover design was used, in which participants served as their own controls (Figure 1). Participants completed three conditions, each of which was 4 days in length:
**Condition** | **Intervention**
--- | ---
1. Baseline | Maintain habitual levels of PA and SED

Following two conditions performed in random order (see Figure 1)

2. Exercise | Participants perform 1 hour of moderate-to-vigorous exercise per day
3. Exercise + sedentary | Participants perform 1 hour of moderate-to-vigorous exercise per day and increase daily SED by 1 hour from baseline.

Prior to beginning the baseline condition cardiorespiratory fitness was measured using a maximal treadmill test (VO$_2$ peak test; Figure 1). All subsequent moderate-to-vigorous exercise was performed at 65% of VO$_2$ peak (see Section 3.3.6).

In the 4-day baseline condition, participants were asked to wear the accelerometer (see Section 3.3.3) for four days to obtain their baseline measure of PA and SED (Figure 1). Participants were asked to remove the accelerometer before getting into bed therefore the amount of time spent sleeping was measured as the time elapsed between removing the device at night and putting it on in the morning. PA was measured from Monday to Thursday so that data was collected only during the workweek as activity levels would likely differ on weekend days. Specifically, the four days of each condition began on a Monday and ended on a Thursday, with measurements of insulin action, insulin sensitivity, and plasma lipids performed in the fasted state on the Friday morning (Figure 1). Alternatively, since not all subjects could be tested on a Friday, some participants wore the accelerometer from Sunday to Wednesday, with blood work completed on a Thursday.
Figure 1: Study Timeline

- **Day 1 (Monday)**
  - VO2 test
  - 4-day baseline

- **Day 5 (Friday)**
  - Exercise + Sedentary

- **Day 8 (Monday)**
  - OGTT #1

- **Day 10 (Monday)**
  - 10-day washout period

- **Day 12 (Friday)**
  - Exercise

- **Day 22 (Monday)**
  - Exercise + Sedentary

- **Day 26 (Friday)**
  - OGTT #3
The purpose of the baseline condition was to: 1) measure habitual sedentary and PA levels for 4 days, and 2) obtain a baseline OGTT which reflects at least 4 days of measured baseline activity. Thus, the average sedentary and PA levels obtained from these 4 days of activity was used to determine the amount of SED and PA required in the subsequent exercise (condition 2) and exercise + sedentary (condition 3) conditions. Just prior to performing the baseline OGTT, habitual levels of SED and PA were confirmed from accelerometry data.

The order in which participant’s completed conditions 2 and 3 was randomized; participants completed the conditions in order 1-2-3 or 1-3-2. A computerized random number generator was used to randomize participants to one of the two treatment orders. In the exercise condition, participants were required to perform one hour of structured MVPA (exercise) under supervision. In the exercise + sedentary condition, participants were required to perform one hour of structured MVPA (exercise) and to increase SED by 1 hour. In each of these conditions participants were asked to sleep for the same amount of time as the average amount of sleep measured at baseline. Similar to the baseline condition, the required amounts of SED and PA were quantified from the accelerometry data just prior to performing the OGTT.

Between the exercise and exercise + sedentary conditions was a washout period of 10 days. Given that the acute effect of exercise on insulin action is diminished within 4 days of the last exercise session [85] it was assumed that 10 days would provide sufficient time for exercise-induced changes in insulin action to return to baseline levels.

3.3.3 Physical activity
PA was measured with the Actigraph GT3X accelerometer (Actigraph, Pensacola, Florida). Although this is a triaxial accelerometer, only the vertical axis was used for analysis. Accelerometers were worn during the baseline, exercise, and exercise + sedentary conditions.
Accelerometers were programmed to collect data in 1-minute epochs over a 4-day period and were worn on an elastic belt positioned over the right hip at all times except during water-based activities. Participants were instructed to remove the accelerometer when they began sleeping at night and to resume wear in the morning when they woke up.

To be included in the analysis, participants were required to wear the accelerometer for at least 10 hours each day during the 4-day monitoring period. Non-wear time (defined as extended period of consecutive zero counts >60 minutes) was excluded from the analysis.

The accelerometer cutpoints used to translate the ‘count’ value into an estimate of PA intensity were those developed by Freedson and colleagues [68]. SED was defined as counts per minute (cpm) <100, LPA as 100 to 1952cpm, and MVPA as ≥1952. Physical activity (LPA and MVPA) and SED accumulated during each day of monitoring was quantified as an average daily duration, in hours:minutes per day (hr:min/day). MVPA was further partitioned into exercise time (defined as average daily supervised exercise time on treadmill) and unstructured MVPA (defined as the difference between total MVPA and exercise time).

3.3.4 Self-monitoring of sedentary and physical activity levels

During the 4 days for each of the 3 conditions, participants wore pedometers to provide feedback using a surrogate measure of time spent engaging in PA and SED. This was required to overcome the limitation of the accelerometer wherein the participants are blind to any measure of PA or SED. During the baseline period, participants were asked to record their accumulated steps for each of the 4 days at two time points: 5pm and prior to sleep.

For the two exercise conditions, participants removed the pedometer while exercising on the treadmill so that the steps taken during baseline would be comparable to those achieved during the two exercise conditions and thus could be used to prescribe step goals for the exercise
conditions. With the assumption that a reduction in the number of steps taken during a given time period equates to an increase in SED of a similar amount of time, step reductions were prescribed in order to help participants meet the required amounts of SED in the two exercise conditions. In the exercise condition, participants were asked to reduce their steps from baseline by the average number of steps taken in an hour during waking hours. This reduction was calculated as the average amount of total steps divided by average total wake hours. For example if someone took an average of 8000 steps during baseline and was awake for on average of 16 hours, this participant would be asked to reduce steps by 500 (8000 steps/ 16 hours) to 7500 steps in the exercise condition. We prescribed this reduction in steps because we believed that the act of coming into the lab to exercise would displace an hour of SED, and therefore an hour increase in SED outside of exercise time would be required to maintain SED at baseline levels.

In the exercise + sedentary condition, participants were asked to reduce their steps from baseline by the average number of steps taken in 2 hours during waking hours. This reduction was calculated as the average amount of total steps divided by average total wake hours multiplied by 2. Using the same example as above, someone with 8000 steps and 16 hours of wake time at baseline would be asked to reduce steps by 1000 [(8000 steps/ 16 hours)x2] to 7000 steps in the exercise + sedentary condition. This reduction was prescribed in order to meet the requirement of a 1 hour increase in SED from baseline.

### 3.3.5 Anthropometry

Body mass and height were measured at baseline to the nearest 0.1 cm and 0.1 kg, respectively. These measures were used to calculate BMI (weight in kg/ height in m$^2$). WC was obtained at the level of the iliac crest. The iliac crest was located by palpating the upper right
hipbone and drawing a line where the uppermost border of the iliac crest was identified. Measuring tape was then positioned horizontally around the abdomen so that the inferior edge of the tape was at the level of the landmarked point. Measurement was taken at the end of a normal expiration.

3.3.6 Cardiorespiratory Fitness

Cardiorespiratory fitness measured as oxygen consumption per unit of time (VO$_2$ peak) was determined using a maximal treadmill test combined with standard open-circuit spirometry techniques (SensorMedics Corp, Yorba Linda, California). Participants began the test by walking at a self-selected speed (one that elicits a heart rate of about 120 bpm after completion of a 5-minute warm up) at level grade, increasing grade to 2% at the 3rd minute, and then further increasing the grade by 2% every 3 minutes thereafter. If the subject did not reach exhaustion after 3 minutes at the maximal incline of 14%, the speed was increased by 0.4 mph every 3 minutes until reaching volitional fatigue. Heart rate was measured continuously throughout the test using heart rate monitors (Polar USA, Stanford, CT) and was recorded on the VO$_2$peak Data Collection Sheet (Appendix B). VO$_2$max was attained when at least three of the four following criteria were achieved: VO$_2$max reached plateau with increase in work rate (i.e. a change in VO$_2$ of less than 0.05L/min over a 40 second period), respiratory exchange ratio (RER) was above 1.1, heart rate exceeded age predicted maximum heart rate minus 12 bpm, and/or participant estimation of exhaustion was ten on the Borg scale. Of the 15 participants, 11 attained at least 3 of the 4 criteria. For the 4 participants that did not attain 3 of the 4 criteria, 1 attained 2 of 4, 1 attained 1 of 4, 1 attained 0 of 4, and 1 was unable to complete the treadmill test due to injury.
3.3.7 Exercise regimen

In the two exercise conditions, participants walked/jogged on treadmills located within our research laboratory. All exercise sessions were supervised. Subjects were asked to walk at a heart rate (± 3 beats) that corresponded to 65% of their VO₂ peak (as determined from the maximal treadmill test) for 1 hour. Heart rate was monitored continuously throughout each exercise session using an automated (Polar) heart rate monitor. An exercise intensity of 65% of VO₂ peak and a duration of 1 hour were selected as it has been shown that this intensity and duration of exercise can improve insulin action following only 1 day of exercise. To help ensure that participants who met one or fewer of the criteria for achieving VO₂max or were unable to complete the treadmill test exercised at an intensity that approximated 65% of their VO₂ peak, exercise was prescribed at an intensity of 75% of age-predicted maximum heart rate (220 – age).

3.3.8 Dietary regimen

Participants were asked to not alter their diet throughout the study. For each day preceding an OGTT, participants recorded their dietary intake in a food journal (Appendix C) and were asked to make best efforts to eat exactly the same foods (and same quantity of food) the day prior to the OGTT in the subsequent two conditions. This was done to help ensure that diet did not influence glucose, insulin, lipid, and lipoprotein measurements and consequently, that we isolated the effects of exercise and SED on each of these outcomes.

3.3.9 Measurement of primary outcome – insulin action

The primary outcome - insulin action – was measured as insulin AUC and was determined using a 2-hour OGTT the morning after an overnight fast (12 hour fast). Blood samples were collected from the antecubital vein in the fasted state just prior to ingestion of 75g of Glucodex and subsequently at 30, 60, 90, and 120 minutes. Following centrifugation and
separation of the serum from whole blood, aliquots of serum were obtained and stored in a fridge between 4 and 6°C. Once blood samples for each participant being tested that morning were obtained, all blood samples were then delivered to KGH Core Lab for analysis.

Plasma glucose was determined using an oxygen rate method on the Synchron LXH Systems (Beckman Coulter, Inc., Brea, CA, USA). Serum insulin was determined using the Beckman Coulter UniCel DxI 800 Access Immunoassay System. Here, the chemiluminescent substrate Lumi-Phos 530 is added to the reaction vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of insulin in the sample. The reported variation for repeated measurements of the same sample using quality control samples within the Core Lab, or coefficient of variation (%CV), for glucose and insulin are 2% and 3%, respectively (Core Laboratory Services, Kingston, ON, Canada).

Insulin area under the curve was calculated as \((\text{fasting insulin}/2) + 30 \text{ min insulin} + 60\text{min insulin} + 90\text{min insulin} + (120 \text{ min insulin}/2)\). Other outcomes obtained from the OGTT include: fasting glucose, 2-hr glucose, glucose AUC, fasting insulin, homeostatic model of assessment of insulin resistance (HOMA-IR), and the Matsuda index. For calculation of HOMA-IR and the Matsuda index please see Appendix E.

**3.3.10 Measurement of secondary outcomes**

**Lipids and lipoproteins**

Blood samples were also obtained in the morning after a 12-hour overnight fast to determine fasting triglycerides (TG), total cholesterol, low density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol. These measurements were taken at the “0” time point of the OGTT, just prior to consumption of the Glucodex. Following centrifugation and separation of serum from whole blood, blood samples were kept in a fridge until delivery of
blood samples to KGH Core Lab for analysis. Serum total cholesterol, TG, LDL cholesterol and HDL cholesterol levels were determined using standard enzymatic methods on the Synchron LXH Systems (Beckman Coulter, Inc., Brea, CA, USA). The %CV for each of these variables is 3%.

**Blood pressure**

Blood pressure (BP) was measured using the automated device BP Tru Blood Pressure Monitor (BPTru Medical Devices, Coquitlam, BC, Canada) in the morning after an overnight fast. The device took 6 readings, each of which was 2 minutes in length. Systolic (SBP) and diastolic blood pressure (DBP) were calculated as the average of the last five blood pressure readings.

### 3.3.1 Statistical analysis

All statistical analyses were executed with SPSS (SPSS 22, Chicago, IL, USA). Descriptive data are expressed as means ± SD. Variables were tested for normality and homogeneity of variance. Repeated measures ANOVA was applied to test for significant differences between the three conditions for all primary and secondary outcomes. If the global test revealed that an outcome differed by condition, conditions were pairwise compared using a Least Significance Difference (LSD) test. As a secondary analysis, a two-way mixed design ANOVA (with independent measures on sex and repeated measures on study condition) was used to determine whether the effects of each condition on insulin action varied by sex. As another secondary analysis, to further explore whether SED was a determinant of insulin action and to determine whether a dose-response relationship exists between a reduction in SED and an improvement in insulin action, we performed two tests for linear trend. First, we divided our sample into tertiles (5 participants per tertile) based on the magnitude of the reduction in SED in
the exercise – sedentary condition as compared to baseline and examined whether a linear trend exists in the relationship between a reduction in SED and an improvement in insulin action. Second, we divided our sample into tertiles based on the magnitude of reduction in SED in the exercise – sedentary condition as compared to exercise and examined whether a linear trend exists in the relationship between a reduction in SED and an improvement in insulin action. Significance for all tests was set at $p \leq 0.05$. 
3.4 Results

Subject characteristics are shown in Table 1 and confirm that our sample represented a homogeneous group of sedentary, abdominally obese men and women. Average sedentary time for participants at baseline was 9:28±0:45hr:min while WC was 106.7±10.6cm.

Despite our intention to recruit inactive (less than 1 hour of planned physical activity per week) 3 of the 15 participants met the Canadian Physical Activity Guidelines of 150 minutes of bouted physical activity per week at baseline, as measured by accelerometry. Since similar responses to the exercise conditions were observed when the 12 inactive participants were analyzed separately as when all 15 participants were analyzed, the 3 active subjects were not excluded and remained included in all analyses.

The interaction between sex and study condition did not reach statistical significance, $F(2, 26) = 1.707, p=0.201$. Therefore all primary and secondary outcomes are shown collapsed across sex.

Despite our protocol that was designed to have participants increase SED by 1 hour in the exercise + sedentary condition and to maintain SED in the exercise condition, we were unsuccessful in achieving this goal. In fact, as indicated in Table 3, as opposed to increasing SED by 1 hour in the exercise + sedentary condition, and maintaining SED in the exercise condition, SED was maintained at baseline within the exercise + sedentary condition and decreased by 1 hour in the exercise condition. Therefore whereas the two conditions involving exercise were initially referred to as ‘exercise + sedentary’ and ‘exercise’, they will herein be referred to as ‘exercise’ and ‘exercise – sedentary’, respectively.
Adherence and compliance data is presented in Table 2. All participants attended each of the 4 exercise sessions in both exercise conditions. Exercise was performed at an intensity of 64.0±2.0% of VO\(_2\) peak and for a duration of 0:59±0:03hr:min in the exercise – sedentary condition, and at 64.0±1.0% of VO\(_2\) peak and for 1:00±0:01hr:min in the exercise condition.

The amount of time spent in each activity category is presented in Table 3. By comparison to baseline, a significant reduction in SED was observed within the exercise – sedentary condition (9:28±0:45 vs. 8:14±1:07hr:min, p<0.001). A significant reduction in SED was also observed between the exercise and exercise - sedentary conditions (9:26±1:06hr:min vs. 8:14±1:07hr:min, p<0.001). Significant increases in the total amount of MVPA performed were observed between baseline and both exercise conditions (p<0.001). Differences in LPA between conditions did not reach statistical significance (p=0.2) and sleep time (p=0.2) did not differ between any of the three conditions.

Results for the primary outcome, insulin AUC, are shown in Table 3. The repeated measures ANOVA test determined that insulin AUC differed by study condition (p<0.01). Pairwise comparisons revealed that in comparison to the baseline condition, insulin AUC was significantly reduced following the exercise – sedentary condition (1601±657pmol·L\(^{-1}\)·2h vs. 1181±419pmol·L\(^{-1}\)·2h , p<0.01) and the exercise condition (1601±657pmol·L\(^{-1}\)·2h vs. 1362±456pmol·L\(^{-1}\)·2h , p<0.05) (Figure 2). The reduction in insulin AUC following the exercise – sedentary condition in comparison to the exercise condition approached statistical significance (1362±456pmol·L\(^{-1}\)·2h vs. 1181±419pmol·L\(^{-1}\)·2h, p=0.058).

A secondary analysis was performed wherein the insulin AUC results for all subjects were divided into tertiles based on the reduction in SED in the exercise – sedentary condition in
comparison to baseline (reduction in insulin AUC tertile 1 = 699±499 pmol·L⁻¹·2h, tertile 2 = 281±281 pmol·L⁻¹·2h, tertile 3 = 281±375 pmol·L⁻¹·2h), a linear trend was not observed in the relationship between the reduction in SED and change in insulin AUC, $F(1, 12) = 3.114$, $p=0.1$ (Figure 3).

When subjects were divided into tertiles based on the reduction in SED in the exercise – sedentary condition in comparison to exercise (reduction in insulin AUC tertile 1 = 11±328 pmol·L⁻¹·2h, tertile 2 = 420±362 pmol·L⁻¹·2h, tertile 3 = 114±224 pmol·L⁻¹·2h), a linear trend was not observed in the relationship between the reduction in SED and change in insulin AUC, $F(1, 12) = 0.273$, $p=0.2$ (Figure 4).

For secondary outcomes, statistically significant changes between conditions were observed for the Matsuda index alone ($p<0.01$). Pairwise comparison revealed that the index improved significantly in the exercise – sedentary condition in comparison to baseline (6.9±3.1 vs. 5.2±2.7, $p<0.01$).
Table 1. Descriptive characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>15 (7M, 8F)</td>
<td></td>
</tr>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>49.7 ± 10.7</td>
<td>(30.0 - 64.0)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>93.0 ± 13.3</td>
<td>(73.7 - 116.9)</td>
</tr>
<tr>
<td>Body mass index (kg/m^2)</td>
<td>32.2 ± 3.1</td>
<td>(27.1 - 31.1)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>106.7 ± 10.6</td>
<td>(88.1 - 124.0)</td>
</tr>
<tr>
<td><strong>Metabolic Variables (mmol/L)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>5.4 ± 0.7</td>
<td>(4.5 - 6.7)</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>59.0 ± 34.5</td>
<td>(19.0 - 149.0)</td>
</tr>
<tr>
<td>2-hour glucose</td>
<td>7.3 ± 1.8</td>
<td>(4.0 - 9.8)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.1 ± 1.3</td>
<td>(0.6 - 5.3)</td>
</tr>
<tr>
<td>Matsuda index</td>
<td>5.2 ± 2.7</td>
<td>(2.2 - 10.6)</td>
</tr>
<tr>
<td>Glucose AUC (mmol·L⁻¹·2h)</td>
<td>32.2 ± 5.4</td>
<td>(22.5 - 40.3)</td>
</tr>
<tr>
<td>Insulin AUC (pmol·L⁻¹·2h)</td>
<td>1601 ± 657</td>
<td>(820 - 2926)</td>
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<tr>
<td>Total cholesterol</td>
<td>5.1 ± 1.4</td>
<td>(2.4 - 7.9)</td>
</tr>
<tr>
<td>TG</td>
<td>1.4 ± 0.9</td>
<td>(0.4 - 3.2)</td>
</tr>
<tr>
<td>HDL</td>
<td>1.3 ± 0.4</td>
<td>(0.8 - 2.0)</td>
</tr>
<tr>
<td>LDL</td>
<td>3.1 ± 1.2</td>
<td>(1.3 - 5.6)</td>
</tr>
<tr>
<td><strong>Blood Pressure (mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>118.1 ± 13.7</td>
<td>(99 - 143)</td>
</tr>
<tr>
<td>DBP</td>
<td>77.8 ± 8.8</td>
<td>(66 - 97)</td>
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<tr>
<td><strong>Cardiorespiratory Fitness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO₂ peak (L/min)</td>
<td>3.0 ± 0.8</td>
<td>(1.5 - 4.4)</td>
</tr>
<tr>
<td>VO₂ peak (mL/kg/min)</td>
<td>31.8 ± 6.5</td>
<td>(17.3 - 46.4)</td>
</tr>
<tr>
<td><strong>Physical Activity Variables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(hr:min/day)</td>
<td>9:28 ± 0:45</td>
<td>(7:35 - 10:36)</td>
</tr>
<tr>
<td>SED</td>
<td>4:09 ± 1:20</td>
<td>(2:15 - 6:50)</td>
</tr>
<tr>
<td>LPA</td>
<td>0:28 ± 0:28</td>
<td>(0:00 - 1:27)</td>
</tr>
<tr>
<td>MVPA(hours:min/day)</td>
<td>8:56 ± 1:27</td>
<td>(6:05 - 11:38)</td>
</tr>
<tr>
<td>Sleep(hours:min/day)</td>
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<td></td>
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Data presented as mean±SD (range).
Table 2. Exercise intervention adherence and compliance data

<table>
<thead>
<tr>
<th></th>
<th>Exercise – Sedentary</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adherence</strong></td>
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<td></td>
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<tr>
<td>Sessions prescribed</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Sessions attended</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Attendance (%)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Compliance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensity prescribed (% VO_2 peak)</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>Intensity performed (% VO_2 peak)</td>
<td>64.0 (2.0)</td>
<td>64.0 (1.0)</td>
</tr>
<tr>
<td>Exercise time prescribed (hr:min)</td>
<td>1:00</td>
<td>1:00</td>
</tr>
<tr>
<td>Exercise time performed (hr:min)</td>
<td>0:59 (0:03)</td>
<td>1:00 (0:01)</td>
</tr>
</tbody>
</table>

Data presented as mean (standard deviation)
**Table 3. Time spent in activity categories, and all primary and secondary outcomes**

<table>
<thead>
<tr>
<th>Physical Activity Variables (hr:min/day)</th>
<th>Baseline</th>
<th>Exercise - Sedentary</th>
<th>Exercise</th>
<th>p-value</th>
<th>B vs. E-S</th>
<th>B vs. E</th>
<th>E-S vs. E</th>
</tr>
</thead>
<tbody>
<tr>
<td>SED</td>
<td>9:28 (0:45)</td>
<td>8:14 (1:07)</td>
<td>9:26 (1:06)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.869</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LPA</td>
<td>4:09 (1:20)</td>
<td>4:28 (1:30)</td>
<td>3:57 (1:08)</td>
<td>0.196</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total MVPA</td>
<td>0:28 (0:28)</td>
<td>1:21 (0:25)</td>
<td>1:14 (0:15)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.142</td>
</tr>
<tr>
<td>Exercise</td>
<td>0:00</td>
<td>0:59 (0:03)</td>
<td>0:59 (0:00)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.530</td>
</tr>
<tr>
<td>Unstructured MVPA</td>
<td>0:28 (0:28)</td>
<td>0:26 (0:18)</td>
<td>0:16 (0:12)</td>
<td>0.032</td>
<td>0.515</td>
<td>0.052</td>
<td>0.014</td>
</tr>
<tr>
<td>Sleep</td>
<td>8:56 (1:27)</td>
<td>8:59 (1:11)</td>
<td>8:31 (1:35)</td>
<td>0.243</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Metabolic Variables (mmol/L)**

<table>
<thead>
<tr>
<th>Insulin AUC (pmol·L⁻¹·2h)</th>
<th>1601 (657)</th>
<th>1181 (419)</th>
<th>1362 (456)</th>
<th>0.001</th>
<th>0.001</th>
<th>0.037</th>
<th>0.058</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose</td>
<td>5.4 (0.7)</td>
<td>5.3 (0.6)</td>
<td>5.3 (0.6)</td>
<td>0.880</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fasting insulin</td>
<td>59.0 (34.5)</td>
<td>48.7 (34.6)</td>
<td>50.3 (40.4)</td>
<td>0.241</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-hr glucose</td>
<td>7.3 (1.8)</td>
<td>6.4 (1.6)</td>
<td>6.6 (1.3)</td>
<td>0.107</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.1 (1.3)</td>
<td>1.7 (1.3)</td>
<td>1.8 (1.5)</td>
<td>0.223</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matsuda index</td>
<td>5.2 (2.7)</td>
<td>6.9 (3.1)</td>
<td>5.9 (2.0)</td>
<td>0.006</td>
<td>0.005</td>
<td>0.125</td>
<td>0.076</td>
</tr>
<tr>
<td>Glucose AUC (mmol·L⁻¹·2h)</td>
<td>32.2 (5.4)</td>
<td>30.2 (5.1)</td>
<td>30.8 (4.5)</td>
<td>0.185</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.1 (1.4)</td>
<td>4.8 (1.3)</td>
<td>4.9 (1.3)</td>
<td>0.093</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>1.4 (0.9)</td>
<td>1.1 (0.5)</td>
<td>1.2 (0.5)</td>
<td>0.077</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>1.3 (0.4)</td>
<td>1.3 (0.4)</td>
<td>1.2 (0.4)</td>
<td>0.330</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>3.1 (1.2)</td>
<td>3.0 (1.0)</td>
<td>3.1 (1.0)</td>
<td>0.539</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Blood Pressure (mmHg)**

<table>
<thead>
<tr>
<th>SBP</th>
<th>118.1 (13.7)</th>
<th>113.8 (11.8)</th>
<th>118.7 (10.6)</th>
<th>0.056</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBP</td>
<td>77.8 (8.8)</td>
<td>75.6 (8.0)</td>
<td>77.7 (6.7)</td>
<td>0.209</td>
</tr>
</tbody>
</table>

Data presented as mean (SD) for the baseline (B), exercise – sedentary (E-S), and exercise (E) conditions. The ‘p-value’ represents the level of significance for the repeated measures ANOVA. The final three columns provide the statistical significance for pairwise comparisons using the Least Significant Differences test if the global test revealed that an outcome differed by study condition.
Figure 2. Insulin action in the baseline, exercise – sedentary, and exercise conditions

Data is presented as mean± standard deviation.

* Exercise - sedentary significantly lower than baseline

** Exercise significantly lower than baseline

p=0.001

p=0.037

p=0.058
Figure 3. Insulin AUC in the baseline and exercise – sedentary conditions, and the reduction in insulin AUC across tertiles of reduction in SED in the exercise – sedentary condition in comparison to baseline.

Data is presented as mean ± standard deviation.
Tertile 1: n=5, 2:07 ± 0:25 hr:min reduction in SED
Tertile 2: n=5, 1:07 ± 0:14 hr:min reduction in SED
Tertile 3: n=5, 0:28 ± 0:14 hr:min reduction in SED
Figure 4. Insulin AUC in the exercise and exercise – sedentary conditions, and the reduction in insulin AUC across tertiles of reduction in SED in the exercise – sedentary condition in comparison to exercise.

Data is presented as mean ± standard deviation.
Tertile 1: n=5, 2.04 ± 0.27 hr:min reduction in SED
Tertile 2: n=5, 1.16 ± 0.25 hr:min reduction in SED
Tertile 3: n=5, 0.17 ± 0.14 hr:min reduction in SED
3.5 Discussion

The principal observation of this study is that among sedentary abdominally obese adults, a 1 hour decrease in SED in combination with 1 hour of moderate-to-vigorous exercise was associated with a non-significant trend for improvement in insulin action by comparison to 1 hour of exercise alone. These preliminary findings add to a growing body of evidence suggesting that both SED and PA are distinct behaviors that should be targeted to decrease health risk. Our results reinforce the public health message that in addition to engaging in 150 minutes of MVPA per week, adults may also benefit from minimizing the time they spend being sedentary each day.

The aim of the present investigation was to explore the hypothesis that a 1 hour increase in SED would mask the acute effects of exercise on insulin action. However participants were unable to increase SED by 1 hour from baseline. Despite the fact that a 1 hour increase would have been a substantially lower increase in SED than was achieved by Duivivier et al. where participants sat for over 13 hours per day, the 1 hour increase was not putative. We suspect that the requirement to come to our facility to exercise on a daily basis required alteration in their daily lifestyle that reduced their overall SED. The fact that it was so difficult for participants to increase SED with the addition of 1 hour of exercise is both insightful and encouraging; suggesting that, by necessity, the performance of 1 hour of daily structured MVPA substantially reduces the opportunity to engage in sedentary behaviour. Therefore in addition to the benefits related to the performance of 1 hour of exercise in and of itself, it is likely that the reduced opportunity to be sedentary secondary to the addition of exercise also provides health benefit.

That the participants in the Duvivier study were able to increase SED so substantively may be explained by the study sample of university students whose lifestyle may have facilitated
compliance, which in this case required extended periods of sedentary behaviour. The large increase in SED achieved by Duvivier et al. also reminds us that our goal of increasing SED by 1 hour, although challenging, is not impossible. Nevertheless having a condition where SED decreased from baseline provided a novel opportunity to investigate the interaction between SED and MVPA, albeit with the interaction being examined in the opposite direction as initially intended. Therefore although we were unable to test our original hypothesis, the modified study conditions allowed us to answer two fundamental questions: 1) “Does exercise performed in combination with a reduction in SED improve insulin action to a greater extent than exercise alone?” and 2) “Does exercise improve insulin action regardless of whether SED is decreased by an hour or maintained at baseline levels?”

Consistent with our hypothesis that SED could attenuate the benefits of exercise on insulin action, we observed that exercise performed in combination with a reduction in SED improved insulin action to a greater extent than exercise alone, albeit not significantly from a statistical standpoint. Given that those who have never had a CHD event or a stroke have a 21% lower insulin AUC than those having a CHD event [2], it seems reasonable to conclude that the 15% reduction in insulin AUC in the exercise – sedentary condition by comparison to exercise alone represents a clinically meaningful difference. These results lend further support to the cross-sectional evidence linking SED to elevated health risk [3, 5, 7, 8, 11]. This observation is also consistent with the findings of Duvivier et al. who noted that a substantive increase in SED was able to completely mask the acute effects of 4 days of exercise on insulin action and plasma lipids [10]. What is particularly striking about our results is that, unlike the Duvivier study which had participants increase SED to levels which are not representative of typical free-living adults (approximately 13 hours per day), a pragmatic 1 hour difference in SED was associated
with a non-significant trend for an attenuation in the exercise-induced improvement in insulin action. Thus the implications of this finding are two-fold: 1) sedentary adults should limit SED and benefit may be achieved by reducing SED by only 1 hour; 2) upon incorporating structured MVPA into their daily lives, sedentary adults should be cautioned to limit the amount of time they sit while they are not engaging in exercise as increases in SED of as little as 1 hour may attenuate the benefits achieved through exercise.

Our results confirm that exercise, either in combination with a reduction in SED or on its own, is associated with improvement in insulin action. These results are not surprising given the plethora of evidence which has established exercise as a powerful means to improve insulin action [48-50]. To what extent SED can increase from habitual values and still permit exercise to improve insulin action cannot be determined from this study. Nonetheless our findings reveal that the adoption of PA consistent with the guidelines provides benefit, even if SED is maintained at baseline status throughout the period of time in which exercise is performed – but not increased.

Secondary analysis of our primary outcome, insulin AUC, revealed that a dose-response relationship does not exist between the magnitude of reduction in SED and the magnitude of improvement in insulin action following the exercise – sedentary condition as compared to baseline. Similarly, a dose-response was not observed between the magnitude of reduction in SED and the magnitude of improvement in insulin action following the exercise – sedentary condition as compared to exercise. Due to the small sample within each tertile and the large variation in insulin response it is difficult to conclude whether a dose-response relationship truly exists. It is unambiguous that the notion of interindividual variability in response to any behaviour was confirmed in this study. Clearly, regardless of SED or PA, the variation in insulin
response was tremendous. These results extend the notion of individual differences in response to physical activity as first demonstrated by Bouchard et al [86].

The lack of significant changes in secondary outcomes between all 3 conditions is not surprising given that our sample, despite being abdominally obese, presented with a cardiometabolic profile at baseline with values well below the criteria for diagnosis of the metabolic syndrome [87]. It is likely for this reason that exercise was unable to further improve secondary outcomes beyond baseline. The normal glucose values observed concomitant with elevated insulin during the OGTT at baseline may be indicative of an early compensatory phase of beta-cell dysfunction whereby insulin secretion increases, compensating for skeletal muscle insulin resistance in order to maintain normoglycemia [88]. Since beta-cell dysfunction often precedes the development of hyperglycemia [89, 90], it is possible that our sample is at risk for the future development of T2D despite normal glucose levels. Given the significant differences observed in insulin AUC, it is not surprising that the Matsuda index, the only secondary outcome which is calculated using post-load insulin measures from the OGTT, was the only secondary outcome to have differed by study condition. The improvements in insulin AUC and the Matsuda index and the lack of reduction in glucose values associated with exercise collectively reflect an improvement in insulin action, whereby a lower amount of insulin is required for the uptake of a given amount of glucose.

The principal limitation of the present investigation was undoubtedly the small study sample. Given the small sample size it is possible that we may have committed a type 2 error by concluding that insulin AUC did not differ between the exercise and exercise – sedentary conditions. In fact, based on the change we observed between the two exercise conditions and the standard deviation, a sample size of 30 would have been required to detect a difference in
insulin AUC. Nonetheless it is encouraging that the trend for an attenuation in the exercise-induced improvement in insulin action we observed was consistent with our a-priori hypothesis and current knowledge regarding the association between SED and impaired insulin action. With the addition of another 15 people and presumable lower standard deviation, we would expect to see a difference that is not only clinically meaningful but also unequivocally statistically significant.

Our sample of predominantly white, abdominally obese adults is rather homogeneous and thus the generalizability of our findings may be limited. However since over a third of Canadians adults possess this high risk form of obesity [15] our findings are applicable to a large segment of the population. The short duration (4 days) of each condition prevents inference of the chronic effects of SED on insulin action. Finally, our measure of insulin action although a clinically useful measure, is a surrogate measure of insulin action and therefore it is possible that different results would have been observed had we used the criterion hyperinsulinemic-euglycemic clamp technique.

In summary, these findings provide preliminary evidence in support of the notion that SED, in addition to the adoption of PA, may be an important behaviour to target for management of insulin action. We have also demonstrated that when incorporating 1 hour of exercise into their daily routine there is little time available for men and women to engage in sedentary behaviour, which may be an important benefit of engaging in structured PA.
Chapter 4 – General Discussion

4.1 Clinical implications

The main findings of the study lend support to the notion that, in addition to PA levels, SED should be considered as a treatment target in the prevention of type 2 diabetes and other related comorbidities. Therefore from a public health message perspective Canadians should be advised to not only meet the Canadian Physical Activity Guidelines, but to also limit SED. While there are currently no evidence-based SED guidelines for adults, the observation that exercise in combination with a 1 hour reduction in SED was associated with a trend for an improvement in insulin action by comparison to exercise alone suggests that small and manageable reductions in SED should be recommended for Canadians, especially for those with sedentary lifestyles.

To our knowledge this study is the first to demonstrate that a pragmatic increase in SED may be associated with an impairment in insulin action. Our findings extend the observations from the large cross-sectional studies and the intervention study conducted by Duvivier et al. which have provided evidence in favour of the relationship between elevated SED and impaired insulin action.

Although the primary public health message “exercise, and sit less” emerging from this study is straightforward, getting the public to actually perform these behaviours is a complex issue. The public has known for many years now that, simply stated, “exercise is good for you”. Yet despite this knowledge there remains a tremendous gap between the knowledge of the behaviour and the performance of the behaviour, as demonstrated by the mere 15% of Canadians who meet the Canadian Physical Activity Guidelines [62]. SED, on the other hand, has only
recently emerged as a distinct behaviour that predicts health risk. It is abundantly clear that a large portion of the population in today’s society is highly sedentary, and as technological advances continue to occur it is likely that opportunities to engage in sedentary pursuits will increase further.

What is interesting to me is whether “sit less” is easier to enforce in society than “exercise more”. For reasons that may be physical, psychological, and/or logistical, exercise represents a serious challenge for many people. Although reducing sitting time for a given amount of time may not provide the same benefit as exercise of equivalent duration, I believe that those following a recommendation to sit less would encounter far fewer barriers than they would with exercise, and therefore I predict more people would be able to meet this recommendation. It may be that a consequence of the public health meassage “sit less” is that people will be more active, certainly in LPA. This may be important as the greatest impact on events shown numerous times prospectively is going from being sedentary and inactive to doing PA which is below the guidelines [91]. I hope that in the near future, as the body of evidence linking SED and health risk continues to grow, that health care professionals will counsel patients to reduce their daily amount of sitting, either as a supplement or as an alternative to exercise.

4.2 Strengths and limitations

The objective measurement of PA and the randomized crossover design (i.e. having each participant complete each study condition) are the two most notable strengths of our study. Additionally, our study population was relatively homogeneous: all participants were sedentary, abnormally obese men and women recruited from Kingston, Ontario, Canada and surrounding
areas. Lastly, exercise intensity was determined from a maximal oxygen consumption test, the criterion measurement of cardiorespiratory fitness.

Despite the strengths of our study, several limitations should be noted.

First and foremost, with a sample size of 15 we cannot conclude with complete confidence that SED was truly masking any acute effects of exercise on insulin action. With such a small sample size and the large variability observed in our primary outcome, insulin AUC, it is difficult to determine conclusively whether any differences or non-differences represent biological truths. In particular, the lack of difference in insulin AUC we observed between the exercise and exercise – sedentary conditions at p=0.058, although clinically meaningful, may represent a type 2 error. Furthermore, as we were only able to separate participants into tertiles of five by change in SED, we lacked the power to conclusively determine whether a dose-response relationship between sedentary time and insulin action exists.

Second, although the present investigation was able to determine whether SED can attenuate the acute effect of exercise on insulin action, the study did not address the unique effect of a pragmatic increase (or decrease) in SED on insulin action, in the absence of the performance of daily exercise. To do so would have required the addition of a fourth condition wherein participants would have increased (or decreased) their daily SED by 1 hour in the absence of exercise. The addition of a forth condition would have significantly increased the complexity of the project as well as the required sample size, and would have been an added burden to our participants.

Third, we did not measure insulin resistance using the euglycemic clamp method, considered the criterion measure of insulin sensitivity. To measure insulin resistance by the
euglycemic clamp method three times in four weeks would have been an incredible burden to the participants, would have increased the cost to perform the trial substantively, and would have made it difficult to compare our findings to previous studies that have employed an oral glucose challenge to measure glucose and insulin response.

Fourth, although the notion that an hour increase in daily SED can diminish the effect of 4 days of exercise on insulin action is powerful, the short duration of the interventions precludes us from making any conclusions regarding the effects of chronic periods of increased sedentary behaviour. Stated otherwise, with interventions that are only 4 days long, it is likely that any impairment in insulin action was transient and does not represent a chronic adaptation to increased sedentary behaviour.

Fifth, since SED and PA levels during each condition were measured as the average over the 4 days of each condition, we did not take into account that the days closer in temporal proximity to the OGTT may have had a greater impact on insulin action. For instance, if someone had their OGTT on a Friday and accumulated 8 hours of SED on Monday and Tuesday and 9 hours on Wednesday and Thursday the average SED would be 8.5 hours. However, since the two days when 9 hours were accumulated were closer to the date of the OGTT, a true measure of SED that is associated with the measure of insulin action obtained from the OGTT would be greater than the average (i.e. between 8.5 and 9 hours).

Finally, since our main inclusion criteria of a sedentary lifestyle and abdominal obesity were quite strict, our sample of men and women was relatively homogeneous and thus limiting the external validity of our findings. Furthermore all but one of our participants was white and thus are findings may not be generalizable to other races. However, given the prevalence of
abdominal obesity in Canada and worldwide our findings are pertinent for a large portion of the adult population.

4.3 Future research

Further research is required to overcome the limitations described above. First, in order to clearly elucidate the effect of SED on insulin action, the effect of a pragmatic increase in SED in the absence of the performance of exercise should be examined. Such an investigation would be insightful as it is possible that in our study the effect of sedentary behavior on insulin action was mediated by the daily bouts of exercise, and therefore we cannot be certain that the magnitude of the impairment in insulin action would have been equivalent following the imposition of a condition that required a change in SED without any exercise being performed. Furthermore, given that such a large proportion of the population is sedentary and that, by definition, these individuals do not meet consensus PA guidelines, determination of the effect of a pragmatic increase in SED on insulin action in the absence of exercise would provide information that would be both generalizable and clinically relevant. However as I learned from this study, having people increase their SED from baseline is not an easy task, and recruiting people to increase sitting time without offering the opportunity to exercise would certainly be challenging.

Second, with intervention durations of only 4 days in length it is difficult to determine whether chronic periods of reduced SED would result in similar improvements in insulin action. A duration of 4 days offers very little time for adaptations related to exercise and altered SED to occur. Furthermore, it may be that chronic periods of reduced SED, like exercise, have both an acute, transient effect on insulin action that can occur after only a single day of reduced SED as
well as a chronic effect that represents adaptation to chronic periods of altered SED. Having measured insulin action the day after completing each condition in our study, we were unable to tease out whether the changes in insulin action we observed were due to adaptations that occurred over the course of the 4 days of each condition or if they were merely related to the act of exercising and/or reducing SED the day prior to the OGTT. Therefore future studies which aim to determine the chronic effects of prolonged periods of altered SED on insulin action should have condition durations greater than 4 days (at least 10 days) and should include the measurement of insulin action 48-72 hours after the completion of any interventions to ensure that any changes in insulin action are due to chronic adaptations to changes in sedentary and PA levels.

Third, a more detailed assessment of insulin action using the criterion hyperinsulinemic-euglycemic clamp technique would allow for determination of possible mechanisms underlying the link between sedentary behaviour and impaired insulin action. Similarly, muscle biopsies should be used to obtain measurements of LPL, ceramides, and glucosylceramides to determine if any of which lie in the causal pathway between elevated SED and impaired insulin action.

Fourth, with the exception of one Asian male, all of our participants were white men and women. Since it is well established that insulin action differs according to race [92, 93], further studies are required to determine how a pragmatic change in sedentary behaviour influences insulin action in a variety of races.
4.4 Conclusions

We have demonstrated that exercise in combination with a reduction in SED was associated with a trend for improved insulin action by comparison to exercise alone. Our findings, although preliminary, are consistent with the large epidemiological studies which have demonstrated an association between SED and impaired insulin action, and add to the small but growing body of evidence from intervention studies which have demonstrated that SED influences insulin action. Our findings support the notion that SED, in addition to moderate-vigorous exercise, may be an important behaviour to target in the prevention of type 2 diabetes and CVD. The impact of a pragmatic change in SED should be examined over a longer period of time to determine the effect of chronic periods of altered SED on insulin action. Furthermore, as our sample comprised mostly of white men and women, the relationship between SED and insulin action should be examined in other races and ethnicities.
REFERENCES


10. Duvivier, B.M., et al., *Minimal intensity physical activity (standing and walking) of longer
duration improves insulin action and plasma lipids more than shorter periods of moderate to
vigorous exercise (cycling) in sedentary subjects when energy expenditure is comparable.* PLoS

activity with metabolic risk factors among people with recently diagnosed type 2 diabetes.*


13. Chaput, J.-P., et al., *Combined associations between moderate to vigorous physical activity and
sedentary behaviour with cardiometabolic risk factors in children.* Applied Physiology, Nutrition,

14. Ekelund, U., et al., *Moderate to vigorous physical activity and sedentary time and
cardiometabolic risk factors in children and adolescents.* JAMA: the journal of the American

15. Janssen, I., et al., *Prevalence and secular changes in abdominal obesity in Canadian adolescents


17. DeFronzo, R.A., *The triumvirate: β-cell, muscle, liver. A collusion responsible for NIDDM.*

p. 2773-2776.


APPENDIX A – CONSENT FORM

CONSENT TO VOLUNTEER FOR PARTICIPATION IN A STUDY

TITLE: Can a 1 hour increase in daily sitting time negate the acute effect of exercise on cardiometabolic health risk?

PRINCIPAL INVESTIGATORS: Robert M.J. Ross, Ph.D.
Queen’s University
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Medicine, Division of Endocrinology and Metabolism
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(613) 533-6583

Mitch Wilson, MSc. Candidate
Queen’s University
School of Kinesiology and Health Studies
Kingston, Ontario, K7L 3N6
(613) 214-4924
You are invited to participate in a research study that will examine the effect of varying amounts of sitting time and physical activity on cardiometabolic health risk. The following document is intended to provide you with the details you should be aware of prior to your consent as a participant in this study. Please read the following information carefully and feel free to ask any questions that you may have.

**BACKGROUND INFORMATION**

A sedentary lifestyle (i.e. a lot of sitting) is associated with negative health consequences. Exercise, on the other hand, is a good treatment strategy for improving a variety of health outcomes. There is currently very little research that has examined whether increased sitting time can negate the improvements in health associated with exercise. Therefore, you are invited to participate in a study to determine whether increased daily sitting time reduces the positive effects of exercise on a series of metabolic variables (e.g., blood fats and blood sugar). The results of the study will hopefully provide a better understanding of the interactive effects of exercise and sitting on metabolic health.

**EXPLANATION OF PROCEDURES**

**Pre-participation screening**

You will complete a physical activity readiness questionnaire (PAR-Q+). If you respond ‘yes’ to one or more questions of the follow-up questions in section 2 of the questionnaire you will be excluded from the study. We will also measure your waist circumference, as well as blood sugar and fats. These measures are explained in further detail on pages three (3) and four (4) of this form. If your waist circumference is measured as less than 102cm at this initial consult you will be excluded from the study.
Study Protocol

The study will be approximately 4 weeks in duration. You will complete 3 activity regimes, each of 4 days in duration. Between the final 2 regimes will be a 10-day period where you will maintain your physical activity levels as they were prior to commencement of the study. The order in which you complete each regime will be selected at random.

Expectations

You will be expected to:

Participate fully in each of the 3 experimental conditions for the duration of the study

Keep all testing appointments

Provide accurate answers on all questionnaires

You can expect:

Full disclosure of all procedures required for participation in this study

To be treated fairly and with respect

Any information that is disclosed will be private and confidential

No one will be coerced or forced to do anything they wish not to do

To have all your questions answered fully and as promptly as possible

To not be penalized for choosing to withdraw from the study for any reason
**Baseline Condition:** You will maintain your usual physical activity levels for 4 days. During these 4 days you will be recording your activity and diet.

**Exercise Condition:** You will exercise for 1 hour per day on a treadmill at our lab located within the School of Kinesiology and Health Studies. You will walk at an intensity that corresponds to 65% of your maximal oxygen uptake (VO$_2$ max).

**Exercise + Sit Condition:** You will exercise for 1 hour on a treadmill at 65% of VO$_2$ max. You will also increase your daily sitting time by 1 hour from baseline (i.e. a 1 hour increase from how much you normally sit).

**Diet Program:** You will be asked to maintain your diet as it was prior to beginning the study. No alterations in diet will occur at any point during the study. You will be required to record the food you eat each day for the duration of the study (excluding days during the washout period). All aspects of the diet plan will be explained to you by a Dietician. The session will take place at the beginning of the study. All of your meetings with the Dietician will be in Dr. Ross’s laboratory within the School of Kinesiology and Health Studies at Queen’s.

**Assessment of cardiovascular fitness**

We will measure your cardiovascular fitness (endurance) using a treadmill (VO$_2$) test. The test will begin at a level you can easily accomplish and will be advanced in stages, depending on your capacity to do so. We may stop the test at any time because of signs of fatigue or you may stop the test because of personal feelings of fatigue or discomfort.

The treadmill test involves risks comparable to any strenuous exercise situation. They include very rare instances of abnormal blood pressure, fainting, disorders of the heartbeat, and heart
attack. Every effort will be made to minimize your risk by preliminary medical examination and observation during the test. A Research Assistant at Hotel Dieu Hospital, with a trained paramedic or medical doctor on-site, will conduct your fitness test. You will perform the exercise test once at the beginning of the study.

**Anthropometry (Circumference, weight and height)**

The circumference of your waist will be measured, along with your height and weight. These measurements can be used to derive estimates of body composition. These measurements require about 15 minutes to complete and will be obtained at Dr. Ross’s laboratory within the School of Kinesiology & Health Studies at Queen’s. We will collect these measurements once at the beginning of the study.

**Assessment of Daily Physical Activity**

How physically active you are throughout the day will be measured by a small device known as an accelerometer. The accelerometer is a small unit that you wear on your belt at the level of your hip. This device measures the amount and intensity of physical activity that you perform each day. You will wear the monitor during all of your waking hours and will remove the monitor when you sleep or participate in water activities such as showering, bathing, or swimming. You will first wear the accelerometer at the beginning of the study for 4 consecutive days so we can obtain a measurement of your usual physical activity levels. The activity pattern recorded on those days will be used to formulate tailor-made instructions on how to change daily activities during the different regimes. You will then wear this device for 4 consecutive days for both the sitting and exercise condition.
In addition to wearing an accelerometer, you will report your time spent walking, standing, or sitting every 30 minutes during waking hours using a stopwatch. This is done to ensure compliance with the prescribed amounts of physical activity, sitting and sleeping time for each condition.

**Laboratory measurements (blood glucose (sugar), lipid (fat) tests) and blood pressure**

The measurement of how much sugar and fat are in your blood will be done at Dr. Ross’s laboratory within the School of Kinesiology & Health Studies at Queen’s. To determine your ability to manage blood sugar you will be asked to perform an Oral Glucose Tolerance Test. You will be asked to arrive at the lab in the morning after an overnight fast (no eating after 7pm the night before). The first step of this test will be the insertion of a saline lock into a vein in your arm. This allows the nurse to take blood at different times without having to re-puncture each time. She will then remove about 30 ml (3 tablespoons) of blood. The only risk from this procedure is possible local pain and bruising at the time of the blood test. In addition, you will be asked to drink a fluid that contains 75 grams of sugar (like an orange drink). At 30-minute intervals for 2 hours after drinking the sugar solution, a small amount of blood will be taken (through the saline lock) for the purpose of measuring the amount of sugar in the blood. Additionally we will measure your blood pressure. These tests will be performed 3 times during the study: at the beginning of the study and the day after completing each of the three four-day experimental conditions.

**Summary of Appointments and Time Requirements**

All appointments will be scheduled at a time that is convenient for you. All testing will be done at Dr. Ross’s laboratory in the School of Kinesiology & Health Studies at Queen’s. This
includes: three 2.5-hour appointments for the oral glucose tolerance test and blood lipid/cholesterol tests (fasting blood draw); a 30-minute nutrition meeting, a 1 hour VO₂ max test, and 8 1-hour bouts of exercise performed on a treadmill within our laboratory.

**Benefits of Participation**

You will gain no direct benefit through participation in this study.

**Risks of Participation**

Participation may involve some risks. The known risks are:

Insertion of a catheter in your arm or hand vein may cause bruising, bleeding, soreness or infection.

Risk of Wearing the Activity Monitor: Some people may experience mild skin irritation at the site where the activity monitor is worn.

Risk of exercise: there is an extremely small risk of cardiac problems occurring while exercising on the treadmill. All exercise will be supervised by CPR-trained graduate students who are instructed to call 911 should a cardiac event occur. It should be noted that several hundred participants have exercised within our laboratory over the last 10 years and not a single major cardiac event has occurred during this time.

The exercise test may cause muscle soreness or fatigue. In any individual, there is a minute risk of a heart attack or death from the exercise test. A trained paramedic or medical doctor will be on-site. If you develop chest pain, the test will be stopped immediately.
You should inform the investigators if you have participated in any other research study during the previous year. This will help to ensure that you have not been exposed to a procedure in another study that may influence your ability or eligibility to participate in this one. You should understand that this study is a research study and may not be of direct benefit to you. If requested, a report will be generated for your medical record, which will include any information important for your medical care.

CONFIDENTIALITY

All information obtained during the course of this study is strictly confidential and your anonymity will be protected at all times. Your information will be kept in locked files and will be available only to Dr. Robert Ross and those working within his laboratory. Your identity will not be revealed in any description or publication.

In the event you that you are injured as a result of the study procedures, medical care will be provided to you until resolution of the medical problem. By signing this consent form, you do not waive your legal rights nor release the investigator(s) and sponsors from their legal and professional responsibilities.

VOLUNTARY CONSENT

I have been given an opportunity to ask any questions concerning the procedures. All of my questions regarding the research project have been satisfactorily answered. I understand that my test results are considered confidential and will never be released in a form that is traceable to me, with the exception of my family physician or myself. I understand that all my lab results will be sent to my family physician. I do understand that I am free to deny consent if I so desire, and may withdraw from the study at any time without prejudicing current or future medical care.
Should I have any questions about the study, I know that I can contact any of the following: Dr. Robert Ross (613 533-6583), Mr. Mitch Wilson, MSc student, School of Kinesiology and Health Studies (613 214-4924), or Dr. Albert Clark, Chair, Queen’s Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (613 533-6081). A copy of this consent form will be provided me for my records. My signature below means that I freely agreed to participate in this study.

_____________________________   _______________________
Volunteer’s Signature       Date:

STATEMENT OF INVESTIGATOR

I, or one of my colleagues, have carefully explained to the subject the nature of the above research study. I certify that, to the best of my knowledge, the subject understands clearly the nature of the study and demands, benefits, and risks involved to participants in this study.

_____________________________   ______________
Principal Investigator’s Signature   Date
APPENDIX B – VO₂ DATA COLLECTION SHEET

Maximal Oxygen Consumption Test – Activity is Best Study
Visit: V00
Mask Size: __________
Date: ____________________
Participant ID: ______________
Height (cm): ______________ DOB: _______________
Weight(kg): ______________ Age Predicted HR: 220-12-____ = _____

Speed: 3.5 mph
Tester: _______________________

Checklist: 1. RQ>1.1 ______ 2. HR ≥ HRmax ______ 3. VO₂ plateau ______ 4. BORG =10 ______

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APPENDIX C – DIETARY REGIMEN

Activity is Best STUDY

Name: _______________________                                         Condition (B/E/E+S): __________
Date: ________________________                                         Day of condition (1-4): __________

Breakfast Time: __

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Lunch Time: __

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Snacks

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APPENDIX D – PHYSICAL ACTIVITY

Physical activity (PA) was measured with the Actigraph GT3X accelerometer (Actigraph, Pensacola, Florida). Accelerometers were programmed to collect data in 1-minute epochs over a 4-day period and were worn on an elastic belt positioned over the right hip at all times except during water-based activities. A complete day was defined as at least 10 hours of wear time during the day. Wear time was calculated after extended periods of consecutive zero counts ≥ 60 minutes, and sleep time was measured as the time elapsed between removing the accelerometer at the commencement of sleep at night and resuming wear upon waking in the morning. The accelerometer cutpoints in this study used to translate the ‘count’ value into an estimate of physical activity intensity were those developed by Freedson and colleagues [68]. Sedentary time was defined as counts per minute (cpm) <100, LPA as 100 to 1952cpm, and MVPA as ≥1952. Activity and sedentary time accumulated during each day of monitoring was quantified as an average daily duration, in minutes per day (hr:min/ for each of sedentary time, LPA, and MVPA. MVPA was further partitioned into exercise time (defined as average daily time on treadmill) and unstructured MVPA (defined as the difference between total MVPA and exercise time).

Participants wore the accelerometers for 4 days during the baseline, exercise, and exercise+sitting conditions. If the required amounts of sedentary and physical activity for the two exercise conditions were not met there are variety of courses of action which can be taken in order to reconcile the accelerometry data: 1) if the exercise+sedentary condition was performed first and the participant was unable to increase their sedentary time beyond baseline levels, this condition was renamed as the exercise only condition and the participant was asked to repeat the exercise+sedentary condition as the third condition. 2) if the exercise+sedentary condition was
performed second and sedentary time was lower compared to the exercise only condition, then these 2 conditions were switched. 3) if the exercise only condition was performed first and sedentary time increased beyond baseline levels, this condition was renamed as the exercise+sedentary condition and the participant was asked to repeat the exercise as the third condition. Ultimately, the exercise condition was determined as the exercise condition with the lower amount of average daily sedentary time, and the exercise+sedentary condition was determined as the exercise condition with the higher amount of average daily sedentary time.

**Measurement Device**

GT3X Actigraph Activity Monitor (Pensacola, FL)

**Measurement Procedures**

*Preparing the Accelerometer for Data Collection*

1. The accelerometer has a rechargeable battery that should be fully charged before being given to a participant. The accelerometer can be recharged via a standard 2.0 USB connection plugged into a computer or a 7-terminal charging hub which is plugged into a regular electrical outlet. A fully depleted battery takes approximately 3 hours to fully recharge. Note: **When the battery is plugged in and recharging a red light will flash. When this red light stops flashing and stays on, the battery is fully charged.**
2. When the accelerometer is charged it can be initialized. Double-click the Actilife software icon on the desktop.

3. When the Actilife program is open on the computer, use a USB connection to connect the accelerometer to the computer (as shown in the picture below – the arrow on the USB cable should be facing up). A new driver has to be installed for each port that the USB + accelerometer is plugged into.

4. Once the accelerometer is plugged in the screen will change to look like the one below. Check the battery of the accelerometer to make sure it is fully charged.

5. Click on the ‘Initialize’ button to initialize the accelerometer. The following screen will pop up. Ensure all boxes checked in the snapshot below are also checked on your screen. Fill in the appropriate start and stop times. The accelerometer should start at midnight on the first day and stop at 11:59 pm on the last day (i.e. the 3rd of baseline or the 4th day of each of the 2 interventions). If the participant picks up the accelerometer on one day the accelerometer will start at midnight that same day and begin recording data for the next day (i.e. day 1 of 4). Therefore participants should begin wearing the accelerometer at
some point the night before the first day of collection so that the participant is wearing the accelerometer when it begins recording at midnight. Note: after the accelerometer has been initialized the red light will flash until it hits the programmed start time. The red light will start flashing again once it reaches the stop time until the data is downloaded. Once the appropriate boxes have been checked and the start and stop times have been set, click “Enter Subject Info…”.

6. After you click “Enter Subject Info…” the following pop up window will appear. Change the subject name field to reflect the participant’s ID and visit number followed by the day number the accelerometer has been initialized for. In the example below W001 is the participants ID, B/E/E+S refers to the condition (Baseline/Exercise/Exercise+Sitting) Once this field is filled in, click “Initialize All”.

![Initialize Devices](image)
7. Once the accelerometer has been successfully initialized the box below will pop up. Double-check that the start date and time are correct. Disconnect the accelerometer from the USB cable.

8. Attach the accelerometer to an appropriately sized elastic band.

9. Record the serial number of the accelerometer in the *Acceleometer Wear Spreadsheets — All Studies* excel file so we can track who was wearing which accelerometer.

   Participants will wear the same accelerometer throughout the duration of the study.

   Below is the Flashing Light Reference for the accelerometer

<table>
<thead>
<tr>
<th>ActiGraph GT1M/ASM/GT3X LED Flashing Reference</th>
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<tbody>
<tr>
<td>ActiGraph GT1M Connected to PC</td>
</tr>
<tr>
<td>1 Flash: Li-Ion Battery is Charging</td>
</tr>
<tr>
<td>2 Flashes: Li-Ion Battery is Faulty</td>
</tr>
<tr>
<td>Steady On: Battery Charged</td>
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</tbody>
</table>

   ActiGraph GT1M/ASM/GT3X Not Connected to PC

<table>
<thead>
<tr>
<th>No Flashes (LED Off)</th>
<th>Actively Taking Data (“Flash Mode” Disabled) or Battery Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Flash:</td>
<td>- Delay before start mode (the GT1M LED always flashes prior to starting data collection)</td>
</tr>
<tr>
<td>2 Flashes:</td>
<td>- Actively taking data (“Flash Mode” Enabled - not recommended)</td>
</tr>
<tr>
<td>3 Flashes:</td>
<td>- Low Battery (use ActiLife Lifestyle software to check for remaining battery life) The unit needs to be recharged</td>
</tr>
</tbody>
</table>

   Notes:
   - The LED will ALWAYS flash to indicate LOW BATTERY regardless of whether "Flash Mode" is enabled or disabled.
   - If ‘stop time’ (optional) has been reached, the LED will stop flashing all together regardless of its previous state.

Preparing the Participant
1. There are no guidelines the participant needs to follow prior to getting the accelerometer.

2. The accelerometer will be attached to an elastic band to be worn around the waist next to the skin (or over a light, tight-fitting shirt if it is too itchy) to minimize extra motion and will be situated directly over the right hip.

3. The participant will be asked to wear the accelerometer for 4 days at baseline and for 4 days during each of the 2 activity regimes, and to remove it only for water activities (eg, swimming, showering, or bathing) and sleep.

**Important Points to Mention During the Initial Meeting with the Participant**

1. We are putting an activity monitor on which will give us an indication of how much physical activity you do. It records how often you move around and how quickly you move around.

2. The activity monitor does not interfere with any medical devices and is not harmful.

3. The activity monitor should be worn on the elastic band around your waist, preferably right next to the skin but it can go over a tight-fitting shirt if it is itchy, or can be looped through belt holes on pants and should be situated directly above the right hip. Please ensure that the elastic is not loose and the activity monitor is not flopping around because it will not collect good data.

4. The activity monitor should be worn at all times for the next 4 days (depending on baseline or intervention) during waking hours except it should be removed for all water-based activities such as swimming, showering, or bathing because it is not waterproof. It is most important that you are wearing it during your waking hours. So, because you are not wearing it to sleep, please put it on as soon as you wake up in the morning and remove it immediately prior to climbing into bed at night.
5. If you have any immediate concerns while wearing the device please contact Mitch Wilson at 8mrw2@queensu.ca or at (613) 533-6000 Ext 78613.

6. Although the activity monitor is very durable, please be careful and gentle with it as it is very expensive.

7. Please remember to bring the activity monitor to your next OGTT.

Data Management

Acquiring Data

1. Retrieve accelerometer and log from the participant.

2. Open the Actilife program, connect the accelerometer to the computer via USB connection and click the ‘download’ button on the left side of the screen. The box below should pop up on the screen.

3. Once the data has been successfully downloaded, the box below will pop up. Select ‘Subject Name’ from the “Download Naming Convention. Ensure that all the boxes and settings are the same as the image below. Then click “Download All Devices”. Note: The data will be stored in the accelerometer until it has been initialized again and at this point it is deleted. So, be sure data has been downloaded before initializing any device.
4. The file (it will be a .AGD file) generated will be saved to the directory chosen
(\DiskStation\GradStudent/Walk Sit\Accelerometry). The pop up below should appear
and indicate that the download is finished.
Initial Data Quality Assessment

1. This will be done by creating graphs of the daily activity within Actilife 6. After you have saved the data, select the graphing tab within the Actilife software as seen in the image below.

![Actilife Graphing Tab](image1.png)

2. Now, you must select the file that you would like to graph. Go and click on the “Select Dataset” button (Seen Below). Find the file in the Accelerometer folder, under the appropriate visit folder.

![Select Dataset Button](image2.png)

3. The ActiLife 6 program will graph the PA data of all 3 axes for each day of wear. In the example shown below, you can see that the graphs will display all collected data on the same set of axis. You can go and unselect the data you do not want to see on the right (see arrow).

![Graph Example](image3.png)
4. Once you have unselected axis 2 and axis 3, the graph will become much clearer and allow you to assess the quality of the data (See below).
5. The program will often recognize if the accelerometer has malfunctioned and a pop-up will appear when the accelerometer is plugged in that provides details about the problem. There should be some periods of zeros if the participant showered. Also ensure that sleep and wake times look logical.

6. There must be a minimum of 10 hours of wear time each of those days to ensure the data can be used.

7. Once the file has been assessed, bin it into the appropriate folder according to whether or not it was worn for the minimum number of required days (4 days: 3 weekday and 1 weekend of at least 10 hours). If a file does not meet these requirements, the data cannot be used and should be placed in the unusable data folder. The participant should be asked to wear the accelerometer again.

**Obtaining Sedentary and Activity Data from Actilife**

1. Click ‘Wear Time Validation’ and then ‘Add Dataset’. Select the appropriate AGD file. On the left side of the screen ensure that you have selected Trojano (2007) and checked off ‘default’. Next click ‘Calculate’ at the bottom of the screen.
2. Click ‘Data Scoring’ and then ‘Add Dataset’. Select the appropriate AGD file. On the left side of the screen it is important that you check off ‘Cut Points and MVPA’ and select Freedson no lifestyle (2013). For filters, it is important that you check off ‘Exclude Non-wear Times from Analysis’. Next click ‘Calculate’ at the bottom of the screen. A row of variables will then appear on the screen.

3. At the bottom of the screen click ‘Export’, Select Export Type as ‘Excel’ (xlsx). Then click ‘Export…’ and save it to the appropriate folder in the Accelerometry sub-folder (Baseline vs Exercise vs Exercise+Sitting). The name of the document should be named
to have the subject ID and condition included at the beginning of the document name ie. W018B Accelerometry.

4. To obtain the hours spent in sedentary, LPA, and MVPA click on the ‘Daily’ within the excel file. Calculate the averages and convert to hours as displayed below. Enter these values in the appropriate cells in the ‘Walk Sit Accelerometry Data’ excel file.
5. Click ‘Sleep Analysis’. Note (i.e. write down) the 3 “in bed” and “out bed” times by clicking on the points on the graphs where data collection ends at night (in bed) and where data collection begins in the morning (out bed). On the right-hand side of the screen click ‘add sleep period’ and do this three times for the three individual sleep periods.

6. Click ‘Export Report’, then ‘Create Report…’
7. Open the pdf file. The average sleep time for the 4 days is listed as the average ‘‘Total Sleep Time (TST) (min)’’. Enter this value in the ‘Walk Sit Accelerometry Data’ excel file located in the ‘Accelerometry’ folder. Save the pdf as subjectIDCondition Sleep i.e. W004 B Sleep.

Sleep Algorithm Used: Sadeh

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<th>Efficiency</th>
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<th>Total Sleep Time (TST) (min)</th>
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1.6.7 Instructions to Participants

1. After having confirmed their commitment to completing the study, participants will pick up an accelerometer from the lab or they will be given the accelerometer at their VO2 peak test.
3. Participants will return the accelerometer when they come in on Thursday or Friday for their 1st nursing appointment. At this point, Mitch will download the accelerometry data and obtain the average number of hours spent sitting, engaging in LPA and MVPA, and sleeping.

4. The **SAME** accelerometer will be returned to the participant at the end of the nursing appointment. It will be initialized to collect for the next 4-day period (Sun-Wed or Mon-Fri).

5. The participant will begin wearing the accelerometer the next Sunday or Monday when they wake up, and will wear it continuously (except while sleeping) for 4 days until midnight of the Wednesday or Thursday.

7. Participants will return the accelerometer when they come in on the Thursday or Friday for their 2nd nursing appointment.

8. Participants will then begin a 10-day washout period where they return their usual activity levels. At some point during this 10-day washout, they will come in to the lab to pick up the **SAME accelerometer** so that they are ready to begin the 2nd activity regime. The accelerometer will be initialized prior to their arrival.

9. Two weeks after completing the previous activity regime, participants will begin wearing the accelerometer when they wake up on Sunday or Monday and remove it at midnight of the Wednesday or Thursday.

10. Participants will return the accelerometer when they come in on the Thursday or Friday for their 3rd and final nursing appointment.
APPENDIX E – CALCULATION OF HOMA-IR AND THE MATSUDA INDEX

HOMA-IR was calculated as \( [(\text{fasting glucose (mmol/L)} \times \text{fasting insulin (mU/mL)})/22.5] \) and the Matsuda index was calculated as:

\[
\frac{10000}{\sqrt{\frac{\text{fasting glucose} \times \text{fasting insulin}}{(\text{mean OGTT glucose concentration (0−120)} \times \text{mean OGTT insulin concentration (0−120)})}}}
\]

*Glucose in mg/dL; insulin in mU/mL.
APPENDIX F – RESEARCH ETHICS BOARD APPROVAL

QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD-DELEGATED REVIEW

November 19, 2013

Mr. Mitchell Wilson
School of Kinesiology & Health Studies
Queen’s University

Dear Mr. Wilson

Study Title: PHE-139-13 Can exercise compensate for the negative metabolic effects of excessive sitting time?
File # 6010959

Co-Investigators: Dr. R. Ross

I am writing to acknowledge receipt of your recent ethics submission. We have examined the protocol, peer review, recruitment letter, questionnaire – PAR-Q+ and revised information/consent form for your project (as stated above) and consider it to be ethically acceptable. This approval is valid for one year from the date of the Chair’s signature below. This approval will be reported to the Research Ethics Board. Please attend carefully to the following listing of ethics requirements you must fulfill over the course of your study:

Reporting of Amendments: If there are any changes to your study (e.g. consent, protocol, study procedures, etc.), you must submit an amendment to the Research Ethics Board for approval. Please use event form: HSREB Multi-Use Amendment/Full Board Renewal Form associated with your post review file # 6010959 in your Researcher Portal (https://eservices.queensu.ca/romeo_researcher/)

Reporting of Serious Adverse Events: Any unexpected serious adverse event occurring locally must be reported within 2 working days or earlier if required by the study sponsor. All other serious adverse events must be reported within 15 days after becoming aware of the information. Serious Adverse Event forms are located with your postreview file 6010959 in your Researcher Portal (https://eservices.queensu.ca/romeo_researcher/)

Reporting of Complaints: Any complaints made by participants or persons acting on behalf of participants must be reported to the Research Ethics Board within 7 days of becoming aware of the complaint. Note: All documents supplied to participants must have the contact information for the Research Ethics Board.

Annual Renewal: Prior to the expiration of your approval (which is one year from the date of the Chair’s signature below), you will be reminded to submit your renewal form along with any new changes or amendments you wish to make to your study. If there have been no major changes to your protocol, your approval may be renewed for another year.

Yours sincerely,

Chair, Health Sciences Research Ethics Board

November 19, 2013

Investigators please note that if your trial is registered by the sponsor, you must take responsibility to ensure
that the registration information is accurate and complete.

QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD

The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards and operates in compliance with the Tri-Council Policy Statement; Part C Division 5 of the Food and Drug Regulations, OHRP, and U.S DHHS Code of Federal Regulations Title 45, Part 46 and carries out its functions in a manner consistent with Good Clinical Practices.

Federalwide Assurance Number: #FWA00004184, #IRB00001173

Current 2013 membership of the Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board:

Dr. A.F. Clark, Emeritus Professor, Department of Biomedical and Molecular Sciences, Queen's University (Chair)

Dr. H. Abdollah, Professor, Department of Medicine, Queen's University

Dr. R. Brison, Professor, Department of Emergency Medicine, Queen's University

Dr. C. Cline, Assistant Professor, Department of Medicine, Director, Office of Bioethics, Queen's University, Clinical Ethicist, Kingston General Hospital

Dr. M. Evans, Community Member

Ms. J. Hudacin, Community Member

Dr. B. Kisilevsky, Professor, School of Nursing, Departments of Psychology and Obstetrics and Gynaecology, Queen's University

Mr. D. McNaughton, Community Member

Ms. P. Newman, Pharmacist, Clinical Care Specialist and Clinical Lead, Quality and Safety, Pharmacy Services, Kingston General Hospital

Ms. S. Rohland, Privacy Officer, ICES-Queen's Health Services Research Facility, Research Associate, Division of Cancer Care and Epidemiology, Queen's Cancer Research Institute

Dr. A. Singh, Professor, Department of Psychiatry, Queen's University

Dr. J. Walia, Assistant Professor and Clinical Geneticist, Department of Paediatrics, Queen's University and Kingston General Hospital

Ms. K. Weisbaum, LL.B. and Adjunct Instructor, Department of Family Medicine (Bioethics)
APPENDIX G – PERMISSION FOR TABLE 1, CHAPTER 2

Order Details

Progress in lipid research

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- **Author(s):** Summers, Scott A.
- **DOI:** 10.1016/J.PLIPRES.2005.11.002
- **Date:** Jan 01, 2006
- **ISSN:** 0163-7827
- **Publication Type:** Journal
- **Volume:** 45
- **Issue:** 1
- **Start page:** 42
- **Publisher:** Pergamon

- **Permission Status:** ✅ Granted
- **Permission type:** Republish or display content
- **Type of use:** reuse in a thesis/dissertation

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APPENDIX H – PERMISSION FOR TABLE 2, CHAPTER 2

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APPENDIX I – SPSS STATISTICAL OUTPUT FOR PRIMARY ANALYSIS

Within-Subjects Factors
Measure: MEASURE_1

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<th>INSauc</th>
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<td>InsAUCE</td>
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Descriptive Statistics

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<th>Sig.</th>
<th>Epsilon(^b)</th>
<th>Greenhouse-Geisser</th>
<th>Huynh-Feldt</th>
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\(^a\) Mauchly’s Test of sphericity
\(^b\) Epsilon
Tests of Within-Subjects Effects

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Pairwise Comparisons

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<th>Sig. b</th>
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