ASSOCIATIONS OF MODIFIABLE RISK FACTORS WITH INSULIN RESISTANCE AMONG INACTIVE, ABDOMINALLY OBESE ADULTS

by

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Abstract

Insulin resistance (IR) is implicated in the development of type 2 diabetes and cardiovascular disease, both of which are leading causes of mortality worldwide. Three modifiable risk factors that are associated with IR include physical activity (PA), sedentary behaviour (SED), and diet. Consensus guidelines state that to obtain health benefit, PA should be performed at a moderate-to-vigorous intensity in bouts of at least 10 consecutive minutes for a minimum of 150 minutes per week. Whether PA that is accumulated below guideline recommendations- such as light PA or sporadic PA (bouts < 10 minutes) - is associated with improvements in IR is uncertain. Irrespective of PA, a growing body of literature suggests that SED may elicit health consequences independent of PA. Furthermore, a dietary pattern in accordance with guidelines may attenuate IR, although the optimal dietary pattern for the prevention of IR remains unclear.

Although the separate associations between IR and objectively measured PA and SED as well as self-reported diet quantity and quality have been reported in the literature, few studies have considered the interaction between these modifiable risk factors and IR. We sought to assess the associations of PA, SED, diet quantity, and diet quality with IR in a sample of inactive, abdominally obese men (n=33) and women (n=69).

SED and light PA displayed a strong inverse relationship with each other. SED was positively associated with IR, whereas light PA was negatively associated with IR. Sporadic, moderate-to-vigorous intensity PA was not associated with IR. Neither diet quantity nor diet quality were directly associated with IR. Together, these findings suggest that the substitution of SED for modest amounts of PA accumulated below guideline recommendations may attenuate IR among inactive, abdominally obese adults.
Co-Authorship

Critical revisions for intellectual content were provided by Dr. Robert Ross. Sara L. Giovannetti was responsible for assisting in the acquisition of data, all statistical analyses of data, and the writing of the manuscript contained within this thesis.
Thesis Contributions

This thesis is a secondary analysis of a 6-month randomized controlled trial (SERENA Study) designed to investigate the separate effects of exercise volume and intensity on cardiometabolic risk factors in abdominally obese men and women. Due to the nature of the study, a collaborative effort was put forth by staff members and graduate students (including myself) for the data collection, management, and cleaning. My primary responsibilities were the acquisition of nutritional data from the dietary records (using ESHA Food Processor software) and their compilation into a diet quality index. Furthermore, I assisted in recording sleep and wake times for the management and cleaning of accelerometer data. Lastly, I performed all statistical analyses.
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<tbody>
<tr>
<td>AHEI-2010</td>
<td>Alternative Healthy Eating Index-2010</td>
</tr>
<tr>
<td>aMed</td>
<td>Alternate Mediterranean Diet</td>
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<tr>
<td>Akt/PKB</td>
<td>Protein kinase B</td>
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<td>BMI</td>
<td>Body-mass-index</td>
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<td>BP</td>
<td>Blood Pressure</td>
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<tr>
<td>cpm</td>
<td>Counts per minute</td>
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<tr>
<td>CRF</td>
<td>Cardiorespiratory fitness</td>
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<tr>
<td>CDA</td>
<td>Canadian Diabetes Association</td>
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<tr>
<td>DASH</td>
<td>Dietary Approaches to Stop Hypertension</td>
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<tr>
<td>FA</td>
<td>Fatty acids</td>
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<td>FFA</td>
<td>Free fatty acids</td>
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<td>GI</td>
<td>Glycemic index</td>
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<td>GLUT</td>
<td>Glucose transporter</td>
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<td>HDL-C</td>
<td>High-density lipoprotein cholesterol</td>
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<td>HEI-2010</td>
<td>Healthy Eating Index-2010</td>
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<tr>
<td>HOMA-IR</td>
<td>Homeostatic model assessment of insulin resistance</td>
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<td>Insulin AUC</td>
<td>Insulin area-under-the-curve</td>
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<tr>
<td>IR</td>
<td>Insulin resistance</td>
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<td>IRS</td>
<td>Insulin receptor substrate</td>
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<td>LDL-C</td>
<td>Low-density lipoprotein cholesterol</td>
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<td>LPA</td>
<td>Light physical activity</td>
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<td>LPL</td>
<td>Lipoprotein lipase</td>
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<td>MetS</td>
<td>Metabolic syndrome</td>
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<td>METs</td>
<td>Metabolic equivalent tasks</td>
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<td>MUFA</td>
<td>Monounsaturated fatty acids</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>MVPA</td>
<td>Moderate-to-vigorous physical activity</td>
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<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
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<td>OGGT</td>
<td>Oral glucose tolerance test</td>
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<td>PI3K</td>
<td>Phosphatidylinositol-3 kinase</td>
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<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>SED</td>
<td>Sedentary behaviour</td>
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<td>SFA</td>
<td>Saturated fatty acids</td>
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<td>trans-fatty acids</td>
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<td>Triglycerides</td>
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<td>TNF-α</td>
<td>Tumour necrosis factor-α</td>
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<td>TPA</td>
<td>Total physical activity</td>
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<tr>
<td>VIF</td>
<td>Variance inflation factor</td>
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<tr>
<td>VLDL</td>
<td>Very-low-density lipoprotein</td>
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<td>WC</td>
<td>Waist circumference</td>
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Chapter 1

Introduction

Physical activity (PA) and diet have been tightly linked throughout human evolutionary history: energy expenditure was required in order to obtain dietary nutrients [2]. Due to the rapid industrialization and technological advances of modern society, humans today have relatively low energy expenditures in comparison to their hunter-gatherer ancestors. Furthermore, highly processed, energy-dense foods are readily available for consumption, requiring little or no preparation. Thus, today’s environment promotes an inactive, obesogenic lifestyle [3].

The health consequences associated with the activity and dietary behaviours commonly exhibited by humans today are a cause for concern. Indeed, inactivity and poor nutrition are linked to cardiovascular and all-cause mortality [4-6]. When unhealthy lifestyle behaviours are engaged in on a regular basis, various physiological abnormalities become apparent prior to the development of overt chronic illness. One such physiological abnormality is insulin resistance (IR). Insulin is an anabolic hormone produced by the pancreas, and under healthy conditions it binds to the receptors of target tissues within the body and mediates glucose uptake [7]. IR, however, is characterized by a defect in insulin action, resulting in hyperinsulinemia to maintain glycemic control [8]. If the pancreas cannot compensate by secreting enough insulin, glucose tolerance deteriorates, increasing one’s risk for type 2 diabetes and cardiovascular disease [9].

The aforementioned modifiable risk factors- PA and diet- have received ample attention in the literature. It is well-established that PA consistent with consensus recommendations- moderate or vigorous activity for 30 minutes per day on most days of the week- is associated with an amelioration in IR [10, 11]. The guidelines further suggest that the moderate and/or vigorous activity be accrued in bouts of at least 10 consecutive minutes. Strikingly, a mere 15% of Canadian adults adhere to these guidelines [12]. Less certain is whether PA that is
accumulated below guideline recommendations confers benefit [13, 14], and this remains an avenue for future exploration.

In terms of nutrition as a modifiable risk factor for IR, dietary habits of Canadians do not adhere to recommended guidelines. According to the 2004 Canadian Community Health Survey, 5 in 10 women and 7 in 10 men had energy intakes that surpassed their caloric requirements [15]. On average, saturated fat intake was above, whereas fibre intake was below the recommendations stated within Eating Well with Canada’s Food Guide [16]. Thus, the dietary habits of Canadians are inconsistent with dietary patterns associated with reduced risk of heart disease and diabetes, such as the Mediterranean and prudent diets [17, 18]. The prudent diet, which is akin to the Mediterranean diet and is commonly recommended for health benefits in Western cultures, consists of high quantities of vegetables, legumes, whole grains, fruit, fish, and poultry [19]. Although a dietary pattern for optimal cardiovascular health has yet to be fully elucidated, mounting evidence exists that the prudent diet confers benefit.

A new and emerging risk factor for type 2 diabetes and cardiovascular disease is sedentary behaviour (SED). SED is thought to be associated with IR independent of PA [13, 20]. Technological advancements over recent years have provided researchers with the ability to objectively assess SED, as well as PA, using accelerometry. Accelerometers have advanced this field of research because they remove the bias associated with self-reported data [21]. Although the use of accelerometers as a PA and SED research tool has proliferated since the 2000s [22], many unanswered questions remain, especially those pertaining to the benefits of accumulating PA below consensus recommendations [10].

Absent from the literature is a careful, simultaneous examination of the modifiable risk factors- PA, SED, and diet- and IR. Whether these modifiable risk factors interact together or are associated with IR independently is uncertain. Given that obesity, particularly within the abdominal region, is a major determinant in the development of IR [23, 24], it is uncertain whether modifiable risk factors are predicting IR independently or via the deposition of
abdominal adipose tissue. With the prevalence of abdominal obesity implicating 35.6% of Canadians [25], this knowledge is highly generalizable to the Canadian population. The following literature review found in Chapter 2 will delve into research relating to IR, as well as its mechanisms and modifiable risk factors. The subsequent manuscript in Chapter 3 investigates the associations between modifiable risk factors and IR. Chapter 4 summarizes the implications of our investigation, and discusses future directions.
Chapter 2

Literature Review

2.1 Introduction

The purpose of this review is to provide a summary of the current literature on IR and its relationship with modifiable risk factors: PA, SED, and diet. These modifiable risk factors may be associated with IR either directly or indirectly through the deposition of excess adipose tissue, particularly in the abdominal region. Given that abdominal obesity is clearly associated with IR [26] and that the prevalence of abdominal obesity has increased threefold among Canadian adults (11.4% to 35.6%) since 1981 [25], the aforementioned topic is highly pertinent at this time. Furthermore, IR is implicated in the pathogenesis of type 2 diabetes and cardiovascular disease, both of which are increasing in prevalence worldwide [9, 27, 28]. A greater understanding of the modifiable risk factors associated with IR may aid in attenuating the development of IR and its progression into chronic disease. The concepts reviewed will establish a foundation for the subsequent investigation in Chapter 3.

2.2 Insulin Resistance

2.2.1 What is Insulin Resistance?

Insulin is an anabolic hormone produced in the pancreas, specifically within the β cells of the islets of Langerhans. Insulin is secreted upon a rise in circulating blood glucose, thus playing a pivotal role in the regulation of carbohydrate as well as lipid metabolism. Described thoroughly in the following section, insulin’s primary role is to bind to receptors of target tissues and mediate glucose uptake. These target tissues include skeletal muscle, adipose tissue, and liver [7].

The ability of insulin to stimulate glucose disposal is referred to as the insulin sensitivity of a tissue. Insulin resistance is the opposite of insulin sensitivity, and refers to the inability of
insulin to stimulate the disposal of glucose, resulting in hyperinsulinemia to maintain euglycemia [8]. If the pancreas cannot compensate by secreting substantial insulin, glucose tolerance will deteriorate. Similar to insulin sensitivity, the term insulin action refers to the ability of insulin to stimulate glucose disposal. In contrast, insulin action also encompasses insulin-related variables that are not considered to be traditional measures of IR - such as 2-hour glucose or insulin area under the curve - in addition to measures of IR itself.

2.2.2 Mechanisms of Insulin Resistance

The mechanisms of IR are complex and multifaceted. To explain broadly, Figure 2-1 depicts the cascade of signalling events that occur upon the binding of insulin with its receptor on the cell surface of a healthy, insulin-sensitive target tissue. First, the insulin receptor is phosphorylated, resulting in the activation of tyrosine kinase [29]. The phosphorylation of numerous intracellular proteins ensues, including insulin receptor substrate (IRS)-1 and IRS-2. IRS-1 is necessary for glycogen synthesis in muscle, whereas IRS-2 mediates the effect of insulin on hepatic glucose production and glycogen formation. Phosphatidylinositol-3 kinase (PI3K) is activated and binds to IRS-1 or IRS-2 via its p85 regulatory subunit [30]. The subsequent activation of protein kinase B (Akt/PKB) results in the translocation of glucose transporter (GLUT)-4 [31] as well as the stimulation of glycogen synthase [32]. GLUT-4 permits glucose to enter the cell via facilitated diffusion.
Figure 2-2 portrays the mechanisms associated with impaired insulin signalling. A reduction in insulin-stimulated tyrosine kinase activity is realized. This defect, however, may be corrected following weight loss and normalisation of fasting plasma glucose within an individual [29]. In addition, the association of IRS-1 with the p85 subunit of PI3K is reduced [33]. These, among other defects such as a reduction in receptor concentration and lowered activity of intracellular enzymes, inhibit the translocation of GLUT-4 and thus glucose uptake [34].

![Diagram](image_url)

**Figure 2-2** Impaired insulin signalling, resulting in the inhibition of glucose uptake. Taken from DeFronzo [1].

The pathophysiology to support the aforementioned mechanisms of impaired insulin signalling- which precipitate IR- is not fully understood. A major contributor to the development of IR is thought to be an excess of circulating free fatty acids (FFAs). This notion encompasses a lipocentric view, and suggests that once adipose tissue has reached its maximal storage capacity, lipids ‘spillover’ from adipocytes and accumulate ectopically [35]. Ectopic sites include skeletal muscle, cardiac muscle, liver, and visceral adipose tissue. This pattern of accumulation is metabolically deleterious in comparison to subcutaneous deposition. For example, Ross and colleagues have demonstrated in men and women that visceral adipose tissue is strongly correlated with insulin resistance, independent of subcutaneous adipose tissue [24, 36].

Although insulin is normally an inhibitor of lipolysis, this characteristic is impaired in an IR state [37]. The resultant elevation in circulating FFAs alters liver metabolism by increasing fat
oxidation and hepatic gluconeogenesis, perpetuating the IR state [38]. Plasma glucose and FFA concentration are heightened, although target tissues are resistant to these substrates. This may contribute to the development of a slew of cardiometabolic risk factors that constellate the 'metabolic syndrome', which will be described in the following section.

The elevation in FFAs may result in the accumulation of triglycerides and fatty-acid derived metabolites, such as fatty acyl-CoA and ceramides, within muscle and liver [39]. This will impair the insulin signalling cascade (as portrayed in Figure 2-2), providing reasoning for the strong, negative correlation between insulin stimulated glucose disposal and the elevation of intramuscular triglycerides [40]. In addition, prolonged exposure to excessive FFA concentration can inhibit the secretion of insulin from pancreatic β-cells. This concept supports the notion that IR involves the loss of in vivo insulin action, as well as pancreatic secretory capacity.

Finally, another mechanism of IR may relate to the inflammatory nature of adipose tissue and FFA. Because the functions of adipose tissue extend beyond that of a storage depot- it is also an endocrine organ- adipose tissue can release pro-inflammatory cytokines that ultimately lead to ‘lipotoxicity’ [41]. Although beyond the scope of this review, one such cytokine released by adipose tissue is tumour necrosis factor-α (TNF-α). TNF-α is expressed more strongly in obese humans, and has been shown to reduce insulin receptor kinase activity, thus contributing to IR [42].

Despite the evidence in support of the lipocentric model, it is important to note that some individuals have a genetic predisposition to IR. There is large variance in the ability of insulin to stimulate glucose uptake among individuals with normal glucose tolerance. Twenty-five percent of individuals with normal glucose tolerance display a defect in insulin action that is similar to those with impaired glucose tolerance and type 2 diabetes [43, 44]. Although it is known that genetics and circulating FFAs are implicated in the development of IR, IR is multi-faceted and its associated mechanisms have yet to be fully elucidated.
2.2.3 Insulin Resistance and its Association with the Metabolic Syndrome

The constellation of metabolic risk factors that predispose an individual to type 2 diabetes and cardiovascular disease was noted as early as 1923 by Kylin, a Swedish physician [45]. In 1988, Reaven coined the term ‘Syndrome X’ to describe the metabolic abnormalities secondary to IR [46]. These included resistance to insulin-mediated glucose uptake, hyperglycaemia, hyperinsulinaemia, elevated plasma concentration of very-low-density lipoprotein (VLDL) triglyceride, decreased plasma concentration of high-density lipoprotein cholesterol (HDL-C), and hypertension. This common clustering of risk factors is now referred to as the ‘metabolic syndrome’ (MetS).

Despite the lack of a universally accepted definition for the MetS, the essential components- abdominal obesity, glucose intolerance, dyslipidemia, and hypertension- have all been agreed upon by various organizations [47-52].

Evidence in support of IR as the central tenet of the MetS comes from the Bruneck Study [53] and the Barilla Factory Study [54]. In the Bruneck Study, IR was assessed using the homeostatic model assessment for insulin resistance (HOMA-IR). A correlation was reported between IR and the number of metabolic risk factors. In addition, when several metabolic risk factors were clustered together, IR was likely present [53]. In the Barilla Factory Study, 647 healthy subjects underwent a glucose challenge, and were divided into quartiles based upon their plasma insulin response. 15 years later, 25% of the subjects within the most insulin resistant quartile displayed significant increases in the incidence of impaired glucose tolerance or type 2 diabetes (8-fold), hypertension (2-fold), and coronary heart disease (3-fold), compared to subjects in the other quartiles [54].

Mechanisms tying IR with various metabolic risk factors are not entirely understood. The elevation in FFA flux to the liver, a characteristic of IR, augments hepatic synthesis of VLDL-triglyceride, contributing to hypertriglyceridemia. IR may also decrease the concentration of lipoprotein lipase (LPL)- an enzyme that hydrolyzes triglycerides- in peripheral tissues [55]. The
reduction in LPL may contribute to hypertriglyceridemia, although by a lesser magnitude compared to the overproduction of VLDL.

Lowered concentrations of HDL-C are also associated with hypertriglyceridemia, and this may be an indirect effect of IR. The reduction in HDL-C levels are primarily attributable to the effects of cholesteryl ester transfer protein, rendering the HDL-C particle smaller and more dense, thus effecting its protective properties [56].

The contribution of IR to hypertension relates to several mechanisms. IR enhances renal sodium reabsorption, resulting in an increase in extracellular fluid volume and thus an elevation in blood pressure [57]. Furthermore, insulin increases the activity of the sympathetic nervous system, which stimulates norepinephrine and leads to an increase in blood pressure [58].

As noted previously, IR inhibits glucose uptake in insulin sensitive tissues. IR also impairs the ability of insulin to suppress hepatic glucose production, resulting in impaired glucose tolerance or elevated fasting plasma glucose. Although the mechanisms of IR are complex and have yet to be fully elucidated, Figure 2-3 depicts the relationships of IR with various metabolic risk factors that were discussed in this section.
2.2.4 Measurement of Insulin Resistance

The gold standard technique for assessing insulin sensitivity is the hyperinsulinemic euglycemic clamp [60]. In brief, insulin is continuously infused into an individual, and euglycemia is maintained at basal levels via a variable glucose infusion rate. Because the exogenous hyperinsulinemia suppresses hepatic glucose production, the glucose infusion rate is equivalent to the glucose disposal within bodily tissues, thus quantifying IR. The hyperinsulinemic euglycemic clamp is primarily used as a research tool because the procedure is invasive and unsuitable in a clinical setting.

The oral glucose tolerance test (OGTT) is an alternative method commonly employed to determine IR. To commence this test, an oral glucose load is administered to a patient under
fasting conditions. Afterward, blood is drawn from the patient at consecutive time periods in order to record plasma glucose and insulin concentration in response to the glucose challenge. Many IR indices have been derived utilizing OGTT results [61-65]. These indices have statistical associations \((r = 0.43 – 0.55)\) with the hyperinsulinemic euglycemic clamp, however, they exhibit substantial error \((\sim 50\%)\). Thus, OGTT derived indices may provide useful information on a population level, but their utility on an individual basis is questionable [66]. A major difference between IR data from the hyperinsulinemic euglycemic clamp versus the OGTT is the contribution of the liver toward whole body insulin sensitivity. Hepatic glucose output is suppressed during the hyperinsulinemic euglycemic clamp, whereas the OGTT reflects both hepatic and muscular contributions to IR. Nonetheless, OGTTs are a practical tool for examining IR in a clinical setting.

### 2.2.5 Insulin Resistance and Risk for Future Chronic Disease

IR is a physiological abnormality that both precedes [67] and is characteristic of type 2 diabetes [68]. IR is also linked to cardiovascular disease [69], and is associated with cardiovascular mortality independent of other risk factors [70]. With the prevalence of type 2 diabetes and cardiovascular disease increasing, the monitoring of IR is important in order to screen for individuals at risk for these diseases, and engage them in preventive treatment strategies. Individuals who are at elevated risk include those who are abdominally obese, men, elderly, and ethnic minorities [71-74]. The prevention of IR is paramount given that cardiovascular disease is the number one cause of global mortality [75].

### 2.3 Physical Activity, Sedentary Behaviour, and Insulin Resistance

#### 2.3.1 Physical Activity and Sedentary Behaviour: Guidelines and Problems

It is established that a single exercise session is associated with reductions in IR that approximate 20%, and this benefit persists for up to 2 days [76]. In addition, chronic exercise in the absence of significant weight loss can improve IR [77, 78]. These findings demonstrate that
PA is a potent modifiable risk factor for IR, hence it is a cornerstone in the guidelines for the treatment and management of risk factors for type 2 diabetes [79].

The PA recommendations by the Canadian Diabetes Association (CDA) are congruent with the Canadian Physical Activity Guidelines, suggesting that adults accumulate 150 minutes of moderate-to-vigorous intensity PA (MVPA) per week on at least 3 separate days, in bouts of at least 10 consecutive minutes [10, 79, 80]. The CDA also states that adults should engage in resistance exercise 3 times per week and limit recreational SED to no more than 2 hours per day.

Although it is known that adherence to the Canadian Physical Activity Guidelines is associated with an improvement in IR, a mere 15% of Canadian adults comply with the recommendations [12]. Furthermore, Canadian adults typically spend 69% of their day engaged in SED, which has been defined as “any waking behaviour characterized by an energy expenditure ≤ 1.5 metabolic equivalent tasks (METs; 1 MET corresponds to the resting metabolic rate or 3.5 ml O₂/kg/min) while in a sitting or reclining posture” [12, 81]. This is worrisome, as SED is associated with an increased risk of mortality and metabolic risk factors [82-84]. 12-year follow-up data from the Canada Fitness Survey exhibits a dose-response relationship between daily sitting time and all-cause and cardiovascular disease mortality [82]. This finding remained significant upon controlling for the respondents’ PA level.

Given the emerging evidence that PA and SED have distinct health consequences, they must be considered separately [85]. In addition, because the vast majority of Canadians fail to meet PA guideline recommendations, the benefits of substituting time spent in SED with light PA (LPA) or PA that is performed in a sporadic fashion (bouts < 10 minutes) should be further investigated. LPA includes activities of daily living, and requires 1.5-3 METs.

The energy required to support the muscular contractions during LPA throughout the day may be far greater than the energy demand for a bout of MVPA [20]. There is also a large degree of variability in daily LPA energy expenditure among individuals. For example, workers
who stand (such as shop assistants and homemakers) expend approximately 1400 kcal/day engaged in LPA, whereas seated workers expend approximately 700 kcal/day [86]. This provokes the question as to whether the accumulation of larger doses of LPA throughout the day will ameliorate IR and other metabolic risk factors. The relationship between these variables, along with MVPA and SED, will be reported in the following section.

2.3.2 Associations of Physical Activity and Sedentary Behaviour with Insulin Resistance

Discrepant findings have been reported in the literature regarding the relationship between PA and SED with IR. Healy et al [13, 87] and Ekelund et al [14, 88] were the first to use accelerometers to objectively assess the relationship between sporadic PA and SED with IR. In a sample of Australian adults, Healy and colleagues reported that, independent of MVPA, LPA and SED were significantly associated with 2-hour glucose, waist circumference, and metabolic risk factors [13, 87]. These findings suggest that rather than emphasizing the importance of increasing MVPA, it may be advantageous to encourage individuals to replace SED with LPA.

In contrast, Ekelund and colleagues [14] reported that sporadic MVPA was associated with IR independent of time spent in SED and LPA in individuals with a family history of type 2 diabetes. These findings are congruent with those from their original study, which demonstrated that the univariate association of SED with metabolic risk factors was attenuated by MVPA and total PA (TPA) [88]. These observations suggest that the intensity of sporadic PA is of great importance in order to achieve health benefits.

Table 2-1 compiles the literature on the relationship between objectively measured PA and SED with IR. Similar to findings from Ekelund and colleagues [14, 88], Balkau and associates [89] reported that time spent in SED and LPA did not impact insulin sensitivity— which was measured by the gold standard hyperinsulinemic-euglycemic clamp— independently of TPA in European adults. However, unlike Ekelund [14], Balkau [89] did not observe an independent relationship between MVPA and insulin sensitivity. This lack of association may have been due
to their methodological decision to solely assess bouted MVPA. Participants were required to be engaged in MVPA for a minimum of 10 consecutive minutes, thus sporadic PA was excluded from their analysis.

A recent study by Peterson and colleagues [90] produced similar findings to Ekelund [14, 88] and Balkau [89]. Among a sample of 5268 Americans ages 20-85 years, they reported that time spent in SED did not predict metabolic risk factors after accounting for MVPA. Individuals accumulating the highest MVPA (approximately 43-48 minutes per day) exhibited similar obesity and metabolic profiles, irrespective of SED. Moreover, LPA was not significantly associated with metabolic risk factors in men or women. These studies suggest that there may be a threshold of PA intensity that individuals must engage in to effectively reduce metabolic risk factors.

In contrast, cross-sectional findings from others [91-95] are consistent with those from Healy and colleagues [13, 87], suggesting that the accumulation of sporadic MVPA is not a major contributor to the development of metabolic risk. For example, Henson et al [93] demonstrated that SED, which was measured using accelerometry in a population presenting risk factors for type 2 diabetes, displayed a stronger association with metabolic risk factors than TPA and MVPA. In a sample of young adult women, Green et al [95] indicated that LPA and HOMA-IR were associated, independent of MVPA. This relationship was attenuated, however, by VO$_2$ peak and body composition, suggesting that LPA may elicit its effect on IR indirectly.

Furthermore, a recent intervention study by Duvivier and colleagues [96] suggests that an hour of daily exercise cannot compensate for the adverse consequences of SED associated with insulin metabolism. Healthy, young adults performed three PA regimes for four days in random order. These regimes included: sitting 14 hours per day, sitting 13 hours per day along with 1 hour of vigorous exercise, and the substitution of 6 hours of sitting with 4 hours of walking and 2 hours of standing. Duvivier et al found that when matching for energy expenditure, increasing the time spent walking or standing beneficially impacted insulin sensitivity more so than engaging in an hour of exercise. These findings are in agreement with the original work by
Healy and colleagues [13, 87], suggesting that lifestyle-based strategies should focus on replacing inactivity with low intensity activities.

Prior studies from our group have suggested that sporadic TPA and SED are not associated with IR among inactive, abdominally obese adults [97, 98]. Accumulation of approximately 10.5 hours of SED, 5 hours of LPA, and 20 minutes of sporadic MVPA per day were not associated with 2-hour glucose or HOMA-IR [97]. Shalev-Goldman and colleagues [98], however, observed an association of sporadic MVPA duration with fasting insulin and the homeostasis model of assessment to estimate insulin sensitivity. This relationship was abolished after adjusting for cardiorespiratory fitness (CRF) and waist circumference (WC). The lack of an independent association by SED and PA with IR demonstrate the need to explore dietary behaviours in order to acquire understanding on the primary determinants of IR and metabolic risk among inactive, abdominally obese adults.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample</th>
<th>Measurement</th>
<th>Adjusted</th>
<th>SED</th>
<th>LPA</th>
<th>MVPA</th>
<th>TPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balkau et al. 2008</td>
<td>346 M, 455 W</td>
<td>Hyperinsulinemic-euglycemic clamp</td>
<td>Age, center, TPA</td>
<td>β [SE]= 0.0005 [0.0022], p=0.8</td>
<td>β [SE]= 0.0001 [0.0019], p=0.9</td>
<td>---</td>
<td>β [SE]= 0.24 [0.04], p=0.0001</td>
</tr>
<tr>
<td>Ekelund et al. 2009</td>
<td>81 M, 111 W</td>
<td>Fasting insulin</td>
<td>Age, sex, smoking, WC, SED, LPA, TV watching</td>
<td>β=0.0004 [95% CI -0.0006 to 0.001], p=0.39</td>
<td>β=0.0002 [95% CI -0.001 to 0.001], p=0.73</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Healy et al. 2011</td>
<td>2378 M, 2379 W</td>
<td>Fasting insulin</td>
<td>Age, sex, race, quartiles of exercise</td>
<td>Adjusted Mean [SD]= 42.1 [24.6, 73.3], p&lt;0.001</td>
<td>---</td>
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</tr>
<tr>
<td>McGuire et al. 2011</td>
<td>43 M, 92 W</td>
<td>HOMA-IR</td>
<td>Accelerometer wear time, WC</td>
<td>β=0.00 [95% CI -0.00 to 0.00], p=0.71</td>
<td>β= -0.01 [95% CI -0.05 to 0.02], p=0.39</td>
<td>---</td>
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</tr>
<tr>
<td>Nelson et al. 2013</td>
<td>213 M, 189 W</td>
<td>HOMA-IR</td>
<td>Adiposity, sex, age, CRF</td>
<td>---</td>
<td>β= -0.0154, p=0.184</td>
<td>β= 0.1457, p=0.035</td>
<td>---</td>
</tr>
<tr>
<td>Green et al. 2014</td>
<td>50 W</td>
<td>HOMA-IR</td>
<td>MVPA, CRF, body mass or composition</td>
<td>---</td>
<td>β= -0.239, p=0.077</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Maher et al. 2014</td>
<td>2402 M, 2216 W</td>
<td>Fasting insulin</td>
<td>Socio-demographic, medical history, smoking, alcohol, diet, TPA</td>
<td>β= 0.02, p&gt;0.05</td>
<td>---</td>
<td>---</td>
<td>β= -0.27, p&lt;0.001</td>
</tr>
<tr>
<td>Shalev-Goldman et al. 2014</td>
<td>141 W</td>
<td>iHOMA2-IS</td>
<td>Age, CRF, WC</td>
<td>---</td>
<td>---</td>
<td>CC= 0.17, p&gt;0.05</td>
<td>CC= 0.12, p&gt;0.05</td>
</tr>
<tr>
<td>Wijndaele et al. 2014</td>
<td>79 M, 92 W</td>
<td>Fasting insulin</td>
<td>Age, sex, socio-economic status smoking, medication, monitor wear time, MVPA, SED</td>
<td>B= 2.85 [95% CI -2.91 to 8.62], p&gt;0.05</td>
<td>B= -17.25 [95% CI -38.34 to 3.84], p&gt;0.05</td>
<td>---</td>
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</tr>
</tbody>
</table>

--- = not assessed, β = standardized regression coefficient, B = unstandardized regression coefficient, CC = correlation coefficient. SED = sedentary behaviour, LPA = light intensity physical activity, MVPA = moderate-to-vigorous physical intensity, TPA = total physical activity. M = men, W = women, WC = waist circumference. CRF = cardiorespiratory fitness. HOMA-IR = homeostasis model assessment of insulin resistance, iHOMA2-IS = homeostasis model of assessment to estimate insulin sensitivity.
2.3.3 Mechanisms Underlying Physical Activity, Sedentary Behaviour, and Insulin Resistance

The mechanisms to explain the transient improvement in IR following an acute bout of PA have not been fully elucidated. They may relate to the increased translocation of GLUT-4 into the sarcolemma and T-tubules of the muscle [102], which occur likely as a result of muscle contraction [103, 104]. The translocation of GLUT-4 during PA in an insulin resistant muscle is not the result of an amendment to the impaired insulin signalling cascade; it occurs via a unique, separate pathway. For example, studies in humans and rats have demonstrated that exercise stimulation, but not insulin stimulation, induce the translocation of GLUT-4 to the plasma membrane in insulin resistant muscle [102, 105]. Although beyond the scope of this review, because exercise does not ameliorate the defects associated with impaired insulin signalling—such as a reduction IRS-1 tyrosine phosphorylation or PI3K activity— it has been speculated that the translocation of GLUT-4 may be mediated by 5'-AMP activated protein kinase [106].

In contrast to acute PA, the improvements in IR elicited by chronic PA may stem from reductions in visceral adipose tissue [107] and enhanced expression of GLUT-4 in skeletal muscle [108, 109]. As noted earlier, chronic PA without weight loss is associated with significant reductions in visceral fat [107, 110]. Weight loss, however, will augment the magnitude of visceral fat reduction, thus increasing the likelihood for a substantive improvement in IR. Furthermore, chronic PA may compensate for defects in insulin signaling and insulin-mediated GLUT-4 translocation by overexpressing the GLUT-4 protein [111]. The abundance of GLUT-4 protein levels can enhance insulin-mediated glucose transport into the muscle, without correcting the deficiencies within the signalling cascade.

The mechanisms linking SED with IR are postulated to be unique from those associated with PA. There is evidence that SED is associated with low levels of LPL - an enzyme that facilitates the uptake of free fatty acids into muscle and adipose tissue- which is linked to
dyslipidemia [20]. As discussed in section 2.2.3, dyslipidemia further perpetuates the severity of IR. In a study examining the effects of 11 days of bed rest in healthy Japanese subjects, an 18% reduction in LPL activity was realized, along with significant increases in plasma triglycerides and decreases in HDL-C [112]. In a study by Bey and Hamilton, a reduction in LPL activity occurred in a dose-response fashion following the elimination of any weight-bearing activity in rats [113]. After 12 hours without PA, LPL was reduced by more than 50% in the rats. LPL activity returned to baseline levels 4 hours post-restoration of the ability to perform PA.

Interestingly, it has been found that the reduction in LPL activity in response to SED occurs in oxidative fibres, whereas improvements in LPL activity elicited by PA occur mainly in glycolytic fibres [114, 115]. This suggests that the effects of SED and PA occur via separate pathways. The relative reduction in LPL activity seen in oxidative fibres following SED are more than 4-fold greater than the increases observed in glycolytic fibres following vigorous PA. This demonstrates the importance of both PA and SED.

In addition to LPL activity, SED may impact GLUT protein content. Studies in individuals with spinal cord injuries have demonstrated substantial increases in GLUT content following the transition from SED to LPA [116, 117]. For example, Chilibeck and colleagues employed 8 weeks of functional electrical stimulation exercise in paralyzed skeletal muscle [116]. This resulted in a 52% increase in GLUT-1 content (responsible for basal glucose uptake), 72% increase in GLUT-4 content, and a significant improvement in insulin sensitivity (determined by an OGTT). These findings suggest that drastic improvements in GLUT content and glucose tolerance may occur when sedentary individuals engage in LPA.

### 2.3.4 Measurement of Physical Activity and Sedentary Behaviour

A plethora of methods exist for measuring PA and SED. These include, but are not limited to: self-report questionnaire, accelerometers, pedometers, heart rate monitors, direct observation, doubly labeled water, and calorimetry. Each of these tools has strengths and weaknesses; however, study designs often dictate the most suitable method. An overview of the
tools most commonly encountered within the studies discussed throughout this review is provided below.

Self-report questionnaires are a common method for assessing PA and SED in large, epidemiological studies. Despite being cost-effective and feasible, they are a subjective measure of PA and SED, predisposing them to recall and social desirability bias [21]. In addition, self-report questionnaires have difficulty capturing sporadic or unstructured activities, whereas structured activities that occur over a period of time (ex. sports, television watching) are captured fairly well [118, 119].

Objective measures of PA, such as pedometers and accelerometers, can overcome the biases inherent to self-reported data. These devices, however, are not without limitations. Pedometers indicate the number of steps that an individual walks within a given time frame. Although feasible for large, epidemiological studies, certain activities may affect the validity of the data. For example, the step count may not be accurate in high speed conditions. If an individual embarks on a run, their PA may be underestimated [120]. In addition, pedometers can only measure walking; they do not provide data on total PA or SED. Despite these limitations, pedometers may be a useful tool for encouraging and monitoring individuals to become more active by developing a ‘steps per day’ goal [121].

Accelerometers are a relatively novel tool for characterising PA and SED. Their use in PA research proliferated in the 2000s [22]. Accelerometers are motion sensors that are commonly worn on the hip. They provide objective, time-sensitive data on the frequency, intensity, and duration of PA and SED. Equations are used to convert their raw counts into physiologically meaningful data, such as intensity levels or energy expenditure.

Although accelerometers have been shown to predict metabolic health more strongly than self-report questionnaires, they are not without limitations [122]. For example, they cannot detect upper body movements, movement up an incline, water-based activities, cycling, or
distinguish between sedentary behaviours [85]. These are not their only limitations- issues arise with data cleaning, reduction, and interpretation.

A standard protocol for accelerometer data cleaning is lacking in the literature. This inconsistency makes it difficult to compare findings from various studies. For example, studies identify non-wear periods using different criteria for the number of minutes of consecutive zero counts [13, 84, 123]. The discrepancy in non-wear time criteria varies by as much as 70 minutes; this can greatly impact the time spent in SED and thus the study’s findings.

Another issue with accelerometers involves the comparison of data between different models. As noted above, the raw data from accelerometers are converted into counts, which are then converted into physiologically relevant units to compare data between studies and determine the time spent in various intensities of PA. There are many different equations for establishing cutpoints to distinguish the intensity of PA. A limitation of Freedson’s cut-points, which are commonly utilized by researchers, is that they were originally developed using a young adult population [124]. The intensity levels may not be generalizable to a population of inactive, abdominally obese adults. Although there are numerous limitations to consider, accelerometer validity and reliability studies have been a focus in the recent literature [125, 126]. These, along with future studies, will equip researchers with the knowledge they need to ensure they are adhering to accurate methods for reporting their data.
2.4 Diet and Insulin Resistance

2.4.1 Diet: Recommendations and Problems

Traditional dietary recommendations for the prevention of risk factors associated with cardiovascular disease involve the consumption of low fat foods, perhaps because fat is the most energy dense macronutrient. Data from the National Health and Nutrition Examination Survey (NHANES) demonstrates that Americans have complied with this suggestion: the percentage of dietary fat has decreased from 36.6% to 33.7% during the years of 1971 to 2006, however the percentage of dietary carbohydrates has increased from 44.0 to 48.7% [127]. Furthermore, total energy intake has increased during this time period, and the obesity epidemic (as well as IR) is escalating. According to the 2004 Canadian Community Health Survey, 5 in 10 women and 7 in 10 men have energy intakes that exceed their caloric requirements [15].

Obesity is a driving force in the development of IR, thus, an elevated quantity of energy (measured in kilocalories, but often abbreviated as ‘calories’) consumed can facilitate the deposition of adipose tissue and impair insulin sensitivity [128, 129]. Because weight loss is almost always associated with an improvement in IR, a balanced, sustainable, hypocaloric diet can reduce IR in obese individuals [130]. The contributions of dietary fats and carbohydrates to IR have also received considerable attention in the literature.

The current CDA dietary guidelines advise individuals to consume a variety of foods specifically, a minimum of 3 out of the 4 food groups at each meal from Eating Well with Canada’s Food Guide [16, 131]. These food groups include vegetables and fruit, grain products, milk and alternatives, and meat and alternatives. Furthermore, the CDA recommends refraining from sweets and opting for foods that are high in fibre and low in fat. Appropriate portion sizes should be eaten in order to maintain a healthy body weight. The following subsections within section 2.4 provide greater detail on the associations between diet and IR, with a particular emphasis on the importance of overall dietary quality.
2.4.2 Dietary Fat and Insulin Resistance

Consumption of a low fat diet was a common strategy employed for the prevention of obesity and related chronic diseases. Equivocal evidence exists, however, on the relationship between fat intake, obesity, and IR. Results from the 2004 Canadian Community Health Survey demonstrate that higher consumption of kilocalories— but not the relative amount of fats— increased the odds of obesity among Canadian adults [132]. In agreement with this notion, several clinical trials have demonstrated that high fat diets (up to 40% of total energy) affect body weight to a similar extent compared to diets consisting of a lower fat content, when energy is restricted [133, 134]. Because body weight reduction is a putative mechanism for ameliorating IR, these findings suggest that a high fat diet may not be detrimental to IR if it does not contain excess calories.

When examining the relationship between dietary fat and IR directly, it appears that the quality of dietary fat is of importance. Dietary fatty acids (FA) vary in terms of their chemical structure and position or configuration of bonds: they may be saturated (SFA), trans (TFA), monounsaturated (MUFA), or polyunsaturated (PUFA; further subdivided as n-3 or n-6). These differences may alter the rates of FA oxidation or deposition, impacting IR [135]. Although definitive evidence does not exist, it appears that MUFAs and PUFAs are associated with various metabolic benefits, whereas SFAs and TFAs may be detrimental [136]. An in-depth discussion of this concept is beyond the scope of this review.

In support of the aforementioned notion, 162 healthy adults were randomized into a controlled, isoenergetic diet containing either a high proportion of SFAs or MUFAs in the 3 month KANWU study [137]. Substitution of SFAs by MUFAs reduced IR by approximately 10%, indicating that the quality of FAs affected IR. Furthermore, when fat intake surpassed 37% of daily energy intake, insulin sensitivity deteriorated independent of the quality of the FAs consumed. This suggests that regardless of FA quality, there is a threshold for the quantity of MUFAs to elicit improvements in IR. Others have
produced similar findings to the KANWU study. Xiao et al demonstrated that acute feeding of SFAs but not MUFAs or PUFAs resulted in decreased insulin sensitivity [138]. In a randomized, crossover study including both healthy and diabetic participants, Summers [139] found that IR improved on a diet rich in PUFAs compared with the diet rich in SFAs.

The mechanisms underlying the effects of FA quality on IR are not entirely understood. SFAs may increase intramyocellular lipid content, thus driving IR [140]. MUFA and PUFA-induced improvements in IR may relate to their effects on cell membranes. They may enhance membrane fluidity, ion permeability, and insulin receptor binding or affinity [141]. MUFAs may also facilitate the up regulation of glucose transporters [140].

Although there appears to be a trend in which MUFAs and PUFAs modulate IR in comparison to SFA, there have been clinical trials in which no difference has occurred [130]. Furthermore, MUFAs are not associated with reduced risk of type 2 diabetes in prospective cohort studies [142, 143]. These inconsistent findings contribute to the lack of definitive associations between dietary fat and IR in the literature.

2.4.3 Dietary Carbohydrates and Insulin Resistance

A diet rich in carbohydrates can be detrimental to those that are insulin resistant. Carbohydrate intake is the primary determinant of post-prandial glycemia [144]. Under such conditions, more insulin is required to maintain euglycemia. The resulting carbohydrate-induced hyperinsulinemia is correlated with hepatic VLDL-triglyceride synthesis, thereby initiating hyperlipidemia [129]. In addition, consumption of a high carbohydrate diet without a concomitant reduction in overall energy intake will favour weight gain, perpetuating IR.

High carbohydrate consumption, defined as ranging from 48 to 63% of total energy intake, has been associated with an increased risk for the MetS in prospective studies [145, 146]. However, large observational studies have produced conflicting evidence surrounding the association between total
carbohydrate intake and diabetes risk [147, 148]. These inconsistent findings highlight the importance of considering the quality of the carbohydrates ingested.

A carbohydrate’s quality is reflective of its glycemic index (GI), fibre content and simple sugar content. The GI refers to the blood glucose response to a carbohydrate portion of food relative to the same carbohydrate quantity of a reference food. High GI foods, such as sugar-sweetened-beverages, result in an abrupt increase in postprandial blood glucose, thereby initiating hyperinsulinemia. A meta-analysis demonstrated a clear relationship between GI and the risk for type 2 diabetes [149]. Furthermore, a high dietary GI has been associated with adverse changes in body weight, body fat, and waist circumference [150, 151]. These consequences will affect IR indirectly, as waist circumference is a strong predictor of glucose and insulin metabolism [24].

Insoluble fibre- found in whole grain foods such as cereals, legumes, and nuts- is inversely related to incident type 2 diabetes [152-154]. In studies employing the gold standard hyperinsulinemic euglycemic clamp technique, cereal fibre intake improved IR [155, 156]. Many low GI diets are rich in cereal fibre, accentuating the difficulty to decipher the independent effects of GI and fibre with IR [157]. This supports the rationale for analyzing dietary patterns rather than single components of complex foods.

### 2.4.4 Dietary Patterns and Insulin Resistance

The conflicting findings from studies that analyzed the effects of single nutrients on IR may be due to the complex interactions among nutrients within whole foods. For example, clinical trials that manipulate the intake of an individual nutrient within an isocaloric diet will inevitably alter the quantity of other nutrients. In addition, many nutrients are correlated- such as GI and fibre- creating difficulty to disentangle their separate effects [157]. The effect of a single nutrient may be insignificant, but the cumulative effect of multiple nutrients may yield a measurable result [158]. Furthermore, people eat meals that consist of foods containing a combination of nutrients; therefore the assessment of patterns is more representative of an individual’s overall diet.
Various dietary patterns have been associated with improvements in IR as well as MetS. The dietary habits of populations from the Mediterranean region- Greece and southern Italy- have received ample attention due to their association with reduced all-cause and cardiovascular mortality [17, 159, 160]. The Mediterranean diet is primarily plant-based, including mostly vegetables, fruits, nuts, legumes, cereals, and whole grains. Red meat is consumed in low quantities, whereas fish and poultry are consumed in moderation. Extra virgin olive oil, which is rich in MUFAs, is used regularly. Modest wine consumption is another characteristic of this dietary pattern [161]. Mediterranean diet may be beneficial for IR, independent of weight loss [162]. In non-diabetic, Greek adults, higher adherence to the Mediterranean diet was associated with lower IR, which was assessed by HOMA-IR [163]. Moreover, in an intervention involving the consumption of a non-calorie restricted traditional Mediterranean diet, a reduction in the incidence of diabetes was realized at 4 years follow-up despite the absence of significant weight loss [164].

The palatability and satiating effect of a diet is extremely important in order to ensure compliance and long-term sustainability [165]. The Mediterranean diet is feasible in this regard, as it is a high volume diet with low energy density. Other dietary patterns can provide similar metabolic benefit. For example, the ‘prudent diet’ is akin to the Mediterranean diet and consists of high quantities of vegetables, legumes, whole grains, fruit, fish, poultry, and low-fat dairy products [19]. The prudent diet is commonly recommended for improved health in Western cultures [136]. It differs from the Mediterranean diet in that it contains a lower fat content. Observational studies have documented that the prudent diet is associated with a reduced risk of diabetes [18, 136, 166]. Nettleton and colleagues demonstrated that this was true even among ethnic minorities, who are known to be at elevated risk for IR [73, 74, 166].

In opposition to such the Mediterranean and prudent diets, a diet consisting of red meat, processed meat, French fries, high fat dairy products, sweets, and refined grains is known as the ‘Western diet’ [19]. This dietary pattern has been associated with elevated risk for type 2 diabetes in
It is noteworthy that the Mediterranean and prudent diets consist of the high quality FAs and carbohydrates described in the subsections above, whereas the Western diet contains nutrients that are lower in quality.

### 2.4.5 Measurement of Diet Quality

Diet quality indices assess dietary variety, and thus the interactions between various nutrients. They follow the notion that nutrients act synergistically, rather than independently, with one another. Upon considering both quantity and quality of foods, diet quality indices produce a score that is reflective of a particular dietary pattern. Numerous indices exist, and it is beyond the scope of this review to critically evaluate them all [169].

A recent study by Reedy and colleagues evaluated the associations between 4 indices- the Healthy Eating Index-2010 (HEI-2010), the Alternative Healthy Eating Index-2010 (AHEI-2010), the Alternate Mediterranean Diet (aMed), and Dietary Approaches to Stop Hypertension (DASH)- with all-cause, cardiovascular disease, and cancer mortality in a prospective cohort study [170]. These 4 indices are similar in that they place emphasis on the same core tenets: consumption of whole grains, vegetables, fruit, and plant-based proteins. Perhaps unsurprisingly, high adherence to each index was associated with a lower risk of mortality outcomes for both men and women [170].

Although there are commonalities among the aforementioned diet indices, subtle differences exist. The HEI-2010 quantifies adherence to the American federal dietary guidelines, and it scores components on a density basis (per 1000 kilocalories) [171]. The AHEI-2010 is representative of Harvard’s Healthy Eating plate, and it was developed to incorporate current scientific evidence on diet quality and health, thus improving the HEI-2010. It scores 11 components- whole grains, vegetables (excluding potatoes), fruit, nuts and legumes, trans fat, n-3 FAs, PUFAs, alcohol, red and processed meat, sugar-sweetened beverages and fruit juices, and sodium- for a total of 110 points. Both the HEI-2010 and the AHEI-2010 resemble the prudent dietary pattern. The aMed score is reflective of a Mediterranean diet that was adapted for use in an American population [172], whereas the DASH
score exemplifies a dietary pattern associated with blood pressure [173]. These 2 indices have greater simplicity in that their scores are produced based on the population's median and quintile-based intakes.

It appears that the AHEI-2010 may be best suited for assessing the relationship between diet quality and IR. In a study by Chiuve and colleagues, the HEI-2005 and the AHEI-2010 were compared among 71,495 women in the Nurses’ Health Study and 41,029 men in the Health Professionals Follow-Up Study [174]. Both indices similarly predicted risk of stroke and cancer, however, the AHEI-2010 was more strongly associated with risk of coronary heart disease and diabetes (p< 0.001). Higher scores on the HEI-2005 were associated with 18% lower risk of diabetes, whereas higher scores on the AHEI-2010 were associated with 33% lower risk of diabetes. Because IR is implicated in the pathogenesis of diabetes and cardiovascular disease, the findings by Chiuve suggest that the AHEI-2010 is more reflective of a dietary pattern preventive of IR. Other studies have also supported this notion [175, 176].

Although diet quality indices capture dietary patterns and variety, it is noteworthy that they are limited by the subjective nature inherent to self-reported data. Common methods for participants to report their dietary intakes include food records, food frequency questionnaires, and 24 hour recalls. Studies have demonstrated that diet is reported with error, and this error is augmented when participants are aware that they are being evaluated [177, 178]. In a study investigating the disparity between reported and actual caloric intakes within the NHANES dataset, it was uncovered that an under-reporting of up to 850 kilocalories occurs per day [179]. Despite the bias associated with self-reported data, these methods are feasible and remain common protocol.
2.5 Interactions Between Physical Activity, Sedentary Behaviour, and Diet with Insulin Resistance

Although uncertainties remain, the separate effects of PA, SED and diet are well documented in the literature. The interaction between these modifiable risk factors is also important to consider as they capture both sides of the energy balance equation. The synergistic effects of PA and diet have yet to be fully elucidated.

Various studies have assessed the interaction between PA, SED, and diet [180-187]. These studies, however, utilize subjective measures of PA and SED, and do not focus on IR per se. A relationship between increased PA levels and a healthful diet has been documented in several studies [180-182, 185-187]. Furthermore, engaging in SED has been associated with consumption of a poor quality diet [182, 185, 186], although the contrary has also been reported [187]. In regards to SED, Charreire and colleagues reported that television viewing time- measured as a surrogate of SED- was related to an ‘unhealthy’ food pattern among French adults [185]. Wagner and colleagues observed a dose-response relationship between SED and the energy-dense dietary pattern with MetS, independent of PA [186]. In contrast, Monfort-Pires found that SED was not predictive of poor dietary quality [187]. These conflicting findings may stem from the use of various methods to assess diet, or the lack of an objective measure for SED. Future research should examine the interaction of these modifiable risk factors using objective data for the activity variables.

The associations of subjectively measured modifiable risk factors with mortality have been documented in the literature [183, 184]. van Dam and colleagues [183] reported that 28% of cardiovascular deaths at follow-up in the Nurses’ Health Study could be attributed to PA below consensus guidelines, 18% to poor diet quality, and 31% to being overweight. In the Aerobics Centre Longitudinal Study, a diet high in processed and red meats, white potato products, refined grains, and added fats was a modest risk factor for all-cause mortality [184]. After controlling for CRF-a marker of PA in recent months- the risk estimate was attenuated by 55%. Thus fitness, which is reflective of
recent PA behaviours, largely confounded the diet-mortality relationship. Because fitness has a genetic component [188], future investigations should use accelerometers to objectively assess the behaviours of PA and SED and determine whether the aforementioned relationship remains. Few studies have reported the interaction between objectively measured PA and SED with diet and IR.

### 2.6 Concluding Remarks

The independent and combined effects of modifiable risk factors- PA, SED, and diet- on IR remain unclear. Given that approximately 40% of the Canadian population is abdominally obese and the vast majority of Canadians do not engage in sufficient PA, the risk for the development of IR and future cardiovascular disease in this population is substantive [12, 25]. SED and PA below guideline recommendations have been inconsistently associated with IR. Whether diet is associated with IR either directly or indirectly through the deposition of abdominal adipose tissue requires clarification. Absent from the literature is an assessment of the interaction between diet and objectively measured PA and SED with IR in an at-risk population. These concepts will be examined in the investigation in Chapter 3.
Chapter 3

Sedentary Behaviour and Light Physical Activity are Associated with Insulin Resistance among Inactive Abdominally Obese Adults
ABSTRACT

**Background:** Physical activity (PA), sedentary behaviour (SED), and diet are 3 modifiable risk factors implicated with insulin resistance (IR). PA in accordance with consensus guidelines is associated with improvements in IR, however, this relationship becomes unclear when PA is accumulated below consensus recommendations. SED is a relatively novel risk factor, and may be associated with IR independent of PA. Although adherence to dietary guidelines may attenuate IR, the optimal dietary pattern for the prevention of IR is uncertain. Few studies have considered the interaction between PA, SED, and diet with IR.

**Objective:** The primary aim was to examine the associations of IR with objectively measured PA, SED, and self-reported diet quantity and quality among inactive, abdominally obese adults.

**Methods:** Participants were 102 inactive, abdominally obese adults recruited from Kingston, Canada. PA and SED were assessed using accelerometers. Dietary intake was determined using self-reported food diaries, and assessed in terms of quantity (total kilocalories consumed and grams per macronutrient consumed) and quality (Alternative Healthy Eating Index 2010). A 75 gram oral glucose tolerance test was administered to measure IR using the homeostasis model of assessment for insulin resistance (HOMA-IR) and insulin area-under-the-curve (insulin AUC) as the two primary outcomes.

**Results:** Participants spent (mean ± SD) 623.9 ± 86.2 minutes per day (min/d) in SED, 287.5 ± 82.4 min/d in light PA, and 20.5 ± 15.4 min/d in moderate-to-vigorous PA. Participants consumed 1907.1 ± 465.7 kilocalories per day, with 35% energy from fat, 47% from carbohydrate, and 17% from protein. The percentage of time spent in SED and light PA were associated with both HOMA-IR (SED: \( r = 0.34, p < 0.01 \); light PA: \( r = -0.32, p <0.01 \)) and insulin AUC (SED: \( r = 0.27, p < 0.01 \); light PA: \( r = -0.25, p ≤ 0.01 \)). Neither diet quantity nor diet quality were associated with HOMA-IR or insulin AUC.

**Conclusions:** Objectively measured SED and light PA were associated with measures of IR, suggesting that the substitution of SED with modest amounts of PA accumulated below consensus recommendations may improve IR among inactive abdominally obese adults.
INTRODUCTION

It is well-established that insulin resistance (IR) is a prominent risk factor for type 2 diabetes and cardiovascular disease, both of which are leading causes of mortality worldwide [69, 75]. Also well documented is that physical activity (PA) consistent with consensus recommendations- 150 minutes of moderate-to-vigorous PA per week in bouts of at least 10 minutes [10]- is associated with marked reductions in IR [11], and this remains true independent of age, gender, and race [189, 190]. In addition, diet quantity and quality are known to influence IR, hence nutrition is paramount within consensus guidelines for the prevention and treatment of type 2 diabetes [131]. Finally, sedentary behaviour (SED) is a novel and emerging risk factor for type 2 diabetes and cardiovascular disease, and it is thought to be associated with IR independent of PA [13, 20].

Although PA in accordance with the guidelines [10] attenuates IR, it is unclear whether PA below guideline recommendations provides benefit. Healy and colleagues [13, 87] were the first to indicate that light physical activity (LPA) and SED predicted 2-hour glucose beyond time spent in moderate-to-vigorous physical activity (MVPA). In contrast, Ekelund et al [14] reported that MVPA duration was associated with IR independent of time spent in SED and LPA among individuals with a family history of type 2 diabetes. Several studies support the notion that MVPA duration elicits a stronger effect on IR than SED or LPA [89, 99], whereas others report the opposite [84, 95]. Although minor differences relating to participant characteristics and accelerometry data reduction techniques exist between studies, reasoning for the discrepant findings is uncertain and the independent contributions of PA and SED remain highly scrutinized [2].

Given that diet is on the opposite side of the energy balance equation from PA, these two modifiable risk factors are interrelated and may act synergistically with one another [191]. Diets rich in whole grains, legumes, fruits, vegetables, and nuts; moderate alcohol consumption; and lower in refined grains, sugar-sweetened beverages, and red or processed meats have been shown to attenuate the risk of type 2 diabetes and enhance glycemic control among individuals with type 2
diabetes [192]. However, the optimal dietary pattern for the prevention of IR remains uncertain at this time [193]. Furthermore, given that obesity- particularly abdominal obesity- is a major determinant of IR [23, 24], whether it may lie within the causal pathway between modifiable risk factors and IR is uncertain. Excess diet quantity combined with inactivity may result in a positive energy balance, contributing to obesity and strongly predicting IR.

Absent from the literature is an assessment of the interactions between objectively measured PA and SED with self-reported diet quantity and quality, and their associations with IR. We sought to improve understanding of the interaction between modifiable risk factors and IR through careful consideration of both sides of the energy balance equation.
METHODS

Participants

Participants were inactive, abdominally obese men and women between 35 and 69 years of age recruited from the general Kingston area. Inclusion criteria were an elevated waist circumference of at least 102 cm in men and 88 cm in women, planned PA ≤ 1 day per week, and weight stability (± 2 kg) within the six months prior to recruitment. Because participants were initially recruited for an exercise intervention [194], they were excluded if they reported any physical impairment that would render physical activity to be unsafe or difficult. Additional exclusion criteria were history of myocardial infarction, stroke, coronary bypass surgery or angioplasty in the last 6 months; peripheral artery disease, unstable angina or ischemia; if they had diagnosed diabetes or were taking glucose-lowering medication; if they consumed > 21 alcoholic drinks per week. All participants provided written informed consent in accordance with the ethical guidelines of Queen’s University.

Anthropometric and Metabolic Tests

Participants were dressed in standard T-shirts and shorts for all anthropometric testing. Body mass was measured to the nearest 0.2 kg on a calibrated scale. Standing height was measured to the nearest 0.2 cm using a wall-mounted stadiometer. These measures were used to calculate body-mass-index (BMI; kg/m²). Waist circumference (WC) was obtained with the participant in a standing position at the level of the superior edge of the iliac crest. The mean of two measures was recorded to the nearest 0.1 cm.

A 2-hour, oral glucose tolerance test (OGTT) was administered to assess glucose tolerance the morning after an overnight fast. A nurse collected blood samples from the antecubital vein of the participant in the fasted state, and then subsequently at 30, 60, 90, and 120 minutes following consumption of 75 grams of Glucodex. Following centrifugation and separation of the serum from whole blood, aliquots of serum were temporarily stored in a refrigerator between 4 and 6 ºC. Blood samples were then delivered to the Kingston General Hospital Core Laboratory for analysis.
Plasma glucose was determined using an oxygen rate method on the Synchron LXH Systems (Beckman Coulter, Inc., Brea, CA, USA). Insulin was also determined through the use of the Beckman Coulter UniCel Dxi 800 Access Immunoassay System. To do so, the chemiluminescent substrate Lumi-Phos 530 was added to the reaction vessel and a luminometer was used to measure light generated by the reaction. The light production is directly proportional to the concentration of insulin in the sample. The coefficient of variation for repeated measurements of glucose and insulin values from the same sample was reported to be 2% and 3%, respectively (Core Laboratory Services, Kingston, ON, Canada).

The homeostasis model of assessment for insulin resistance (HOMA-IR) was used as a measure of IR and was calculated as fasting plasma glucose (mmol/L) x fasting serum insulin (µU/mL) / 22.5. Insulin area under the curve (AUC) was calculated as (fasting insulin/2) + 30 min insulin + 60 min insulin + 90 min insulin + (120 min insulin/2). Other outcomes obtained from the OGTT include: fasting glucose, 2-hour glucose, and fasting insulin.

Blood samples to determine fasting triglycerides (TG), total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were also obtained in the morning after a 12- to 14- hour overnight fast. These measurements were taken just prior to consumption of the Glucodex for the OGTT. Following centrifugation and separation of serum from whole blood, blood samples were frozen until delivery to the Kingston General Hospital Core Laboratory for analysis. Serum total cholesterol, TG, LDL-C, and HDL-C levels were determined using standard enzymatic methods on the Synchron LXH Systems (Beckman Coulter, Inc., Brea, CA, USA). The coefficient of variation for repeated measures of each of these variables is 3%.

Resting systolic blood pressure (BP) and diastolic BP were measured using the automated BP Tru Blood Pressure Monitor (BPTru Medical Devices, Coquitlam, BC, Canada) in the morning after an overnight fast. The device recorded 6 readings, and systolic blood pressure and diastolic blood pressure were calculated as the average of the last five readings.
Physical Activity by Accelerometry

Physical activity was measured using the Actigraph GT3X accelerometer (Actigraph, Pensacola, Florida, USA). The accelerometers were programmed to record data in 1 minute epochs over 7 consecutive days. Although the Actigraph GT3X is a triaxial accelerometer, only the vertical axis was used for analysis. The participant wore the accelerometer on an elastic belt over their right hip at all times except during water-based activities. Sleep time, wake time, and times the accelerometer was removed were indicated by the participant in a written log.

To be included in our analysis, the participant must have worn the accelerometer for at least 4 days, including one weekend day. A day is defined as at least 10 hours of wear time. Wear time was calculated after extracting extended periods with consecutive zero counts ≥ 60 minutes and sleep time (determined using both the participant logs and visual examination of the data).

The cutpoints developed by Freedson and colleagues [124] were used to translate the raw ‘count’ values recorded by the accelerometer into an estimate of physical activity intensity. Total physical activity (TPA) was defined as >100 counts per minute (cpm), and then further subdivided into light physical activity (LPA) as 100-1951 cpm, and moderate-to-vigorous physical activity (MVPA) as ≥1952 cpm. Sedentary behaviour (SED) was defined as < 100 cpm. Physical activity was not required to be accumulated in ‘bouts’, thus it was accumulated in a ‘sporadic’ fashion. Physical activity was expressed in both absolute (duration in minutes per day) and relative (percentage of accelerometer wear time per day) terms.

Diet

Diet quantity (total caloric intake) and quality (Alternative Healthy Eating Index-2010; AHEI-2010) data were derived using the participants’ daily food diaries. Prior to completing the food diaries, all subjects met with a nutritionist to receive detailed instruction regarding how to record their dietary intake. All participants were instructed to record their dietary intake for 7 consecutive days. To be
included in our analysis, subjects must have provided a minimum of 4 days of dietary records, including one weekend day.

To determine total caloric and macronutrient intake, the food diaries for all subjects were entered and analyzed using ESHA Food Processor software (ESHA, Salem, Oregon, USA). Diet quality was assessed using the AHEI-2010 score, a score that assesses the consumption of foods and nutrients that are associated with chronic disease [174]. The score comprises of 11 components: whole grains, vegetables (excluding potatoes), fruit, nuts and legumes, trans fat, omega-3 fatty acids, polyunsaturated fatty acids, alcohol, red and processed meat, sugar-sweetened beverages, and sodium. Each component is scored between 0 (worst) and 10 (best) points, and summed to reach a maximum of 110 points. See Appendix E for greater detail on scoring methodology.

**Cardiorespiratory Fitness**

Cardiorespiratory fitness (CRF) was measured as oxygen consumption per unit of time (VO$_2$ peak). A graded maximal treadmill test was employed using standard open spirometry techniques (SensorMedics Corp., Yorba Linda, California). A modified Bruce protocol was followed [195]. The test began with the subject walking at a self-selected pace on a level grade. The grade was increased to 5% at the third minute, and by 2% every 2 minutes thereafter. If the subject did not reach exhaustion after 2 minutes at the maximal incline of 15%, the speed was increased by approximately 0.2 miles per hour. Heart rate was monitored and recorded continuously throughout the duration of the test using heart rate monitors (Polar USA, Stanford, CT).

**Statistical Analyses**

All statistical analyses were performed using the SPSS version 22.0 software (SPSS, Chicago, IL, USA). Statistical significance was set at an alpha level of 0.05 (p < 0.05). Descriptive characteristics were summarized as mean ± standard deviations (SD). Associations between variables were examined using Pearson correlation coefficients. Gender differences were assessed using Independent Student’s T-tests.
Simple linear regression analyses were conducted to examine whether PA, SED, and dietary variables predict HOMA-IR and insulin AUC. Gender differences in the relationship between activity and dietary variables with HOMA-IR and insulin AUC were tested by adding the interaction terms into regression models. If no differences were detected, analyses were collapsed across genders. After, stepwise multiple linear regression analyses were performed to establish the associations between predictor variables and HOMA-IR or insulin AUC while controlling for the remaining predictor variables.

Correlation analyses were conducted to determine whether the variables age, WC, BMI, and CRF should be included as covariates. Because WC was the only variable significantly associated with the predictor and outcome variables (HOMA-IR and insulin AUC), it was the only covariate retained. Regression models were adjusted for WC in secondary analyses.

Multicollinearity was screened for in all regression models using the variance inflation factor (VIF) and the tolerance statistic. A VIF < 10 and a tolerance statistic > 0.2 indicated that multicollinearity was not present. To ensure the homoscedasticity assumption was met, a scatterplot was visually examined for each outcome variable. The regression standardized predictor variable was plotted on the x-axis, and the regression standardized residual was plotted on the y-axis. A lack of relationship confirmed the homoscedasticity assumption was met. Furthermore, a histogram was visually inspected to ensure errors were normally distributed. The regression standardized residuals were plotted on the x-axis, and their frequencies on the y-axis.
RESULTS

The participant characteristics are shown in Table 3-1. Men had a higher waist circumference, HOMA-IR, fasting insulin, diastolic blood pressure, CRF, caloric intake, fat intake, and protein intake compared to women (p < 0.05). Women had higher HDL-C and relative carbohydrate intake than men (p < 0.05). Only 8 individuals from our sample of 102 presented with impaired fasting glucose, of which 6 were women.

Accelerometers were worn for a median of 7 days, and for an average of 15.5 hours each day. Participants spent 67% of their waking hours engaged in SED, 31% in LPA, and 2% in MVPA. These percentages translate to over 10 hours of SED, almost 5 hours of LPA, and approximately 20 minutes of sporadic MVPA. Inspection of the accelerometry data revealed that despite a self-reported physically inactive lifestyle, 10 participants from our sample of 102 achieved the Canadian Physical Activity Guidelines, accumulating ≥ 150 minutes of MVPA per week in bouts ≥ 10 minutes. Because observations were similar after excluding the active participants from our sample, the data were collapsed for all analyses.

Dietary logs were completed for a median of 7 days. Women self-reported that they consumed approximately 1800 kilocalories (kcal) per day, whereas men averaged 2100 kcal per day. Carbohydrates comprised 47% of total kcal intake, fat 35%, and protein 17%. On average, participants scored 50 out of the possible 110 points on the AHEI-2010 diet quality index.

The associations between modifiable risk factors- PA, SED, and dietary variables- with HOMA-IR and insulin AUC are shown in Table 3-2. SED was positively correlated with HOMA-IR (r = 0.34, p < 0.01, Figure 3-1a), whereas LPA (r = -0.32, p < 0.01) was negatively correlated with HOMA-IR. No association was found between sporadic MVPA and HOMA-IR (r = -0.12, p > 0.1). Similar associations existed between all activity variables and insulin AUC, albeit with a lower magnitude; see Figure 3-1b. Due to a violation of multicollinearity (tolerance statistic < 0.2, VIF > 10), a multiple linear regression to determine the independent associations of SED, LPA, and MVPA with HOMA-IR and
insulin AUC could not be performed. Multicollinearity was present because SED and LPA are highly correlated ($r = -0.98$, $p = 0.00$), as shown in Figure 3-2.

WC was included as a covariate in secondary regression analyses because it was significantly associated with predictor and outcome variables. SED ($r = 0.24$, $p \leq 0.01$), LPA ($r = -0.23$, $p < 0.05$), and AHEI-2010 ($r = -0.22$, $p < 0.05$) were the modifiable risk factors that were correlated with WC. Furthermore, WC was correlated with both HOMA-IR ($r = 0.52$, $p < 0.01$) and insulin AUC ($r = 0.33$, $p < 0.01$). As shown in Table 3-3, observations remained unchanged after controlling for WC, with the exception of the association between LPA and insulin AUC. This relationship approached significance ($p = 0.058$).

Neither diet quality (AHEI-2010) nor diet quantity (energy intake in kcal) were associated with HOMA-IR or insulin AUC. Analyses on macronutrients as well as other nutrients- such as saturated fat, monounsaturated fat, fibre, and glycemic index- did not reveal any significant findings (data not shown). Furthermore, none of the dietary variables were associated with the activity variables.

Secondary correlation analyses did not reveal significant associations between the activity or dietary variables and fasting glucose, 2-hour glucose, blood pressure, HDL-C, LDL-C, or TG.
<table>
<thead>
<tr>
<th>Table 3-1 Participant Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variable</strong></td>
</tr>
<tr>
<td><strong>Anthropometric</strong></td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
</tr>
<tr>
<td><strong>Metabolic</strong></td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
</tr>
<tr>
<td>2-hour glucose (mmol/L)</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
</tr>
<tr>
<td>Insulin AUC</td>
</tr>
<tr>
<td>HOMA-IR</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
</tr>
<tr>
<td>Low-density-lipoproteins (mmol/L)</td>
</tr>
<tr>
<td>High-density-lipoproteins (mmol/L)</td>
</tr>
<tr>
<td><strong>Blood Pressure</strong></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
</tr>
<tr>
<td><strong>Physical Activity</strong></td>
</tr>
<tr>
<td>Sedentary Behaviour (min/d)</td>
</tr>
<tr>
<td>Sedentary Behaviour (% wear time)</td>
</tr>
<tr>
<td>Light PA (min/d)</td>
</tr>
<tr>
<td>Light PA (% wear time)</td>
</tr>
<tr>
<td>Moderate-to-Vigorous PA (min/d)</td>
</tr>
<tr>
<td>Moderate-to-Vigorous PA (% wear time)</td>
</tr>
<tr>
<td>Total PA (min/d)</td>
</tr>
<tr>
<td>Total PA (% wear time)</td>
</tr>
<tr>
<td><strong>Cardiorespiratory Fitness</strong></td>
</tr>
<tr>
<td>VO₂ peak (L/min)</td>
</tr>
<tr>
<td>VO₂ peak (ml/kg/min)</td>
</tr>
<tr>
<td><strong>Dietary</strong></td>
</tr>
<tr>
<td>Energy (kcal)</td>
</tr>
<tr>
<td>Fat (g)</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
</tr>
<tr>
<td>Protein (g)</td>
</tr>
<tr>
<td>Fat (% total kcal)</td>
</tr>
<tr>
<td>Carbohydrate (% total kcal)</td>
</tr>
<tr>
<td>Protein (% total kcal)</td>
</tr>
<tr>
<td>Diet Quality Score (AHEI-2010)</td>
</tr>
</tbody>
</table>

Data are means ± SD. Significant difference between sex: *(p < 0.05). BMI, body mass index; HOMA-IR, homeostasis model assessment for insulin resistance; PA, physical activity; min/d, minutes per day; % wear time, percentage of accelerometer wear time; kcal, kilocalorie; g, grams; AHEI-2010, Alternative Healthy Eating Index 2010.
Table 3-2 Simple regression analyses for modifiable risk factors with HOMA-IR and insulin AUC.

<table>
<thead>
<tr>
<th></th>
<th>HOMA-IR (n = 102)</th>
<th>Insulin AUC (n = 102)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unstandardized B</td>
<td>R²</td>
</tr>
<tr>
<td>Physical Activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SED</td>
<td>0.061 (0.017)</td>
<td>0.113</td>
</tr>
<tr>
<td>LPA</td>
<td>-0.061 (0.018)</td>
<td>0.103</td>
</tr>
<tr>
<td>MVPA</td>
<td>-0.098 (0.083)</td>
<td>0.014</td>
</tr>
<tr>
<td>Dietary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Kcal</td>
<td>0.000 (0.000)</td>
<td>0.020</td>
</tr>
<tr>
<td>Fat</td>
<td>-0.012 (0.029)</td>
<td>0.002</td>
</tr>
<tr>
<td>Carbohydrate#</td>
<td>W=0.041 (0.023)</td>
<td>W=</td>
</tr>
<tr>
<td></td>
<td>M=-0.073 (0.048)</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M=</td>
</tr>
<tr>
<td></td>
<td>0.070</td>
<td>0.137</td>
</tr>
<tr>
<td>Protein</td>
<td>0.039 (0.048)</td>
<td>0.007</td>
</tr>
<tr>
<td>AHEI-2010</td>
<td>-0.018 (0.012)</td>
<td>0.023</td>
</tr>
</tbody>
</table>

Physical activity (PA) measured as percentage of accelerometer wear time. Diet measured as percentage of caloric intake.

*Significant association (p < 0.05). # Gender interaction; W, women (n = 69); M, men (n = 33).

HOMA-IR, homeostasis model for insulin resistance; insulin AUC, insulin area under the curve; SED, sedentary behaviour; LPA, light physical activity; MVPA, moderate-to-vigorous physical activity; TPA, total physical activity; Kcal, kilocalories; AHEI-2010, Alternative Healthy Eating Index 2010.

Table 3-3 Regression analyses for modifiable risk factors with HOMA-IR and insulin AUC; adjusted for WC.

<table>
<thead>
<tr>
<th></th>
<th>HOMA-IR (n = 102)</th>
<th>Insulin AUC (n = 102)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unstandardized B</td>
<td>R²</td>
</tr>
<tr>
<td>Physical Activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SED</td>
<td>0.041 (0.016)</td>
<td>0.319</td>
</tr>
<tr>
<td>LPA</td>
<td>-0.040 (0.016)</td>
<td>0.315</td>
</tr>
<tr>
<td>MVPA</td>
<td>-0.054 (0.072)</td>
<td>0.276</td>
</tr>
<tr>
<td>Dietary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Kcal</td>
<td>0.000 (0.000)</td>
<td>0.276</td>
</tr>
<tr>
<td>Fat</td>
<td>-0.013 (0.025)</td>
<td>0.274</td>
</tr>
<tr>
<td>Carbohydrate#</td>
<td>W=0.024 (0.022),</td>
<td>W=</td>
</tr>
<tr>
<td></td>
<td>M=-0.032 (0.043)</td>
<td>0.193</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M=</td>
</tr>
<tr>
<td></td>
<td>0.313</td>
<td>0.463</td>
</tr>
<tr>
<td>Protein</td>
<td>0.052 (0.041)</td>
<td>0.284</td>
</tr>
<tr>
<td>AHEI-2010</td>
<td>-0.005 (0.011)</td>
<td>0.276</td>
</tr>
</tbody>
</table>

Physical activity (PA) measured as percentage of accelerometer wear time. Diet measured as percentage of caloric intake. WC, waist circumstance.

*Significant association (p < 0.05). # Gender interaction; W, women (n = 69); M, men (n = 33).

HOMA-IR, homeostasis model for insulin resistance; insulin AUC, insulin area under the curve; SED, sedentary behaviour; LPA, light physical activity; MVPA, moderate-to-vigorous physical activity; TPA, total physical activity; Kcal, kilocalories; AHEI-2010, Alternative Healthy Eating Index 2010.
**Figure 3-1a** Association between sedentary behaviour and HOMA-IR ($r = 0.34$, $p = 0.00$).

**Figure 3-1b** Association between sedentary behaviour and insulin AUC ($r = 0.27$, $p < 0.01$).
Figure 3-2 Association between sedentary behaviour and light physical activity ($r = -0.98$, $p = 0.00$).
DISCUSSION

The primary finding of this study was that the percentage of time spent in SED and LPA were significantly associated with HOMA-IR and insulin AUC among inactive, abdominally obese men and women, whereas neither diet quantity nor diet quality were associated with measures of IR. Our observations add to the growing body of literature on the deleterious association between SED and IR, and support the notion that the substitution of SED with PA accumulated below guideline recommendations may attenuate IR.

The aim of the present investigation was to explore the interaction of modifiable risk factors with IR. The associations of SED and LPA independent of one another could not be assessed due to a violation of multicollinearity within the regression model. SED and LPA were almost perfectly inversely related, suggesting that replacing SED with LPA - which includes occupational tasks, household chores, etc. - may beneficially impact IR.

Inconsistent findings are prevalent in the literature surrounding the associations between SED, PA, and IR [13, 14, 88-90, 93, 95, 97, 100]. Although Ekelund et al [88] and Balkau et al [89] reported a univariate association between SED and IR, this relationship was abolished following statistical control for sporadic MVPA or TPA. Thus, the results from Ekelund and colleagues [88] differed from ours in that sporadic MVPA was associated with IR; this finding was subsequently replicated in a follow-up study by the same group [14]. Furthermore, a recent study by Peterson et al [90] found that time spent in SED was not a predictor of IR when MVPA was accounted for. Together, these studies indicate that PA intensity may be an important determinant of IR. In our study, however, modest amounts of sporadic MVPA (~20 minutes per day) were not associated with improvement in IR. Because our participants were recruited as inactive and spent a large portion of their day in SED, perhaps they were not engaging in sufficient MVPA to elicit an association with IR.

In contrast, numerous authors report that SED and/or LPA predict IR beyond MVPA [13, 93, 95], whereas others have demonstrated a complete lack of association between any of the activity
variables and IR [97]. Healy and colleagues [13] were the first to provide objective evidence that SED and LPA were significantly associated with IR in the general Australian population after controlling for MVPA. Green et al [95] also reported that in a sample of young healthy women, LPA was associated with IR independent of MVPA. However, body composition—measured by air displacement plethysmography—attenuated the relationship, suggesting that adiposity may lie within the causal pathway between LPA and IR. Henson and colleagues [93] provide evidence indicating otherwise: SED was detrimentally associated with IR following adjustment for adiposity in a sample of participants presenting risk factors for type 2 diabetes. Furthermore, SED was more strongly related to IR than time spent in MVPA or TPA. Our findings are consistent with those by Henson et al [93] rather than those by Green and colleagues [95], because the associations between SED and LPA remained significant following control for WC in our sample, suggesting that SED and LPA may be directly related to IR.

An explanation for the disparate findings within the literature is difficult to reconcile. Although participant characteristics vary from one study to another, similar results have been reported in samples presenting different characteristics. For example, Healy and colleagues [13] indicated that SED predicted IR in the general Australian population, whereas Henson and colleagues [93] reported a similar finding among individuals displaying risk factors for type 2 diabetes. The inconsistent findings may relate to limitations associated with accelerometers [122]. Because accelerometers are a relatively novel tool for characterising PA and SED, models are frequently updated and a standard protocol for accelerometry data cleansing is lacking in the literature. For example, studies identify accelerometer non-wear time using different criteria for the number of minutes with consecutive zero counts [13, 84, 123]. The lack of a consistent non-wear time definition can greatly impact the time spent in SED, and thus the study’s findings. Furthermore, uniaxial accelerometers were initially used in PA research, whereas triaxial accelerometers are currently becoming more common. This shift necessitates the validation of new cutpoints to quantify PA intensity [125, 126]. Because
accelerometer cutpoints have yet to be standardized, a variety of cutpoints are commonly employed [13, 90, 95]. Future standardization of cutpoints as well as accelerometer data reduction techniques will enable direct comparisons between studies, and provide insight on the relationships between PA, SED, and IR.

Neither diet quantity nor diet quality were associated with measures of IR in our sample of inactive, abdominally obese adults. Given that obesity largely predicts IR [23, 36], that diet quantity (measured in kilocalories) was not associated with IR was unexpected. Elevated caloric consumption should promote a positive energy balance, thereby increasing fat deposition and thus IR. Our findings contrast those reported from the 2004 Canadian Community Health Survey, which indicated that caloric consumption increased the odds of obesity among Canadian adults [132]. A plausible explanation for the lack of an association between caloric intake and IR is the error inherent to self-reported dietary records, which is exacerbated when participants are aware that they are being evaluated [177, 178]. In support of this notion, a recent American study suggested that an underreporting of up to 850 kilocalories occurs per day [179].

The lack of an association between diet quality and IR is also contrary to current evidence [144, 192]. The use of dietary indices to assess diet quality rather than focusing on single dietary components is supported by epidemiology studies because nutrients are consumed in combination and are often correlated with one another [169, 196]. We used the AHEI-2010 [174] to quantify diet quality because it is based upon current scientific evidence to include foods and nutrients predictive of chronic disease risk. Furthermore, Chiuve and colleagues [174] reported that higher scores on the AHEI-2010 were associated with a 31% lower risk of coronary heart disease and 33% reduced risk of type 2 diabetes in two large prospective cohorts. Because IR is a major risk factor for type 2 diabetes [67, 68], these findings suggest that the AHEI-2010 would be well-suited to predict IR within our sample.
Although the AHEI-2010 scores were not associated with IR among our participants, they were negatively correlated with WC. This finding suggests that diet quality and abdominal obesity may be inversely related. Given the strong correlation between WC and IR, these findings together indicate that abdominal adiposity may lie within the causal pathway between diet quality and IR. Reinforcing this notion, a strong association between poor diet quality and greater weight gain among men and women was reported in systematic review comprising prospective cohort studies [197].

To our knowledge we are among the first to assess PA and SED objectively while simultaneously examining both diet quantity and diet quality [198]. The interaction of these modifiable risk factors is important to consider as they capture both sides of the energy balance equation. Various studies have assessed the interaction between PA, SED, and diet [180-187]. These studies, however, utilize subjective measures of PA and SED, and do not focus on IR per se. For example, van Dam and colleagues [183] reported that 28% of cardiovascular deaths in a large prospective study could be attributed to PA below consensus guidelines, 18% to poor diet quality, and 31% to being overweight. Future investigations should use accelerometers to objectively assess the behaviours of PA and SED, which will aid in disentangling the interaction between PA, SED, diet, morbidity, and mortality.

Neither activity nor dietary variables were related to fasting glucose, 2-hour glucose, blood pressure, HDL-C, LDL-C, or TG. However, our participants consumed a diet within the macronutrient distribution range suggested by the Canadian Diabetes Association- 45-60% carbohydrate, 15-20% protein, and 20-35% fat- perhaps attenuating their risk for an abnormal metabolic profile [131]. Furthermore, our study population of predominantly white, inactive, abdominally obese adults may have been too homogenous, limiting our ability to detect associations between modifiable risk factors and IR or metabolic variables. Given that over a third of Canadian adults are abdominally obese [25] and 85% fail to meet the activity guidelines [12], our sample is highly generalizable to the Canadian population.
The cross-sectional design of our study precludes discussion of causal inference. However, we employed sound methodology to establish our observations. PA and SED were measured objectively using accelerometers rather than by self-reported questionnaires, which are prone to recall and social desirability bias and have difficulty capturing sporadic or unstructured activities [21, 118, 119]. Based upon the correlation coefficients, the relationships between SED and LPA with measures of IR displayed effect sizes that were in the moderate range [199]. Although dietary data were self-reported and subject to bias [177, 178], we used a 7 day food diary, which has been shown to have a stronger relationship with dietary intake than other commonly used methods such as a 24-hour recall or food frequency questionnaire [200]. We used the AHEI-2010 to quantify diet quality: this diet index has been associated cardiovascular disease mortality more strongly when compared to 3 other commonly employed indices [170].

Finally, we used surrogate measures of IR: both HOMA-IR and insulin AUC. Perhaps our observations would have differed if we used a criterion method such as the hyperinsulinemic euglycemic clamp [60]. However, our measures were sensitive enough to determine variability in insulin action among participants, and they are feasible from a clinical perspective. Furthermore, HOMA-IR has predicted cardiovascular disease in various studies [201, 202], and insulin AUC has been associated with increased all-cause and cardiovascular mortality [70].

In summary, the percentage of time spent in SED and LPA were significantly associated with HOMA-IR and insulin AUC among inactive abdominally obese adults. In contrast, diet quality was associated with WC but not with measures of IR, indicating that abdominal obesity may lie within the causal pathway between diet quality and IR. That SED and LPA were almost perfectly negatively correlated suggests that the substitution of SED for LPA may attenuate IR.
Chapter 4

General Discussion

4.1 What are our contributions?

To our knowledge we are among the first to assess the associations between objectively measured PA and SED with IR while simultaneously examining self-reported diet quantity and diet quality with IR. Rather than analyzing one component of the energy balance equation, we carefully considered both energy intake and energy expenditure. While there is ample literature focusing solely on PA, SED, and diet, we sought to investigate all modifiable risk factors because activity and nutrition are highly interrelated and synergistic [191].

Findings from the study in Chapter 3 indicate that SED and LPA were associated with IR, whereas neither diet quantity nor diet quality were related to IR in a sample of inactive, abdominally obese men and women. While the health benefits of accumulating PA in a manner consistent with consensus guidelines are well-established, we have added to the literature suggesting that SED should be considered as a risk factor independent of PA [13, 84, 93, 95]. Specifically, our results reinforce the notion that the substitution of SED with PA accumulated below guideline recommendations may attenuate IR.

The Canadian Physical Activity Guidelines for adults focus solely on the performance of 150 minutes of MVPA per week in bouts ≥ 10 minutes, and do not mention the benefits of PA that is accumulated during daily activities [10]. A revision of this message to state the importance of replacing SED with LPA, in concert with the current recommendation to engage in 150 minutes of MVPA per week, may be beneficial for the Canadian population. Given that 85% of Canadian adults fail to adhere to the activity guidelines [12], perhaps a message suggesting the inclusion of LPA into daily routine may be more achievable for those under time constraints, or for those intimidated by guideline recommendations. We, along with others, have indicated that improvements in IR may start to accrue
at low volumes and intensities of PA. Thus, we believe this should be conveyed to the general population.

With respect to diet, although dietary variables were not directly related to IR in our investigation, we did observe an association between diet quality and WC. This finding suggests that diet quality may be related to IR indirectly through the deposition of abdominal adipose tissue. Given that activity variables were correlated with WC as well, these findings reinforce the notion that PA, SED, and diet are interrelated and may act synergistically with one another. The following sections will address limitations and recommendations for future studies in order to develop a clear message relating to the interaction between PA, SED, and diet with IR.

4.2 Strengths and Limitations

As noted within the manuscript in Chapter 3, inconsistent findings are prevalent in the literature surrounding the associations between SED, PA, and IR. Reasons for the discrepant findings are uncertain, but may relate to the use of accelerometers in quantifying PA and SED. Despite overcoming the biases inherent to self-reported data [21], accelerometers are a relatively novel tool in this field of research, hence they are not without limitations. As indicated earlier, the lack of a standard protocol for accelerometry data cleaning, in combination with challenges arising when interpreting results from different accelerometer models and/or cutpoints, may all contribute to the disparities in the literature. These limitations are the focus of various validity and reliability studies, and will likely resolve in time. However, it is important to note that accelerometers have improved upon the ability for researchers to quantify PA and SED in free-living humans, and are a technological advancement from self-reported questionnaires and diaries.

Similar to accelerometers, the use of diet quality indices has proliferated in the literature. Although researchers are recognizing that humans eat mixed diets rather than isolated foods or nutrients, the optimal method for assessing diet quality is unknown. This is apparent by the number of diet quality indices that exist. For example, although the dietary habits from the Mediterranean region
are protectively associated with mortality, 8 of the 10 studies assessing this relationship used different versions of the ‘Mediterranean diet score’ [170]. These versions essentially alter the definition of the Mediterranean diet, thereby increasing the difficulty to make comparisons across studies. Similar issues arise for diet quality indices catered to North American populations.

We used the AHEI-2010 to quantify dietary quality in the investigation in Chapter 3. The AHEI-2010 was chosen due to its stronger association with the risk for type 2 diabetes and cardiovascular mortality compared to other diet quality indices [170, 174]. Because IR is a risk factor for type 2 diabetes and cardiovascular disease, we believe that our decision to use the AHEI-2010 was well-suited to predict IR.

IR was quantified in our study using surrogate measures: both HOMA-IR and insulin AUC. Although the hyperinsulinemic euglycemic clamp is the criterion method for assessing IR [60], our choice of methodology is more practical from a clinical perspective.

The participant characteristics in our study may be considered a limitation, as the inclusion criteria required participants to be inactive, abdominally obese adults from the Kingston community. Because the population of Kingston is primarily Caucasian, this was reflected in our sample. Thus, we cannot generalize our findings to other races or ethnic populations. However, it is important to note that our participants are representative of a growing, high risk group within the Canadian population. Given that 85% of Canadian adults fail to adhere to the Canadian Physical Activity Guidelines, and upwards of 35% of Canadian adults are abdominally obese [25], our results are generalizable to a large portion of Canadians who are at substantial health risk. Furthermore, it must be acknowledged that despite strict inclusion criteria and a relatively small sample size in comparison to epidemiologic studies, variation in modifiable risk factors- PA, SED, and diet- was present. For example, the amount of SED (mean = 623.9 ± 86.2 min/d; range = 457 min/d) accrued over the average day varied amongst participants, as did the accumulation of LPA (mean = 287.5 ± 82.4 min/d; range = 434 min/d). Variation in diet quality (mean = 50.0 ± 12.0; range = 65.1), as quantified by the AHEI-2010, was
apparent too. The variations in modifiable risk factors allowed for us to detect associations between modifiable risk factors and IR.

Finally, the cross-sectional design of our study eliminates our ability to make causal inference. Although this is a limitation, we are among the first [198] to examine objectively measured PA and SED while simultaneously considering diet quantity and diet quality. Section 4.3 will discuss potential research avenues to explore and address the limitation of a cross-sectional study design.

4.3 Future Directions

In a commentary by Sparling and colleagues [191], it is stated that the diet versus physical activity question is “passé”. The importance of recognizing the interrelationship between PA, SED, and diet may enhance public health messages and enable the development of effective behavioural change strategies. With the advent of novel methodology for quantifying activity and assessing dietary quality, this research field is ripe with questions. Research opportunities extend beyond those facilitating the standardization of accelerometry protocols and diet quality indices to ease comparisons amongst studies. Large epidemiological studies are necessary to conduct analyses similar to ours, but with larger, more diverse populations. As evidence accumulates, intervention trials are required to further investigate and establish causality among relationships noted in cross-sectional examinations. Although the possibilities for future intervention trials are numerous, potential ideas include: identifying the magnitude of improvements in IR following an intervention in which SED is substituted with LPA, or determining the optimal dietary pattern for attenuating IR in which adherence is high.
Summary and Conclusions

While it is well-documented that PA performed in accordance with consensus guidelines is associated with a reduction in IR, the results from this thesis contribute to a growing body of literature that suggest the substitution of SED with PA accumulated below guideline recommendations may attenuate IR. Although our findings do not indicate a direct relationship between dietary variables and IR, they support the notion that PA, SED, diet, and abdominal obesity are interrelated. Future studies will improve upon the understanding of the interaction between modifiable risk factors and IR, thereby enhancing public health efforts and ultimately preventing risk for future chronic illnesses such as type 2 diabetes or cardiovascular disease.
References


15. Canada, H., *Do Canadian Adults Meet their Nutrient Requirements through Food Intake Alone?* 2012.


Appendix A

Consent Form

CONSENT TO VOLUNTEER FOR PARTICIPATION IN A STUDY

TITLE:  Dose-response effects of exercise on abdominal obesity and risk factors for cardiovascular disease in women and men

PRINCIPAL INVESTIGATOR:  Robert M.J. Ross, Ph.D.
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Miu Lam, Ph.D.
Queen's University
Department of Community Health and Epidemiology
Kingston, Ontario, K7L 3N6
You are invited to participate in a research study on the influence of different doses (amounts) of exercise on abdominal fat and related health risk. The following brief 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 question that you may have.

BACKGROUND INFORMATION

Obesity is a major risk factor for disease and a public health problem. Recent information suggests that body fat located in the upper body region (abdominal fat) conveys a very strong health risk. Exercise is thought to be a good treatment option for reducing both abdominal fat and cardiovascular risk factors (e.g., blood fats (cholesterol), blood sugar and blood pressure). However, the specific exercise strategy or program required to achieve optimal benefit continues to be the source of considerable debate. At present, health professionals are unsure of the specific type, amount, pattern, and intensity of exercise that provides optimal health benefits. Therefore, you are invited to participate in a study to assess the relationships between exercise dose (how much) and intensity (how hard) on abdominal fat, and cardiovascular risk factors (e.g., blood sugar and fats). The results of the study may have important implications for development of public health messages and clinical guidelines for prevention and treatment of obesity and associated health risks through exercise.

EXPLANATION OF PROCEDURES

Pre-participation screening

You will be required to complete a medical questionnaire and make an appointment with your family physician prior to participation in this study. Your physician will also complete a medical questionnaire and may perform a medical examination on you. If your family physician charges you for completion of this exam, an invoice can be faxed to the Project Coordinator 613-533-2580 for payment or, the study investigators will reimburse you fully. In addition to the medical exam, you will have a fasting blood test (done by a finger prick) to measure your blood fat and sugar levels. We will also measure your waist circumference and take height and weight.

Study Protocol

The exercise study will be approximately 7 months in duration. The 6-month exercise period will begin and end with a 1 to 2 week weight maintenance period. By volunteering to participate in this study, your name will be selected by chance and placed into one of the following four groups: (1) Control - no exercise, (2) Low volume-Low intensity exercise, (3) High volume- Low intensity exercise, (4) Low volume-High intensity exercise. You will have a 1 in 4 chance of being placed in one of the four study groups. You will not be able to choose which group you will be in.
The follow-up study will take place during Months 7-13. During this part of the study, you will be asked to continue the same exercise routine that you followed for the first six months. The reason for the 6-month follow-up is to find out whether you have been able to maintain the exercise level prescribed at start of the study.

**Expectations**

You will be expected to:

Accept your group assignment

Participate fully in your assigned groups 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

**Control Group:** For the entire study the men and women in this group will consume a healthful diet. Thus there will be no weight loss or exercise.

**Low volume-Low intensity group:** As a participant in this exercise group you will be asked to perform walking type exercise on a motorized treadmill for around 30 minutes, 5 times per week, at about 50% of your maximum fitness level (e.g., low-to-moderate paced walking) for the duration of the 6 month treatment period. During each exercise session we will measure your heart rate every 5 minutes using an automated heart rate monitor. All of your exercise sessions will be by appointment and performed under supervision of a trained professional within our laboratory at Queen’s.
**High volume-Low intensity group:** As a participant in the aerobic exercise group you will be asked to perform walking type exercise on a motorized treadmill for around 60 minutes, 5 times per week, at about 50% of your cardiovascular fitness level (e.g., low-to-moderate paced walking) for the duration of the 6 month treatment period. During each exercise session we will measure your heart rate every 5 minutes using an automated heart rate monitor. All of your exercise sessions will be by appointment and performed under supervision within our laboratory at Queen’s.

**Low volume-High intensity group:** As a participant in the aerobic exercise group you will be asked to perform walking and/or jogging type exercise on a motorized treadmill for around 30 minutes, 5 times per week, at about 75% of your cardiovascular fitness level (e.g., brisk walking) for the duration of the 6 month treatment period. During each exercise session we will measure your heart rate every 5 minutes using an automated heart rate monitor. All of your exercise sessions will be by appointment and performed under supervision within our laboratory at Queen’s.

**Diet Program:** All participants in each group will eat the same type of foods. The diet will consist of regular foods that you will buy and prepare yourself. All aspects of the diet plan will be explained to you by a nutritionist. The session will take place at the beginning of the study, with several additional sessions planned throughout to help you follow the diet plan. If someone else shops for your food or prepares your meals, or if you share those tasks with someone else, that person is invited to meet with the nutritionist as well. You will be required to record the food you eat each day for the duration of the study. All of your meetings with the nutritionist will be in Dr. Ross’s laboratory within the School of Kinesiology & Health Studies at Queen’s.

At the beginning of the study, using the diet records that you complete, the number of calories required to maintain your body weight will be determined. During the study the nutritionist will work with you to help you to maintain this caloric (number of calories) intake. In other words, the nutritionist will help you eat an amount of food that would normally maintain your body weight. Thus any weight loss you experience will be the result of an increase in exercise.

**Magnetic Resonance Imaging**

Magnetic resonance imaging (MRI) is a method for creating pictures of body structures or organs. MRI gives pictures (images) in slices comparable to those produced by x-ray tomography (e.g., CT scan). One of the primary advantages of MRI is that it does not use x-rays or other forms of radiation. Instead, a large magnet, a radio transmitter/receiver and a computer are used to gather information from the body, and to produce pictures of internal anatomy. No harmful effects have been associated with MRI under existing conditions of use. However, if you feel claustrophobic during the scan you can end the test immediately.

As mentioned, the MRI procedure is very similar to a scanner examination. You will be placed on a table and moved smoothly into the scanner. A loud-speaker within the magnet makes it possible for you to
keep in constant contact with the staff. At all times the operator can see and hear you and if you need help or have questions, you can be removed from the machine if necessary. The whole procedure takes about 30 minutes and will be performed by appointment at Kingston General Hospital once at the beginning of the study and one at the end of the exercise study (week 24).

**Anthropometry (Skinfolds and Circumferences)**

Many circumference measurements will be taken at numerous places on your body. These measurements can be used to derive estimates of body composition. Skinfold callipers (skinfold thickness) will be measured at 4 different places on your body. Circumferences measurements using a measuring tape will also be obtained at different places on the body. These measurements require about 45 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 five times throughout the study: at the beginning (week 0), then after two months (week 8), four months (week 16), at the end of the exercise training period (week 24), and at follow-up (week 48, six months after the end of the exercise training period).

**Assessment of Cardiovascular Fitness**

We will measure your cardiovascular fitness (endurance) using a treadmill (VO\textsubscript{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 6 times: at the beginning (Week 0), after one month (week 4), after two months (week 8), after four months (week 16), at the end of the exercise training period (week 24), and at follow-up (week 48, six months after the end of the exercise training period).

**Assessment of Daily Physical Activity**

How physically active you are throughout the day will be measured by two small devices known as accelerometers: one is worn on your arm (armband) and one is worn on your hip (Actigraph). The armband involves wearing a monitor that is worn on your upper right arm that will track the amount of energy you burn and the amount of physical activity that you perform. The Actigraph is a small unit that you wear on your belt at the level of your hip and this device also measures the amount of physical activity that you perform. You will wear these monitors 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 wear this device for 7 consecutive days at 0, 8, 16, and 24 weeks.

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

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. This test will be performed four times during the study: at week 0, after four months (week 16), at the end of the exercise period (week 24) and at the end of the follow-up (week 48).

**Summary of Appointments and Time Requirements**

All appointments will be scheduled at a time that is convenient for you. For the testing you will be required to make six 45-minute appointments at the Hotel Dieu Hospital to complete the cardiovascular fitness (VO₂ max). We will also arrange two 30-minute appointments to complete the MRI (Kingston General Hospital). The other testing will be done at Dr. Ross’s laboratory in the School of Kinesiology & Health Studies at Queen’s. This includes: four 2.5-hour appointments for the oral glucose tolerance test and blood lipid/cholesterol tests (fasting blood draw); and five 45-minute anthropometric measurement appointments. In addition, we will ask you to make appointments for dietary counselling and for exercise (if you are randomized into one of the exercise groups). The total time commitment for all testing appointments and exercise sessions over the total 13-month study will be between 86 and 149 hours.
## Time commitment per participant

<table>
<thead>
<tr>
<th>Measure/Task</th>
<th>Time per session</th>
<th>Number of sessions</th>
<th>Total time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthropometrics</td>
<td>1 hr</td>
<td>5</td>
<td>5 hr</td>
</tr>
<tr>
<td>Fitness (VO$_2$) test</td>
<td>1 hr</td>
<td>6</td>
<td>6 hr</td>
</tr>
<tr>
<td>OGTT</td>
<td>2.5 hr</td>
<td>4</td>
<td>10 hr</td>
</tr>
<tr>
<td>MRI</td>
<td>0.75 hr</td>
<td>2</td>
<td>1.5 hr</td>
</tr>
<tr>
<td>Dietary Counsel</td>
<td>0.5-1 hr</td>
<td>6-12</td>
<td>3-6 hr</td>
</tr>
<tr>
<td>Exercise</td>
<td>0.5-1 hr</td>
<td>120</td>
<td>60-120 hr</td>
</tr>
</tbody>
</table>

**TOTAL** 86-149 hours

## 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.

For MRI, there are certain conditions that would exclude you from participating in this study. These include cardiac pacer, aneurysm clip, cochlear implant, intra-uterine device (IUD), shrapnel, neurostimulators or other metal devices. Metal objects present in the body could be moved by the large magnet involved in the MRI, and such movement could cause serious injury. Fear of closed spaces (claustrophobia) is also a reason you would be excluded from the study. No serious biological effects have been reported from being in a magnet. If you experience a fear of the confined space while in the magnet, you can terminate the study. Trained personnel are always in attendance during these studies.

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.
Risk of Wearing the Activity Monitor: Some people may experience mild skin irritation at the site where the activity monitor is worn. One cause of skin irritation has already been identified in people who wear the armband for extensive periods of time (i.e., more than 24 hours). Specifically, the build-up of sweat that can be trapped between the skin and the armband can cause pink pustules or pimples to appear. This condition is named miliaria, or prickly heat. This condition is common and occurs in 10% to 25% of people (10 to 25 out of 100 people) that wear the armband. To help to prevent this condition you should clean your arm using rubbing alcohol before putting on the activity monitor. Also, you should use soap and water to clean the elastic strap that attaches the monitor to your arm before each use. You should also wipe off the monitor using rubbing alcohol and allow this to dry before putting it on your arm.

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. Financial remuneration ($100) for parking, gas, and other costs associated with participation in the study will be provided to you.

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), Dr. Jean Coté, Head, School of Kinesiology and Health Studies (613 533-6601), 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

Protocol for Glucose Metabolism, Blood Lipids and Lipoproteins

List of Measured Variables

- Fasting glucose
- Fasting insulin
- 2-hour glucose
- 2-hour insulin
- Serum total cholesterol
- High density lipoprotein cholesterol
- Total triglycerides

Measurement Equipment

- 2 X 6 mL mauve, 3 X 6ml red, 1 X 3ml gold/yellow and 1 X 3ml grey top tubes: labelled 0
- 1 red and grey top 3 mL tubes labelled 30, 60, 90 and 120. These are the times you need to take the blood.
- 20-gauge angio
- 1 interlink cap used to put on the end of the angio
- 1 X 4.4cm X 4.4cm Tegaderm
- 1 X10 mL normal saline flush
- Alcohol swab
- 4 x 4 gauze
- 7 blunt cannulas
- 6 BD Vacutainer Multiple Sample Luer Adapters
- 1 Vacutainer
- 1 x 5 mL syringe with blunt plastic cannula attached to end for discarded blood
- 1 op-site to apply over angio
- Tape
- COLD Glucodex (75 g 300 mL) with glass
- Timer
- Paper and pen to keep record of times for blood draw

Measurement Procedures

The 2-hour oral glucose tolerance test (OGTT) was acquired at our laboratory facilities in the Kinesiology and Health Studies Building on Queen’s campus. At the time of the OGTT, blood lipids were also measured.

Preparing the participant: Participants were instructed to eat a normal meal the evening before, followed by a 12-hour fast prior to testing. Upon arrival to the laboratory, participants were introduced to the study nurse, who would seat them and briefly explain the test.

OGTT Procedure: The participant was asked when they last had something to eat or drink. If they had not fasted for at least 12 hours the test was not completed. The procedure for taking blood was then explained to the participant.

1. Start IV with 20 angio with Interlink (cap) in the antecubital vein.
2. Have a 10mL syringe of flush ready.
3. The order of blood draws were as follows: Gold/yellow, red, mauve and grey
4. Take your first blood sample (time 0) with the multiple adapter and blunt cannula attached to the vacationer. Gently invert the tubes 5 to 10 times to enhance the clotting or anticoagulation.
5. Inject 2.5mL of flush in the angio to keep the line patent.
6. Give the participant Glucodex 75g 300mL in a cup. They have only 5 minutes to drink it. They must drink ALL of it within the 5 minutes.
7. As soon as they are finished start the timer for 30 minutes. This will start the OGTT. From this point onwards, blood samples will be taken every 30 minutes.
8. Allow the yellow/gold and red top tubes to sit at room temperature for 30 minutes to clot.
9. Prepare centrifuge to spin mauve, grey and red top tubes. These tubes should be put in the centrifuge immediately after blood is taken.
10. After each blood draw follow #8’s instructions.
11. When you have taken your last sample (at 120 minutes), offer juice, crackers and cheese or peanut butter.
12. Explain to the participant the importance of having some food after the OGTT.
13. Explain hypoglycaemia and the symptoms to the participant. Explain there might be a small chance of feeling weak after the OGTT.
14. Each mauve 6 mL top tube requires two aliquots (4 in total) and the 3 mL requires one aliquot for each time taken.
15. Make sure blood tubes are labeled with code number and times (0 30 60 90 120).

Storage and Handling of Blood Samples

Preparing the Blood for the Lab and Centrifuge:
1. All blood tubes should be kept in a vertical position in a blood tray. This position promotes complete clot formation and reduces agitation of the tube contents, which in turn reduces the potential for hemolysis.
2. The yellow and red top tubes should remain at room temperature for 30 - 60 minutes to allow for clotting. The mauve top tubes should be put in the centrifuge at 16°C immediately.
3. Once the yellow top has clotted, prepare to send to the lab by making sure the tube has been labeled and there is a requisition. The tube must be transported in a Biohazard bag. The tube should reach the final lab within 2 hours sitting at room temperature.
4. The mauve top tubes should be spun and separated within 2 hours. (The sooner the better). The tubes should be stored in a ~80°C freezer labeled with the code and study.

Operating the Centrifuge:
1. Keep the centrifuge temperature at 20°C.
2. The speed (rpm) should be at 4000 - 4250.
3. The blood should be spun for 10 minutes.
4. When putting the tubes in the centrifuge the tubes must be balanced for size and volume. If the volume is not equal you must fill an empty tube of the same size with water to match the volume. BALANCE IS A MUST.
5. Close the lid and hit the start button.
6. When the centrifuge is done a green light will come on.

**Separating the Blood:**

1. Remove the tubes from the centrifuge VERY carefully to a test tube rack.
2. Fill out the required aliquots with the proper study and code number.
3. Very carefully separate the serum from the whole blood making sure no blood cells enter the pipette. Distribute the serum evenly amongst the aliquots.
4. If some red blood cells enter the pipette the tubes must be spun again.
5. Put the proper coloured caps on the aliquots that match the study.
6. Dispose the blood tubes in the proper biohazard bucket or container.
7. Store aliquots immediately in the -80°C.

**Analysis of Blood**

After separating out the plasma by centrifugation, blood samples from the OGTT were sent to the Kingston General Hospital CORE Lab for analysis. Plasma glucose was determined using enzymatic methods on the Synchron LX® Systems (Beckman Coulter, Inc., Brea, CA, USA). Serum total cholesterol, triglyceride, and high density lipoprotein levels were determined using standard enzymatic methods on the Synchron LX® Systems (Beckman Coulter, Inc., Brea, CA, USA). Insulin was determined with a chemiluminescent immunoassay using the Beckman Coulter UniCel Dxl 800 Access® Immunoassay System (Beckman Coulter, Inc., Brea, CA, USA).

The two most important samples for diagnosing Type 2 diabetes are the fasting or “0-time” sample and the sample taken 120 minutes after ingestion of the glucose drink. Fasting samples which are greater than 7.0 mmol/L or a 2-hour glucose level greater than 11.1 mmol/L are diagnostic of diabetes, according to the 1999 World Health Organization diabetes criteria. The blood lipids were also measured with the fasting blood samples.
Appendix C

Accelerometry Protocol

List of Measured Variables
• Average daily intensity (counts per minute; cpm)
• Sedentary behaviour (duration and intensity)
• Light physical activity (duration and intensity)
• Moderate to vigorous physical activity (duration and intensity)
• Bouted (accumulated in ≥ 10 consecutive minutes) activity
• Sporadic (accumulated in < 10 minutes) activity
• Total physical activity (duration and intensity)
• Incidental physical activity (duration and intensity)
• Sleep duration (minutes)

Measurement Device
• GT3X Actigraph Activity Monitor (Pensacola, FL)

Measurement Procedure
The accelerometer was given to the participant either immediately before or after the anthropometric appointment and was worn for 7 consecutive days. Participants were encouraged to wear the accelerometer for the full 7 days (except when participating in water-based activities such as showering or swimming) including at night. However, the participants were reassured that if the device was disruptive to normal sleep habits or if they were uncomfortable wearing the device to bed, it would be sufficient to wear the accelerometer for the full waking period of the day only (i.e., the participant was asked to put the accelerometer on as soon as they woke up in the morning and remove it immediately before returning to bed in the evening).

Preparing the Accelerometer for Data Collection
1. Ensure the battery is fully charged before being given to a participant. The accelerometer can be recharged via a standard 2.0 USB connection plugged into a computer.
   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 to begin collecting data in 60 second epochs on midnight of the day data collection begins and end exactly 7 days later at midnight. When initializing also include the participant ID and check off the boxes for: 1) Activity, 2) Step Count, 3) Enable Stop Time, 4) Dual Axis, 5) 3rd Axis, and 5) Inclinometer. Finally, ensure that the GT3X Mode is selected.
   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.
3. Attach the accelerometer to an elastic band and record the serial number of the accelerometer on the Accelerometer Wear Spreadsheet.

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 top if it is too itchy) to minimize extra motion and will be situated directly over the right hip.

3. The participant will be wearing the accelerometer for a 7 day period and removing it only for water activities (e.g., swimming, showering, or bathing).

4. The participant will also be given a log (see below) where they will record when and why the accelerometer was removed, when sleep started and stopped, and provide comments.

### Activity Monitor Log

<table>
<thead>
<tr>
<th>Participant ID:</th>
<th>Monitor ID:</th>
<th>Return Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day One</td>
<td>Day Two</td>
<td>Day Three</td>
</tr>
<tr>
<td>Dates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time Awake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time Asleep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monitor Off ___ to ___ ___ to ___ ___ to ___ ___ to ___ ___ to ___ ___ to ___</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Why?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monitor Off ___ to ___ ___ to ___ ___ to ___ ___ to ___ ___ to ___ ___ to ___</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Why?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monitor Off ___ to ___ ___ to ___ ___ to ___ ___ to ___ ___ to ___ ___ to ___</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Why?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any problems? Please explain</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Retrieving Data

1. Download raw data and then using proprietary software from Actigraph, convert the raw file into an excel file (which gives a minute-by-minute recording of the data and a graphical representation of each day) to visually examine the data for compliance with wearing instructions and to ensure the accelerometer was functioning properly.

2. When saving the file use the following format: IDVisit_startday, where Monday = 1, Tuesday = 2, and so on. For example: S001V00_2

3. Wash the elastic band in a hypo-allergenic formula and hang to dry.

### Data Analysis

1. Using both the sleep log and the excel file, determine sleep stop (i.e., wake time) and sleep start (i.e., sleep time) for each day and record in a separate excel spreadsheet. The following formula will calculate the exact epoch when each sleep stop time occurs: 
   
   ```excel
   =IF((AND(D8<>"",D8>=0)),((($C8-1)*60*24)+D8*60+E8),"")
   ```
   
   A similar formula was used for sleep start times. The Table below is an example of what the log should look like for each participant. This spreadsheet will then be used by custom designed software to separate daytime and nighttime, and calculate all daytime activity variables.
<table>
<thead>
<tr>
<th>ID</th>
<th>Visit</th>
<th>Weekday</th>
<th>Sleep Stop Hour</th>
<th>Sleep Stop Min</th>
<th>Sleep Stop Hour</th>
<th>Sleep Stop Min</th>
<th>Sleep Stop Hour</th>
<th>Sleep Stop Min</th>
<th>Sleep Stop Hour</th>
<th>Sleep Stop Min</th>
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<th>Sleep Stop Hour</th>
<th>Sleep Stop Min</th>
<th>Sleep Stop Hour</th>
<th>Sleep Stop Min</th>
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<td></td>
</tr>
<tr>
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<td>V00</td>
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<td>4</td>
<td>25</td>
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<td>3</td>
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</tr>
<tr>
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</tr>
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<td>5</td>
<td>18</td>
<td>18</td>
<td>6858</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>7</td>
<td>32</td>
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<td>6</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>V00</td>
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<td>8</td>
<td>13</td>
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<td>7</td>
<td>23</td>
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<td>10079</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Custom designed software was used to determine whether participants met inclusion criteria for accelerometry. Participants were required to wear the accelerometer for 4 full days (defined as at least 10 hours of wear during the waking hours), including at least 1 weekend day. This was determined by the software after extended periods of zeros (i.e., 60 minutes) were removed from the data. The software also calculated all variables necessary for statistical analysis.
Appendix D

Nutritional Assessment & Dietary Records

Personnel

- Nutritionist

Measurement/Assessment Equipment

- Blank Dietary Records
- Food models
- Canada’s Food Guide (Health Canada)
- Nutrition Facts leaflet (Health Canada)
- Instruction sheet summarizing the steps required to fill out the food records

Assessment Procedures

In the SERENA Study, each participant will meet with the study Nutritionist and will be asked to submit daily diet records for the duration of the program.

*During the first session with the Nutritionist the following will take place:*

1. The participant will meet with the study Nutritionist to discuss the expectations of the nutrition component of the study. This participant will be made aware of the following points:
   
   a. Participants must not make any changes to their current way of eating unless advised by the study Nutritionist
   
   b. Participants are expected to maintain weight during the baseline period

2. The Nutritionist will introduce the concept of self-monitoring and instruct the participant on the proper way to fill out the food records provided. The participant will be given a nutrition binder and taught basic tools for portion size estimation. The participant will be instructed not to change anything in terms of diet composition for the first week that they fill out the food records. They will hand in their first set of food records 6 days after the first session.

Food Record Instructions:

- Explore the participant’s knowledge and experience monitoring their food intake. *Have you ever recorded your food intake before? How did you find that? What did you notice?*
- Show them what the food records look like (see below).
**Instructions:**

a. Each day you are required to fill out this form and write down everything you have eaten (this includes butters, spreads, dressings and the little bites of food you may eat while cooking).

b. Time - You must fill out the time you’ve eaten. This is pretty self-explanatory. It is helpful to write down your food just before or after eating to ensure accurate recording (so that you don’t forget anything you have eaten).

c. Amount/Portion – It is important to estimate the portion sizes carefully. (We will go over these in a minute)

d. Food – Fill out exactly what you have eaten. Be as descriptive as possible. Include brand names and the cooking method so that we can look up some of the material if we need to. (e.g. Equality mild cheddar cheese, PC raspberry vinaigrette dressing, Parmalat skim milk, broiled vs. roasted chicken from butcher, etc.)
e. Calories & Fat - Record the calories as well as the total fat (found on the label, in the booklet given or Internet sources).

f. Source – Write down where you found the info for calories and fat (e.g. website, label etc.)

g. 7) Add up calories

3. The Nutritionist will review measurements and proper portions

**Measurements and Portions:**

- Try to make this component as interactive as possible.
- Use poster/handout out with thumb/finger guidelines
  - thumb tip = a teaspoon (helpful for butter, mayo servings, etc.)
    - note: 3 thumb tips = tablespoon (useful for peanut butter, dressings etc.)
  - thumb = 25g therefore 2 thumbs = 50 grams which is cheese serving
  - palm = 3-4 oz serving of meat
  - fist = a cup
- Use measuring cups and spoons to remind people what ⅛ cup, 1 cup, 1 tsp and 1 tbsp look like (use food models here to show 1 tsp of butter and 1 tbsp of peanut butter)
- Using dishware may want to show participant different sizes of glasses (1/2 cup, 1 cup and very large glass) so they can estimate liquids
- Other measurements that might be useful: Open slot ladle = ¼ cup; heaping = ½ cup, Closed ladle = ½ cup liquids
- Some tips that people find helpful while trying to estimate the amount:
  - Pour/ put what usually have in bowl/ plate/ glass, then transfer to measuring cup to see how much you are having
  - Measure out 1 cup of cereal/ juice for example and then put it in a bowl/ glass/ plate and notice where it fills the bowl up (can eye ball it after)
- If they can’t find it anywhere they may have to compare it to a similar item and make an educated guess (they can also leave it and ask you when they see you but try to encourage them to fill out everything).

4. The Nutritionist will explain where to find the calories and fat that needs to be recorded on the food diaries by introducing the Nutrition Facts tables, and using internet calorie counting sites (if relevant to the participant).

**How to read labels:**

- Make sure they know that the information in the Nutrition Facts table is based on the specific amount of food listed. They then need to compare this to the amount they have eaten.
- Go through some examples with the participant having them tell you how many calories they would have gotten if they had half the serving or double the serving etc. (Make sure they are comfortable with this).
• The serving size amount may be listed in grams, cups, ML or pieces of the item (example bread, crackers, cookies, chips etc). Watch for some snack items that don’t include the whole package.
• The information is always listed in the same order so they will always be able to find calories and fat in the same place (point them both out). Explain that we want them to record the Total Fat which is the first fat # listed.
• If they are eating a food with a label have them use that information (don’t look it up in booklet or on internet) as the table will be the most accurate

How to use Internet sites to obtain calorie and fat info (if applicable):
• Can give them a list of websites that you recommend, e.g. http://www.calorieking.com/
• Look up some examples with them (if you have access to a computer)
• Note that those types of websites are “dieting” type sites – remind participant that this is not a dieting study!
# Appendix E

## Diet Quality Index

The Alternative Healthy Eating Index (AHEI-2010) scoring method

<table>
<thead>
<tr>
<th>Component</th>
<th>Criteria for minimum score (0)</th>
<th>Criteria for maximum score (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetables,² servings/d</td>
<td>0</td>
<td>≥ 5</td>
</tr>
<tr>
<td>Fruit,³ servings/d</td>
<td>0</td>
<td>≥ 4</td>
</tr>
<tr>
<td>Whole grains,⁴ g/d</td>
<td>0</td>
<td>Women: 75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Men: 90</td>
</tr>
<tr>
<td>Sugar-sweetened beverages and fruit juice,⁵ servings/d</td>
<td>≥ 1</td>
<td>0</td>
</tr>
<tr>
<td>Nuts and legumes,⁶ servings/d</td>
<td>0</td>
<td>≥ 1</td>
</tr>
<tr>
<td>Red/processed meat,⁷ servings/d</td>
<td>≥ 1.5</td>
<td>0</td>
</tr>
<tr>
<td>Trans fat, % of energy</td>
<td>≥ 4</td>
<td>≤ 0.5</td>
</tr>
<tr>
<td>Long-chain n-3 fats (EPA + DHA),⁸ mg/d</td>
<td>0</td>
<td>250</td>
</tr>
<tr>
<td>PUFA, % of energy</td>
<td>≤ 2</td>
<td>250</td>
</tr>
<tr>
<td>Sodium,⁹ mg/d</td>
<td>Highest decile</td>
<td>Lowest decile</td>
</tr>
<tr>
<td>Alcohol,¹⁰ drinks/d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>≥ 2.5</td>
<td>0.5-1.5</td>
</tr>
<tr>
<td>Men</td>
<td>≥ 3.5</td>
<td>0.5-2.0</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>110</td>
</tr>
</tbody>
</table>


²Includes all vegetables except for potatoes. One serving is 0.5 cup of vegetables or 1 cup of green leafy vegetables (1 cup = 236.59 g).

³One serving is a medium piece of fruit or 0.5 cup of berries (1 cup = 236.59 g).

⁴One serving of a 100% whole grain product (e.g. 0.5 cup of oatmeal or brown rice) contains ~15-20 g of whole grains (per dry weight).

⁵One serving is 8 oz (1 oz = 28.35 g).

⁶One serving is 1 oz (1 oz = 28.35 g) or 1 tablespoon (15 mL) of peanut butter.

⁷One serving is 4 oz of unprocessed meat or 1.5 oz of processed meat (1 oz = 28.35 g).

⁸The cut-off for optimal intake (250 mg/d) is equivalent to ~2-4 oz servings of fish/week.
The cut-offs for sodium were based on deciles of distribution within the sample, due to a lack of brand specificity to accurately estimate absolute intake.

One drink is 4 oz of wine, 12 oz of beer, or 1.5 oz of liquor. Non-drinkers received a score of 2.5.
Appendix F

Anthropometry Protocol

List of Measured Variables
• Weight
• Standing Height
• Waist (iliac crest)

Measurement Devices
• Anthropometric tape (Gullick II) - contains a tension indicator device
• Detecto Weight Scale
• Stadiometer

Measurement Procedures:
Anthropometric measurements were completed at one of two locations: 1) In our laboratory in the School of Kinesiology and Health Studies building on Queen’s campus or 2) In our space at Hotel Dieu Hospital.

Weight (kg): measured on the Detecto scale with shoes removed, wearing the ‘Greys’ clothing provided.

Standing Height (cm): measured with shoes removed, standing with heels close to the wall, feet together, eyes looking straight ahead, back, and buttocks touching the back rest of the stadiometer. The head may or may not touch the back rest, depending on the size of the participant. Some participants may have to lean back in order to have the head touch; this would result in an inaccurate height measurement. Instruct participant to stand tall and take a normal breath in, record measurement given on dial.
**Waist Circumference**
The iliac crest landmark is used.

1. Clear the participant’s abdomen of all clothing and accessories. If you find resistance to the suggestion to fully remove shirt, roll up the shirt to allow free access to measurement sites and hold in place with a clip (i.e., hair clip).

2. Position the participant with feet shoulder width apart and arms crossed over the chest in a relaxed manner.

3. Take a position to the right side of the participant’s body on one knee.

4. Position the tape directly around the abdomen so that the inferior edge of the tape is at the level of the landmarked point. Use a cross-handed technique to bring the zero line of the tape in line with the measuring aspect of the tape. Ensure that the measuring tape is positioned in a horizontal plane around the abdomen. Apply tension to the tape to ensure it is snug, without causing indentation to the skin. Walk around the participant to ensure the tape is straight all around the abdomen. Alternatively, if a mirror is available – use this to ensure proper tape alignment.
5. At the end of a normal expiration, take the measurement at the top of the iliac crest. To find this landmark, palpate the upper right hipbone and draw a line where you locate the uppermost lateral border of the iliac crest.
Appendix G

Example of Statistical Output

Test of gender interaction for the association between light physical activity (LPAperc) and HOMA-IR. genLPAperc= interaction term. No gender interaction present.

Variables Entered/Removed

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables Entered</th>
<th>Variables Removed</th>
<th>Method</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>genLPAperc, LPAPerc, Gender</td>
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a. Dependent Variable: HOMAIRV00
b. All requested variables entered.

Model Summary

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<th>R Square</th>
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<tr>
<td>1</td>
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<td>.159</td>
<td>.133</td>
<td>1.321285771</td>
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</table>

a. Predictors: (Constant), genLPAperc, LPAPerc, Gender

Coefficients

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<td>(Constant)</td>
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a. Dependent Variable: HOMAIRV00

Simple linear regression analysis to determine the association between LPAperc and HOMA-IR.

Regression

Variables Entered/Removed

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<th>Model</th>
<th>Variables Entered</th>
<th>Variables Removed</th>
<th>Method</th>
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<tr>
<td>1</td>
<td>LPAPerc</td>
<td>.</td>
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a. Dependent Variable: HOMAIRV00
b. All requested variables entered.
### Model Summary

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<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
<th>Change Statistics</th>
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<td>.094</td>
<td>1.3509917 73544928</td>
<td>.103</td>
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a. Predictors: (Constant), LPAPerc
b. Dependent Variable: HOMAIRV00

### ANOVA

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<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
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<td>1</td>
<td>20.849</td>
<td>11.423</td>
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<tr>
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<td>192.518</td>
<td>100</td>
<td>1.825</td>
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<td>Total</td>
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a. Dependent Variable: HOMAIRV00
b. Predictors: (Constant), LPAPerc

### Coefficients

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<th>Standardized Coefficients</th>
<th>95.0% Confidence Interval for B</th>
<th>Collinearity Statistics</th>
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<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td>t</td>
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a. Dependent Variable: HOMAIRV00
Appendix H

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