Do Cardiorespiratory Fitness and Abdominal Obesity Mediate the Exercise-Induced Change in Insulin Sensitivity in Older Adults?

By

Gifferd Ko

A thesis submitted to the School of Kinesiology and Health Studies in conformity with the requirements for the degree of Master of Science

Queen’s University

Kingston, Ontario, Canada

September 2013

Copyright © Gifferd Ko, 2013
Lay abstract

Aging is associated with increased insulin resistance, a condition in which the tissue response to insulin-stimulated glucose uptake is reduced. Insulin resistance is a strong predictor of disease and mortality. Aging is also associated with a decline in physical activity, lower cardiorespiratory fitness (ability to deliver oxygen to active muscles during exercise), and increase in abdominal fat. Both low cardiorespiratory fitness (CRF) and excess abdominal fat are associated with reduced insulin sensitivity in older adults. Improvements in CRF and abdominal obesity through exercise training may be responsible for improvement in insulin sensitivity.

Several investigations have reported that changes in CRF and abdominal obesity through exercise are associated with changes in insulin sensitivity. To our knowledge, no prior study has assessed whether change in CRF or abdominal fat alone explains the association between exercise and improvement in insulin sensitivity in older adults. Our findings suggest that improvement in CRF may not explain the exercise-induced change in insulin sensitivity. The improvement in insulin sensitivity from exercise is explained through a decrease abdominal fat that also occurs with exercise. Additionally, improvements in waist circumference, a surrogate measure for abdominal obesity, and body mass index together explained a large portion of exercise-induced change in insulin sensitivity compared to either variable alone.

Our findings suggest that exercise combined with a healthy diet will improve insulin resistance, a risk factor for development of type 2 diabetes and cardiovascular disease in older adults. Our findings suggest that the reduction in abdominal obesity is the conduit by which exercise improves insulin sensitivity in older adults. Although CRF is not related to exercise-induced change in insulin sensitivity, change in CRF from exercise has been reported to decrease risk for other health conditions, such as hypertension and all-cause mortality. Our findings
suggest that clinicians should measure both waist circumference and body mass index when evaluating the effectiveness of a lifestyle-based treatment strategy for improving insulin resistance and its associated health outcomes in older adults.
Co-authorship

This is a secondary analysis using data from an original investigation conducted by Dr. Lance E. Davidson and colleagues. I performed all statistical analyses on the data and wrote the manuscript. Andrea Brennan edited the manuscript. Dr. Miu Lam provided statistical expertise and assistance on mediation analysis. Dr. Robert Hudson performed the hyperinsulinemic-euglycemic clamp procedures. Dr. Robert Ross reviewed, edited and co-wrote the manuscript. The manuscript in Chapter 3 is sent to Diabetologia and is currently being reviewed and under consideration.
Thesis contributions

The data obtained for my thesis was from an original investigation conducted by Dr. Davidson and colleagues. Data was collected by research staff and graduate students. 80 individuals were included in this secondary analysis.

To understand the data acquisition performed by Davidson and colleagues from his original investigation, several similar methods were taught by Dr. Robert Ross’ laboratory staff. Proper measurement of anthropometric variables and conducting a graded maximal VO$_2$ treadmill test were provided by Christine Dibblee and John Clarke. They also guided me through the analysis of an MRI, and the differentiation of tissues with the use of Slice-O-Matic software. I was taught how to acquire blood plasma and serum from our lab nurse, Tammy Scott-Zelt. My education on dietary food records was provided by Jackie LeSarge. I observed recruitment processes conducted by Pamela Asselstine. Although our lab did not conduct the 3-hr hyperinsulinemic-euglycemic clamp during my stay, Dr. Ross and Ms. Scott-Zelt educated me regarding methodology.

I was responsible for conducting and performing all statistical analyses. I thank Paula Stotz for teaching me how to use the custom module by Preacher and Hayes on SPSS to perform mediation analyses. To understand our mediation analysis results, I thank Ms. Stotz and Dr. Shane Sweet for relevant instruction.
Acknowledgements

I would first like to thank my supervisor Bob, for his contribution and consideration towards my master degree. I am wholeheartedly grateful for the time you have invested in me. I am also thankful for the opportunity to work with you, which I feel is a privilege considering that I see you as a genius in the field of exercise physiology. You have definitely taught me to think more critically, and I will not forget that. I would also like to thank you for taking the chances and accepting me as your student. I have come a long way, and it was with your help, as my mentor, in guiding me.

To any other staff and participants from Dr. Ross’ lab who have helped me when I was lost or confused, thank you. Most of you were great listeners with exuberant thoughts in guiding me through the last two years. This group has definitely made my experience at Queen's University fantastic. A special thanks to Christine Dibblee, Pamela Asselstine, Katie Mattiuz, Veronica Lloyd, and Sean Hakimi, for always brightening up my day.

To the graduate students who have walked this journey with me, cheers. It has definitely been a pleasure to work or hang out with you guys. This is especially true for Brenton Button, Brad Hiebert, Sara Giovannetti, Trevor O'Neill, Einat Shalev-Goldman, Mitch Wilson, John Clarke, and more. To Andrea Brennan, who has gone above and beyond to help others (myself included), I thank you. There are more than I can name, but you know who you are. To the Queen’s Medicine friends I have met and paddled with, it has most certainly been a pleasure.

Next I would like to thank my partner, Sarah Haines. Thanks for being your wonderful self. I appreciate your unwavering support and positive attitude towards my study. Aside from academia, I hope we have both learned from one another, and may that never end.
To my friends back home in Toronto, I thank you for being there for me. Beyond the scope of my last two years, I have always valued our friendship. Thank you all for your support. A special thanks to Yegor Puzanov, David Kwok, Duncan Gawel, Adam Okashimo, Vladymyr Martsinkovskiy, Stefan Rak, David Skoryk, and Andrew Nguyen. Last but certainly not least, I thank Iskender Piyale-Sheard for being there for me when I needed it most.

A special thanks to my family. I could not have asked for better parents and brother. I wish for the best for you guys, as I am sure you have for me. I appreciate all the support you guys have given. To my grandmother who passed away last year, you are loved. I'm sorry I haven't visited Taiwan in ages, and I thank you for having been a caring grandmother. I hope I am making you proud.
## Table of contents

Lay abstract........................................................................................................................................... ii

Co-authorship.......................................................................................................................................... iv

Thesis contributions.............................................................................................................................. v

Acknowledgements............................................................................................................................... vi

Table of contents................................................................................................................................... viii

List of tables............................................................................................................................................ xi

List of figures........................................................................................................................................... xii

List of abbreviations............................................................................................................................... xiii

Chapter 1: General introduction........................................................................................................... 1

Chapter 2: Literature review.................................................................................................................. 3

  2.1.1 Introduction................................................................................................................................. 3

  2.2.1 Aging........................................................................................................................................... 3

  2.3.0 Age-related decline in insulin sensitivity.................................................................................... 4

    2.3.1 Insulin......................................................................................................................................... 4

    2.3.2 Measuring insulin sensitivity..................................................................................................... 5

    2.3.3 Aging and insulin sensitivity..................................................................................................... 6

  2.4.0 Aging and abdominal obesity...................................................................................................... 7

    2.4.1 Abdominal obesity..................................................................................................................... 7

    2.4.2 Abdominal adipose tissue depots.............................................................................................. 7

    2.4.3 Age-associated change in adiposity......................................................................................... 9

    2.4.4 Identification of adiposity....................................................................................................... 10

  2.5.0 Aging and cardiorespiratory fitness............................................................................................ 13

    2.5.1 Cardiorespiratory fitness.......................................................................................................... 13
2.5.2 Age-associated change in cardiorespiratory fitness ......................... 13
2.5.3 Low cardiorespiratory fitness and insulin resistance ....................... 14
2.6.0 Exercise .................................................................................. 14
  2.6.1 Exercise reduces risk of morbidity and mortality ......................... 14
  2.6.2 Exercise utilization of carbohydrate and lipid ............................ 15
  2.6.3 Exercise-induced change in insulin sensitivity ........................... 16
  2.6.4 Aging, exercise, cardiorespiratory fitness, abdominal obesity, and insulin
      sensitivity .................................................................................. 21
2.7.1 Gap in our current knowledge .................................................... 24
2.8.1 Mediation analysis .................................................................... 24
2.9.1 Summary .................................................................................. 25

Chapter 3: Manuscript ......................................................................... 26
  3.1.0 Abstract .................................................................................. 27
  3.2.0 Introduction ............................................................................. 29
  3.3.0 Methods ................................................................................ 30
  3.4.0 Results ................................................................................. 34
  3.5.0 Discussion ............................................................................. 36
  3.6.0 Figure 1 ................................................................................ 40
  3.6.0 Table 1 ................................................................................ 41
  3.7.0 Table 2 ................................................................................ 42

Chapter 4: General discussion .............................................................. 44
  4.1.0 Further interpretation ................................................................ 44
  4.2.0 Clinical implications ................................................................. 45
  4.3.0 Limitations ............................................................................. 47
Summary and conclusions ................................................................. 49
References.................................................................................................................................50
Appendix A, Manuscript tables and figures.................................................................62
Appendix B, Hyperinsulinemic-euglycemic clamp measurements.................................68
Appendix C, Maximal treadmill test.........................................................................70
Appendix D, Dietary intake.........................................................................................71
Appendix E, Anthropometric measurements.............................................................72
Appendix F, SPSS mediation analysis.........................................................................73
Appendix G, Full statistical score................................................................................77
Appendix H, MRI analysis.........................................................................................85
Appendix I, Participant consent...............................................................................88
Appendix J, Ethics approval.......................................................................................101
List of tables

Chapter 2: Literature review

Table 1. Intervention studies demonstrating whether exercise-induced change in CRF and/or abdominal obesity is associated with change in insulin sensitivity in older adults………………..18

Table 2. Cross-sectional studies demonstrating whether change in CRF and/or abdominal obesity is associated with change in insulin sensitivity in older adults………………………………20

Chapter 3: Manuscript

Table 1. Participant baseline and post-intervention changes……………………………41

Table 2. Simple and multiple mediation…………………………………………………….42
List of figures

Chapter 2: Literature review

Figure 1. Age-associated change in intra-abdominal AT.................................10

Chapter 3: Manuscript

Figure 1. Simple mediation.................................................................40
**List of abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>Adipose Tissue</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
</tr>
<tr>
<td>CRF</td>
<td>Cardiorespiratory Fitness</td>
</tr>
<tr>
<td>FFA</td>
<td>Free Fatty Acid</td>
</tr>
<tr>
<td>T2D</td>
<td>Type 2 Diabetes</td>
</tr>
<tr>
<td>WC</td>
<td>Waist Circumference</td>
</tr>
</tbody>
</table>
Chapter 1: General introduction

The older adult population is increasing, as the cohort aged 65 and older is predicted to increase from 12% in 2000 to 20% by 2030 in the United States (66). It is well established that aging is associated with reduction in insulin-stimulated glucose disposal (125), which is related to increased morbidity and mortality (16, 29, 151). Insulin is a hormone involved in regulating glucose disposal primarily in skeletal muscle, as well as lipid metabolism within the liver and adipose tissue (21, 92). The apparent age-related decrease in insulin sensitivity is correlated with cardiovascular disease (CVD), type 2 diabetes (T2D), and other metabolic abnormalities (109, 110, 125). There is also an age-related decline in cardiorespiratory fitness (CRF) (140) and increase in abdominal obesity (76), which further increase the risks associated with morbidity and mortality, independent of age, gender or ethnicity. Prevalence of abdominal obesity in older adults has increased globally from 32% in 2000 to 37.4% in 2010 (2). More than half the older adults in the United States are considered to be abdominally obese, as measured by waist circumference. Complications resulting from obesity will account for more than one-third of all healthcare spending, and is expected to increase in the foreseeable future (32).

Aging is associated with increase in visceral adiposity and reduction of abdominal subcutaneous fat (2, 16, 76, 148, 149). It is established that visceral fat is inversely associated with insulin sensitivity (24). Therefore, aging-associated increase in abdominal obesity is positively correlated with several metabolic risk factors which themselves are associated with an increase in risk of CVD and T2D (31, 35, 36). Aging is also correlated with a non-linear decline in cardiorespiratory fitness (CRF) (52). The decline in CRF begins at the age of 45, and occurs at an accelerated rate throughout subsequent years. Low CRF is a strong, independent marker of morbidity and mortality (9, 81, 152).
Numerous studies have reported that excess abdominal obesity and/or low CRF are associated with adverse outcomes such as insulin resistance and premature mortality (79, 82, 115, 119). Prior investigations have shown that physical activity combined with a healthy diet is associated with improvements in various risk factors, such as insulin sensitivity (139), CRF (96, 139), and reduced abdominal obesity in older adults (18, 139). However, whether the exercise-induced change in CRF and/or abdominal obesity independently mediates change in insulin sensitivity in older adults is unclear.

The manuscript contained in Chapter 3 describes a study in which the primary objective was to investigate whether and to what extent change in CRF and abdominal obesity are independently associated with exercise-induced change in insulin sensitivity in older adults. To our knowledge, no other study has assessed the independent effect of change in CRF and abdominal obesity from exercise on change in insulin sensitivity in older adults. Since low CRF and elevated levels of abdominal obesity are associated with insulin resistance in older adults, and exercise may improve both variables, they are potential candidates for mediating the association between exercise and improvement in insulin sensitivity.
Chapter 2: Literature review

2.1.1 Introduction

This review will address the current literature on various issues associated with aging, such as low CRF, elevated abdominal obesity, and insulin resistance. It will address the risks associated with a sedentary lifestyle, and the benefits from structured physical activity for older adults. There are but a handful of studies that have attempted to investigate whether exercise-induced change in CRF and/or abdominal obesity mediates change in insulin sensitivity in older adults. This review will summarize existing literature on exercise-induced change in insulin resistance in older adults and discuss the importance of their work, strengths, and weaknesses that warrant our current investigation (18, 96, 103, 139, 147, 154).

2.2.1 Aging population

It is well established that the older adult population is increasing, as the cohort aged 65 and older is predicted to increase from 12% in 2000 to 20% by 2030 in the United States (2). While improved healthcare has extended lifespan and longevity, a better quality of life is not necessarily guaranteed for older adults (149). Older adults are reported to be more susceptible to impairment of insulin sensitivity when leading a sedentary lifestyle (22, 26, 125, 148). Chronic exposure to impaired insulin-stimulated glucose uptake increase the risk of developing type 2 diabetes (T2D) and other cardiovascular disease (CVD) risk factors associated with morbidity and mortality (21, 108–110). It is reported that by the age of 75, twenty percent of older adults will develop T2D (45). Prevalence of insulin resistance in older adults will decrease quality of life, as well as increase dependence on the healthcare system (2).
Aging is also reported to be associated with a non-linear decline in CRF (52) and an increase in abdominal obesity (29, 148). Both low CRF and excess abdominal obesity have been reported to be associated with insulin resistance in older adults (50, 106). They are also associated with morbidity and all-cause mortality (30, 152).

2.3.0 Age-related decline in insulin sensitivity

2.3.1 Insulin

Insulin is a crucial hormone for carbohydrate and lipid metabolism (92). Secreted by β-cells of the pancreatic islets of Langerhans, insulin stimulates plasma glucose uptake into muscle cells, liver and adipocytes to maintain a homeostatic plasma glucose concentration (131). This occurs when insulin binds with insulin receptors on cell membranes, which rapidly phosphorylates tyrosine residues of insulin receptor substrate-1/2 (IRS-1/2). The phosphorylation of tyrosine residues on IRS-1/2 causes a cascade of events to stimulate glucose transporter (GLUT-4) translocation. This will migrate GLUT-4 from storage vesicles to the cell membrane, which will allow glucose uptake to occur (15, 113).

Insulin can also promote free fatty acid (FFA) re-esterification to form triglycerides within adipocytes, therefore reducing intracellular lipolysis and FFA secretion (127). Prior to entering the systemic circulation, insulin is substantially cleared by the liver, which acts as a filter to regulate the systemic plasma concentration of glucose (37). Insulin sensitivity is defined as the physiological ability of tissues, particularly muscle cells, to respond to insulin and subsequently store plasma carbohydrate and lipids.
2.3.2 Measuring insulin sensitivity

In 1979, DeFronzo et al. were the first to develop a method of assessing peripheral insulin sensitivity (20). Peripheral insulin sensitivity is defined as the ability for tissues (primarily skeletal muscle) to manage insulin-stimulated glucose disposal. Peripheral glucose disposal contributes to the majority of whole-body glucose disposal, or insulin sensitivity as a whole ($r = 0.70, p < 0.001$) (19). The hyperinsulinemic-euglycemic clamp technique is now considered the gold standard for measuring peripheral insulin resistance (38). Although not feasible for clinical practice, this clamp technique serves as a tool for investigators to observe how insulin sensitive a given individual is. The clamp technique suppresses hepatic glucose production, such that a given extraneous infusion of glucose can be measured to determine insulin response to glucose uptake in skeletal muscle (21).

Another method to determine an individual’s insulin response is with an oral glucose tolerance test (OGTT). From OGTT results, an index of insulin sensitivity can be derived (21). OGTT is easier to conduct and obtain results compared to the clamp procedure. As well, an OGTT result is highly correlated with results from the hyperinsulinemic-euglycemic clamp for determining insulin sensitivity ($r = 0.73, p < 0.0001$) (91). Although OGTT is another method to measure insulin response through glucose tolerance, it does not completely suppress hepatic glucose production. Therefore, OGTT results are affected by both liver and skeletal muscle glucose responses. This gives the clamp procedure an advantage, because it can specifically measure skeletal muscle insulin sensitivity more accurately (21). However, both serve the purpose of measuring glucose uptake and tissue response to insulin.
Other measures of insulin sensitivity include the insulin sensitivity index (ISI) (91) and quantitative insulin sensitivity check index (QUICKI) (62). These measurements are calculated from algorithms of fasting plasma glucose and insulin concentrations, or from OGTT results.

2.3.3 Aging and insulin sensitivity

It has been recognized for almost a century that human aging is associated with impaired glucose tolerance (137). As adults age, there is a decline in insulin response towards glucose disposal. This age-associated decline in insulin sensitivity has been reported by several investigators (22, 125). DeFronzo (22) observed a negative correlation between aging and peripheral insulin sensitivity. He reported that this decline is not because of pancreatic β-cell dysfunction, but rather attributed to impaired glucose tolerance in tissues. Swerdloff et al. (142) reported that every decade of aging is associated with an increase in fasting plasma glucose concentration of approximately 1 mg/dL, which further suggests the inability to dispose plasma glucose and impaired insulin function (23).

Potential mechanisms by which an age-associated insulin resistance have been reported from several investigations (107, 131, 144). It may be from excessive FFA secreted from adipose tissues (AT) that compete with glucose for substrate utilization (107, 131). It may be through complex biochemical interactions of cytokine metabolism that increase with age and obesity (144, 145), or impaired receptor function with age (125). While the primary mechanism to induce insulin resistance is still unclear, potential mechanisms are extensively reviewed elsewhere (144). These metabolic changes are recognized to impair the age-related changes in insulin function to control carbohydrate and lipid metabolism.
Although aging is associated with insulin resistance from a sedentary lifestyle (148, 149), Kohrt et al. reported that older adults matched for abdominal obesity had similar glucose disposal as compared to younger adults (70). Therefore, these results suggest that it may not be aging that induces insulin resistance, but rather the sedentary lifestyle and decline in physical activity that leads to the obese conditions which are associated with insulin resistance.

2.4.0 Aging and abdominal obesity

2.4.1 Abdominal obesity

It is well established that obesity is a major health concern that is associated with the older adult population (2, 16, 29, 148). Excessive abdominal adiposity from obesity is associated with morbidity and mortality (26, 27), and has adverse effects on quality of life for individuals, independent of age, gender, or ethnicity. This can result from a sedentary lifestyle, modification of the quantity and quality of our diet, or reduction in structured physical activity (102). Aging is associated with an increase in abdominal obesity (148), and abdominal obesity is associated with insulin resistance (3, 75).

2.4.2 Abdominal adipose tissue depots

Adipose tissues (AT) within the abdominal region are visceral and abdominal subcutaneous AT. Visceral AT are discriminated by omental and mesenteric AT, depending on its anatomical position within the abdominal region (112). Omental and mesenteric AT reside
within the peritoneum, with mesenteric AT situated posterior to omental AT. Visceral AT are directly connected to the intestinal tract through mesenteric arteries and drained by the hepatic portal vein to the liver (61). Visceral AT are reported to have strong lipolytic characteristics of FFA secretion (7, 112), which are approximately 2-4 times greater than peripheral subcutaneous fat (111, 112).

Visceral AT that acquire excess FFA from high dietary intake and/or lack of physical activity become dysfunctional in storage capacity, eventually secreting FFA that deposit in the liver as triglycerides. There is a very strong association between excessive liver fat and metabolic risk factors that predict T2D and CVD (71, 72). This association occurs because the liver is crucial for carbohydrate and lipid metabolism, and it is a major site for insulin and FFA uptake and degradation (101). It has been suggested that excess liver fat is negatively associated with hepatic extraction of insulin, and may result in insulin resistance (89).

Abdominal subcutaneous AT can be divided into deep and superficial layers, depending on their location beneath or above Scarpa’s fascia, respectively (86). Deep subcutaneous AT has a strong association with obesity-related health risks, as they are characteristically more similar to visceral AT than superficial subcutaneous AT (64). Kelley et al. used computed tomography to delineate abdominal subcutaneous AT depot into deep and superficial depots by anatomical demarcation of the fascia. Using this method, they reported that deep subcutaneous AT is correlated more to insulin resistance and visceral AT than superficial subcutaneous AT (64). This would suggest that deep abdominal subcutaneous AT contributes a larger role to insulin resistance than does superficial peripheral adipose tissues.
2.4.3 Age-associated change in adiposity

Aging is associated with loss of fat free mass and an increase in abdominal fat mass, particularly visceral adiposity (76, 116). Several investigations have reported that visceral adiposity is associated with chronic low-grade systemic inflammation, and leads to several metabolic risk factors. Visceral AT is associated with dyslipidemia (95), insulin resistance (3, 24, 67, 110), hypertension (63), T2D (13), CVD (33), morbidity (69), and mortality (75). Banerji et al. reported that visceral fat is a strong independent predictor of insulin resistance and prevalence of T2D and CVD in Asian Indians (3). This is consistent with several other findings that have reported the association between visceral fat with T2D and CVD in other ethnicities (13, 33). The age-related physiological change in adipose tissue distribution impairs the homeostatic regulation of carbohydrate and lipid metabolism.

Kuk et al. have reported in an extensive review that aging is associated with changes in AT distribution. An age-related increase in visceral AT occurs in proportion to total AT, despite the absence of changes in waist circumference size (76). As shown in Figure 1, despite the matching of waist circumference between younger and older adults, frank differences in intra-abdominal AT are apparent. Changes in abdominal adiposity increase risks associated with insulin resistance and other risks mentioned above (7). Therefore, an age-associated increase in visceral AT will increase the risk of metabolic complications that predict morbidity and all-cause mortality (8). The biological reasons for which AT redistribution occurs from aging in older adults are not fully elucidated.
2.4.4 Identification of adiposity

Adiposity can be measured from indirect anthropometric measures or direct measures such as computed tomography (CT) and magnetic resonance imaging (MRI). In clinical practice, a common obesity assessment tool is body mass index (BMI), which is calculated as weight in kilograms divided by height in meters squared (68). Several studies have observed a J-shaped relationship between an individual’s BMI with morbidity and/or mortality risk factors (6, 88). Therefore, a very low BMI is associated with health risk, as well as a very high BMI. Established general BMI categories are < 18.5: underweight, 18.5-24.9: normal weight, 25.0-29.9: overweight, 30.0-34.9: Class 1 obese, 35.0-39.9: Class 2 obese, and ≥ 40.0: Class 3 obese (84, 144). Although BMI is a useful tool to report the epidemiological trends of obesity, body fat distribution cannot be differentiated in two individuals with the same BMI score (25).
Although BMI is a strong independent predictor of morbidity and mortality on an epidemiological scale (153), an anthropometric measure more suitable in clinic is the measurement of waist circumference (WC) (26, 116). WC is more suitable in a clinical setting because WC predicts obesity-related health risks after statistical control for BMI, but the opposite is not true (59). WC has been reported to be very reliable as a surrogate marker of intra-abdominal fat, and therefore a good predictor of risks associated with abdominal obesity (76, 116). The standard threshold of WC to consider an individual as obese is $\geq 88$ cm for women and $\geq 102$ cm for men. Even though anthropometric measurements are useful surrogate markers for the identification of obesity-related risk factors, they vary considerably by different age and ethnic groups (26, 116).

Although WC measurements are reliable measures of abdominal obesity, Janssen et al. have reported that the combined use of WC with BMI is a better predictor of body fat distribution than either alone (58). Both independently contribute to prediction of body fat distribution, but when combined they are able to explain a greater variance of the distribution of AT. In another study, Janssen et al. reported that a higher WC after adjusting for BMI results in greater mortality risk, whereas a higher BMI when adjusted for WC results in a lower mortality risk in older adults (60). These findings support that the measurement of WC and BMI should be taken together when assessing obesity-related health risks in older adults.

Borkan et al. were the first to assess the distribution of abdominal AT using CT (12). They reported that a single CT image is highly correlated ($r = 0.89$, $p < 0.001$) with the same information on adiposity as a series of scans around the abdominal region. Their findings support the use of a single image, preferably around the umbilicus region to obtain measurement of abdominal AT distribution. This method was later reproduced by Fujioka and colleagues who
used CT scan to report that a large accumulation of visceral AT around the abdominal region is associated with an impaired glucose and lipid metabolism observed in obese patients (34). During this time, Sparrow et al. (136), as well as Krotkiewski and colleagues (73) reported the reliability of CT scans for measuring obesity.

Magnetic resonance imaging (MRI) has also been used to anatomically discriminate adipose tissue from other tissues in human subjects (47). Staten and group first measured AT distribution by MRI in 1988 (138). During this time, several studies tested the reliability and reproducibility of MRI, as reported by Ross and colleagues (115, 119, 122–124) and Sobol et al. (134). While WC provides an indirect measure of abdominal fat, MRI and CT scan provide direct measures of different AT depots. CT and MRI have provided precise and reliable measures for determining body fat distribution, as well as identifying different types of AT that encompass the abdominal region. MRI provides greater specificity for scientists and physicians to differentiate between subcutaneous and visceral AT.

Even though an age-related change in adiposity occurs, abdominal obesity is also associated with lack of physical activity and excessive caloric intake (24). Perhaps insulin resistance in older adults stems from a lack of physical activity and low cardiorespiratory fitness (CRF), rather than aging alone. A low CRF suggests that the individual participates in very little physical activity, and may therefore lead to exposure of risks associated with a sedentary lifestyle such as insulin resistance.
2.5.0 Aging and cardiorespiratory fitness

2.5.1 Cardiorespiratory fitness

Blair and colleagues from the Aerobics Research Centre in Texas, coined the term cardiorespiratory fitness to define the attribute that represents fitness through regular physical activity, since physical activity is a habitual behavior that cannot easily be measured (5, 9, 11, 92). CRF is explained as the ability to deliver oxygen to muscle cells during physical activity (92). The major determinant of CRF is cardiac output; the ability to increase blood flow output to increase oxygen delivery and extraction (5). An individual may be able to improve their CRF through chronic adaptations that occur through frequent exercise (121). Resulting from regular aerobic exercise training, the observed change in CRF is approximately 20% from baseline in sedentary individuals, although the results may vary depending on genetics, age, sex, race, and initial fitness (133).

2.5.2 Age-associated change in cardiorespiratory fitness

As reported by Blair et al., a high CRF is inversely correlated with morbidity and all-cause mortality (9, 10, 80, 141). With an increase in abdominal obesity, several investigators have also reported an age-related decline in CRF (52, 141). The decline in CRF accelerates past the age of 45 (52). Sui et al. reported that the association between abdominal obesity and mortality risk is abolished after control of CRF in older adults (141). It is also reported that CRF has a strong inverse association with metabolic risk factors (83) after controlling for adiposity. This would suggest that CRF is a variable to consider for risks associated with morbidity and all-cause mortality, especially in older adults.
2.5.3 Low cardiorespiratory fitness and insulin resistance

A cross-sectional study from Sui et al. reported that a decline in CRF in older adults is associated with impaired glucose disposal (140). Large-scale prospective cohort studies from Sawada et al. (128) and Lee et al. (82) have reported similar findings; that low CRF is associated with impaired fasting glucose and incidence of developing type 2 diabetes. These findings are consistent with Larsen et al. (78), who reported that CRF is associated with insulin sensitivity in a cross-sectional investigation of young adults. Since low CRF is associated with lack of physical activity, these findings suggest that individuals who develop T2D are likely to be physically inactive. High CRF suggests exercise-induced muscle adaptations for improvement or maintenance of glucose tolerance.

Reduction in CRF and increase in abdominal obesity are suggested to cause insulin resistance in older adults. Although low CRF and elevated abdominal obesity are demonstrated to be associated with morbidity and mortality in older adults, exercise may attenuate several risk factors, as well as improve CRF and reduce abdominal obesity.

2.6.0 Exercise

2.6.1 Exercise reduces risk of morbidity and mortality

For more than a century, it has been reported that exercise is associated with reduction in risks associated with morbidity and mortality. In 1843, Dr. Guy from King’s College noticed that vigorous exercise from labor intensive occupations are favorable to health, and that a sedentary
lifestyle affected both men and women (44). These observations were noticed by several other studies in subsequent years (48, 97). In 1953, Morris et al. identified a dose-response relationship between exercise and health (93, 94). In parallel with Morris and colleague’s work, Paffenbarger et al. extended their observation and reported that energy expenditure from exercise is associated with reductions in CVD and other risk factors (98).

2.6.2 Exercise utilization of carbohydrate and lipid

It is well established that energy expenditure from exercise changes the utilization of fat as a substrate for energy production. Exercise intensity is the main factor for determining the degree of carbohydrate or lipid use. Relative fat oxidation is highest at an exercise intensity of 25% VO₂max (92). Fat oxidation accounts for approximately 50% energy expenditure at an exercise intensity of 65% VO₂max. However, since the energy demand to produce physical exertion at 65% VO₂max is greater than low-intensity exercise, the absolute rate of fat oxidation is greater (92, 114). Low-intensity (25% VO₂max) exercise utilizes FFA from AT lipolysis to sustain activity. Chronic exercise at a moderate intensity (50-70% VO₂max) will condition muscle cells to use intra-myoellular triglyceride, as well as plasma FFA (114). High-intensity exercise utilizes glucose and muscle glycogen more than fat. Adaptation to exercise training at moderate intensity will shift substrate utilization towards lipids, and it has been reported that muscle glycogen is preserved and lactate production is reduced in working muscles through chronic exercise (92). This allows for glucose and muscle glycogen stores to be conserved for high-intensity bouts of exercise energy demand.
The shift towards lipid substrates instead of carbohydrate use through exercise training and adaptation will improve insulin sensitivity and reduce obesity. Martin et al. reported that exercise training reduces AT secretion of fatty acid and increased plasma FFA clearance at rest (90). This is consistent with other studies that have reported that reduction of fasting plasma FFA is associated with exercise-induced change in insulin sensitivity (129). Solomon et al. reported that exercise with a reduction in caloric intake reduces resting plasma FFA (135). Regardless of diet, they also noticed an increase in intra-myocelellar FFA oxidation, which is associated with improvement in insulin sensitivity in older adults. Henriksson reported that a submaximal exercise trained leg utilizes more fat for energy production than the untrained leg in young adults (49). This metabolic shift towards fat oxidation and conservation of muscle glycogen for high-intensity exercise provides a potential mechanism by which exercise may improve insulin sensitivity. These reports also explain the physiological mechanism by which exercise may reduce obesity, through reduction of excess FFA.

2.6.3 Exercise-induced change in insulin sensitivity

Exercise has been shown to improve insulin sensitivity in older adults (18, 103, 135). Physical training programs improve insulin action in some insulin-resistant individuals (28, 118). The improvement in insulin sensitivity through chronic exercise allows for a refined ability for insulin-mediated glucose disposal. Improvement in insulin sensitivity reduces risks associated with morbidity and mortality.

Numerous studies show that exercise is associated with reduction in abdominal obesity in older adults (18, 103, 139). Reduction in abdominal obesity is associated with attenuation of risk
factors such as insulin resistance. Exercise has also been shown to improve CRF in older adults (96, 103, 139, 147, 154). It may be that changes in CRF and/or abdominal obesity are the means by which exercise improves insulin sensitivity in older adults. Although the mechanisms are not well known, these changes may be through enhanced insulin receptors, change in substrate utilization or reduction in cytokines (144). Tables 1 and 2 summarize the current literature of exercise-induced improvement of insulin sensitivity through change in CRF and/or abdominal obesity in older adults, by intervention or cross-sectional design, respectively.
Table 1. Intervention studies demonstrating whether exercise-induced change in CRF and/or abdominal obesity is associated with change in insulin sensitivity in older adults.

<table>
<thead>
<tr>
<th>Author</th>
<th>M/F</th>
<th>Age</th>
<th>Ethnicity</th>
<th>Study Design</th>
<th>Adjustment</th>
<th>Insulin Sensitivity Determination</th>
<th>Strengths and Weaknesses</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>O'Leary et al. 2006</td>
<td>5/11</td>
<td>63 ± 1</td>
<td>N/A</td>
<td>12 week exercise intervention, no control ~250-300 min exercise per week</td>
<td>None</td>
<td>OGTT</td>
<td>-Insulin sensitivity measured 16-18 hours after last exercise bout -Exclusion criteria of medication users -Dietary intake maintained at baseline -No control group</td>
<td>Significant negative correlation ($r = -0.48$, $p &lt; 0.05$) between CRF and insulin resistance Reduction in abdominal obesity, particularly visceral adiposity is correlated with change in insulin resistance ($r = 0.66$, $p &lt; 0.05$)</td>
</tr>
<tr>
<td>Yassine et al. 2009</td>
<td>9/15</td>
<td>65.5 ± 5</td>
<td>N/A</td>
<td>12 week exercise intervention. Randomized to exercise, or exercise with diet. No control group ~250 min exercise per week</td>
<td>None</td>
<td>Hyperinsulinemic-euglycemic clamp OGTT</td>
<td>-Insulin sensitivity measured 16 hours after the last exercise session -Abdominally obese older adults -Several participants had T2D glucose intolerance -No control group</td>
<td>Change in CRF is correlated with change in glucose disposal rate ($r = 0.47$, $p = 0.02$) Change in visceral fat is correlated with change in insulin resistance ($r = 0.48$, $p = 0.04$) Subcutaneous fat is not correlated with glucose disposal rate</td>
</tr>
<tr>
<td>Stewart et al. 2005</td>
<td>51/53</td>
<td>55-75</td>
<td>Mostly Caucasian</td>
<td>6 month randomized control trial exercise intervention ~150-200 min exercise per week</td>
<td>None</td>
<td>QUICKI</td>
<td>-Blood samples obtained from an overnight fast -Participants with certain medication were excluded -Maintained caloric intake</td>
<td>No correlation between change in CRF with change in insulin sensitivity Change in abdominal obesity is inversely correlated with change in insulin sensitivity ($r = -0.27$, $p &lt; 0.01$)</td>
</tr>
<tr>
<td>Study</td>
<td>Group</td>
<td>Age</td>
<td>Ethnicity</td>
<td>Duration</td>
<td>Exercise Intensity</td>
<td>Intervention Details</td>
<td>Other Details</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------</td>
<td>------</td>
<td>------------</td>
<td>----------</td>
<td>--------------------</td>
<td>--------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Pratley et al. 2000 (103)</td>
<td>17/0</td>
<td>59 ± 2</td>
<td>N/A</td>
<td>9 month</td>
<td>Exercise intervention, no control, ~120-150 min exercise per week</td>
<td>Multiple regression to assess the independent effects of CRF and body fat at 59 ± 2 N/A</td>
<td>9 month exercise intervention, no control, ~120-150 min exercise per week, OGTT Hyperinsulinemic-euglycemic clamp, Insulin sensitivity measurement time not mentioned, Non-obese healthy older adults, No control group, Middle and older age adults. No correlation between change in CRF with change in insulin sensitivity. Change in WC is correlated with change in insulin response ($r^2 = 0.68$, $p &lt; 0.001$) after adjusting for body fat percentage and CRF.</td>
<td></td>
</tr>
<tr>
<td>Tonino 1989 (147)</td>
<td>6/5</td>
<td>69 ± 2</td>
<td>Caucasian</td>
<td>12 week</td>
<td>Exercise intervention, no control, ~150 min exercise per week</td>
<td>None</td>
<td>12 week exercise intervention, no control, ~150 min exercise per week, OGTT Hyperinsulinemic-euglycemic clamp, Non-obese healthy older adults with normal glucose tolerance, Waist circumference not measured, Clamp measurement made 12-h after overnight fast, Did not maintain caloric intake, No control group. No correlation between exercise-induced change in CRF with change in insulin sensitivity. Baseline CRF is correlated with insulin sensitivity ($r = 0.69$, $p &lt; 0.02$). No attempt in abdominal obesity measurement was made.</td>
<td></td>
</tr>
<tr>
<td>Davidson et al. 2009(18)</td>
<td>47/70</td>
<td>60-80</td>
<td>Caucasian</td>
<td>6 month</td>
<td>Randomized control trial exercise intervention, ~150 min exercise per week</td>
<td>None</td>
<td>6 month randomized control trial exercise intervention, ~150 min exercise per week, OGTT Hyperinsulinemic-euglycemic clamp, Abdominally obese older adults, Clamp measured 36-48 hours after last exercise session, Participants with certain medication were excluded, Maintained caloric intake. Change in abdominal obesity is associated with change in insulin sensitivity ($p &lt; 0.05$). No attempt in assessing CRF in correlation to insulin sensitivity was made.</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: M/F = male/female participants, WC = waist circumference, CRF = cardiorespiratory fitness, OGTT = oral glucose tolerance test, QUICKI = quantitative insulin sensitivity check index
Table 2. Cross-sectional studies demonstrating whether CRF and/or abdominal obesity is associated with insulin sensitivity in older adults.

<table>
<thead>
<tr>
<th>Author</th>
<th>M/F</th>
<th>Age</th>
<th>Ethnicity</th>
<th>Adjustment</th>
<th>Insulin Sensitivity</th>
<th>Strengths and Limitations</th>
<th>Findings</th>
</tr>
</thead>
</table>
| Racette et al. 2006     | 117/290 | 69 ± 11 | N/A       | Multiple linear regression to control for WC, BMI, CRF, and percent fat     | ISI                 | -Controlled for sex, WC, BMI, CRF, body fat percentage  
-Did not exclude participants who were taking medication  
-Insulin sensitivity measured 10-12 hours the morning after an overnight fast  
-Healthy middle-aged and older adults  
-Cross-sectional                                                   | CRF is a significant correlate of insulin sensitivity (r = 0.22, p < 0.0001)  
After controlling for WC, BMI, and percent fat, CRF remained significantly correlated with insulin sensitivity (r = 0.12, p = 0.014)  
WC was the strongest independent correlate of insulin sensitivity (r = -0.52, p < 0.0001)  
WC remained a significant correlate of insulin sensitivity after control of BMI, percent fat, and CRF (r = -0.27, p < 0.0001) |
| (106)                   |      |         |           |                                                                             | OGTT                |                                                                                                              |                                                                          |
| Hollenback et al. 1984  | 20/0 | 66 ± 3  | N/A       | Partial correlation when adjusting for BMI and percent body fat             | OGTT                | -Sample size, and all men  
-BMI < 30 for inclusion criteria  
-Normal glucose tolerance  
-Insulin sensitivity measured > 72 hours after the last exercise session  
-Cross-sectional                                                  | CRF is correlated with in vivo insulin action after adjustment for BMI and percent body fat (r = 0.70, p < 0.001)  
No attempt was made between establishing a correlation between BMI and insulin sensitivity |
| 1984(50)                |      |         |           |                                                                             |                     |                                                                                                              |                                                                          |

Abbreviations: M/F = male/female participants, ISI = insulin sensitivity index, OGTT = oral glucose tolerance test, CRF = cardiorespiratory fitness, BMI = body mass index, WC = waist circumference
2.6.4 Aging, exercise, cardiorespiratory fitness, abdominal obesity, and insulin sensitivity

While it is well established that older adults are more susceptible to low CRF (52) and excessive abdominal obesity (76), perhaps it is not aging that has an influence on insulin sensitivity, but rather the sedentary lifestyle associated with aging that causes the risk. Insulin resistance may lead to development of type 2 diabetes (108), and whether the onset of the disease is caused by aging or risks associated with it have been investigated by Amati et al. (1). They reported, in a cross-sectional study, that when younger and older individuals are adjusted for obesity and fitness, age is not significantly correlated with insulin resistance. This would suggest that obesity and fitness have an influence on insulin sensitivity, independent of age. This is consistent with observations from other studies (70). However, cross-sectional studies are difficult to determine a causal inference on whether exercise-induced change in insulin sensitivity occurs from change in abdominal obesity and/or CRF.

The investigations shown in Table 1 and 2 provide observations that look at whether an exercise-induced change in CRF or abdominal obesity mediates change in insulin sensitivity in older adults. They have extended our understanding of risks associated with insulin resistance in the elderly, and have explored the potential mechanisms by which exercise-induced changes in CRF and abdominal obesity may attenuate this risk. These investigations do support abdominal obesity in mediating exercise-induced change in insulin sensitivity, but CRF findings are inconsistent. As well, there are several strengths and weaknesses that should be noted.

The prospective intervention studies above often lack a large sample size and several lack a control group (96, 103, 147, 154). This makes it rather difficult to interpret the results since the
study may not be powered to answer the research question and findings cannot be compared to a non-exercise group. The inconsistent findings with CRF being associated with exercise-induced change in insulin sensitivity are reported by several studies. Some have reported that CRF is associated with exercise-induced change in insulin sensitivity (96, 154), but others have suggested no association (103, 139, 147). This may be due to the participant’s weekly exercise time being much longer in the former studies. Another weakness from several investigations was through conducting insulin sensitivity tests within 24 hours after the last exercise session (96, 154). Thus, the acute effects of the last exercise session may still be present.

It is well established that exercise elicits an acute physiological response and a chronic adaptation to training (42, 113, 146, 155). An acute bout of physical activity at moderate intensity will cause a physiological response in skeletal muscle, such that skeletal muscle-mediated glucose uptake will occur (19, 113). Uptake of glucose may be stored as glycogen, or oxidized for ATP production for further energy expenditure (92). The acute response to exercise has been reported to last for hours to several days, as there has yet to be an established time range for this response (42). Chronic adaptations to exercise involve increased translocation of glucose transporters to cell membranes, increased mitochondrial biogenesis for enhanced substrate oxidation, or other muscle adaptations (51, 113). This will result in improvements on insulin-mediated glucose uptake. Chronic adaptation to exercise training take several weeks of continuous physical activity to see improvements in insulin sensitivity (146). It is known that if the measurement of insulin sensitivity is taken within several hours after the last exercise session in an exercise training program, there may still be acute responses for glucose uptake. This can overestimate the contributions of changes in CRF and/or abdominal obesity on exercise-induced change in insulin sensitivity.
Several studies were not conducted in obese individuals (103, 147). This would suggest that the older adults were metabolically healthy to begin with, and this would therefore provide different results from older adults who are abdominally obese. Pratley et al. (103) was the only group who attempted to isolate the independent effects of CRF and abdominal obesity on exercise-induced change in insulin sensitivity from the list of intervention studies. They found that CRF is not associated with exercise-induced change in insulin sensitivity, which contradicts findings from several investigations (96, 154).

Racette et al. (106) observed that abdominal obesity is a stronger predictor of insulin sensitivity than CRF in adults aged 50-95 years. They report that WC, a surrogate measure of abdominal obesity, has the strongest association with insulin sensitivity after controlling for BMI and CRF. Their study design used a multiple regression to delineate independent effects of CRF and obesity on insulin sensitivity. However, their participants included healthy middle-aged and older adults. Hollenback et al. reported that higher habitual physical activity (CRF) is associated with insulin-stimulated glucose disposal in older adults (50). They did conduct a partial correlation analysis between CRF with insulin sensitivity when controlling for BMI and body fat percentage. A noted strength in their study is that measurement of insulin sensitivity was 72 hours after the last exercise session. However, this study was also on healthy non-obese older adult males, with a small sample size of 20. Both Racette et al. (106) and Hollenback et al. (50) are cross-sectional investigations, so they are unable to investigate a causal relationship on whether an exercise-induced change in abdominal obesity and CRF is associated with change in insulin sensitivity. As well, these cross-sectional studies do not take into account the lifestyle of the individuals, such as diet and medication use, which may have an influence on insulin sensitivity.
2.7.1 Gap in our current knowledge

Aging is associated with increase in abdominal fat, specifically visceral AT (76), and a non-linear decline in CRF (52). Abdominal adiposity has been reported to be associated with insulin resistance (24, 26). Cross-sectional studies have reported that a high CRF is associated with insulin sensitivity (50, 78). Numerous studies have supported the notion that exercise is associated with improvement in insulin sensitivity (28, 63, 82, 115, 117) and CRF (9, 11), as well as a reduction in abdominal obesity (55, 56, 74, 150).

There has yet to be a study that has sought to investigate whether an independent effect of exercise-induced change on abdominal obesity and/or CRF mediate the change in insulin sensitivity in older adults. Thus, a secondary analysis from of data from a randomized controlled trial conducted by Davidson et al. (18) will be completed to investigate this question. Mediation analysis is required to establish this association.

2.8.1 Mediation analysis

Mediation analysis is a statistical tool used to assess whether a proposed mediator variable is associated with the exposure (exercise) and outcome (insulin sensitivity) variable. Mediation was originally proposed by Baron and Kenny (4) in 1986, and has progressed in recent decades. Preacher and Hayes (104) extended the practical use of mediation analyses, as well as interpretation of statistical results. A diagram to demonstrate mediation analysis can be found in Chapter 3, Figure 1. Mediation analysis is used to identify whether the independent effect of exercise-induced change on CRF and/or abdominal obesity mediates the change in insulin sensitivity in older adults.
2.9.1 Summary

Our population is getting older, and there is an increase in those following a sedentary lifestyle, which represents a challenge in reducing risks associated with morbidity and mortality. Sedentary older adults suffer the risks associated with low CRF, abdominal obesity and impaired insulin sensitivity. There have been several investigations on exercise-induced change in insulin sensitivity, abdominal obesity, and CRF. However, this thesis aims to establish whether the independent effect of exercise-induced change on CRF and/or abdominal obesity mediate the association between exercise and insulin sensitivity. We hypothesize that abdominal obesity independent of CRF mediates exercise-induced change on insulin sensitivity in older adults. CRF, as determined by VO$_2$peak during a maximal exercise test will not mediate the association, since the exercise-induced change in CRF and insulin sensitivity occur through different physiological mechanisms. To our knowledge, this is the first study that will assess this association with mediation analysis in older adults. Be it from the effects of change in substrate or cytokine secretions (144), change in abdominal obesity from exercise will effect change in insulin sensitivity in older adults.
Chapter 3: Manuscript

Title: Do Cardiorespiratory Fitness and Obesity Mediate the Exercise-Induced Change in Insulin Sensitivity in Older Adults?

Ko, Gifferd¹, Davidson, Lance E¹,², Lam, Miu³, Brennan, Andrea¹, Hudson, Robert⁴, Ross, Robert¹,⁴

Affiliation:

1. School of Kinesiology and Health Studies, Queen’s University, Kingston, ON, Canada
2. Department of Exercise Sciences, Brigham Young University, Provo, Utah, United States
3. Department of Epidemiology and Community Health, Queen’s University, Kingston, ON, Canada
4. Department of Medicine, Division of Endocrinology and Metabolism, Queen’s University, Kingston, ON, Canada
3.1.0 Abstract

**Purpose:** Both abdominal obesity and low cardiorespiratory fitness (CRF) are significantly associated with insulin resistance in older adults. Although it is established that exercise is associated with improvement in insulin sensitivity, whether this association is determined by change in CRF and abdominal obesity is unclear. Therefore, we question whether and to what extent change in CRF and abdominal obesity mediate the exercise-induced change in insulin sensitivity in older adults.

**Methods:** Subjects were 80 sedentary, obese older adults (60-80 years) randomized to an exercise (N = 59) or control group (N = 21) for 6 months. The exercise group participated in approximately 150 minutes of supervised exercise per week. CRF (VO₂peak) was measured using a maximal treadmill test, total and abdominal adipose tissue (AT) was measured using magnetic resonance imaging, and insulin resistance was assessed using a hyperinsulinemic-euglycemic clamp procedure. Waist circumference (WC) was measured at the level of the iliac crest. Simple and multiple mediation were used to assess whether and to what extent total and abdominal AT and/or CRF mediated the exercise-induced change in insulin sensitivity.

**Results:** Marked reduction in all AT depots as well as WC and body mass index (BMI) was observed in the exercise group compared to controls (p < 0.05). By comparison to control, exercise was associated with improvement in insulin sensitivity and CRF (p < 0.05). Simple mediation revealed that with the exception of CRF, all AT variables, BMI and WC were mediators of the exercise-induced change in insulin sensitivity (p < 0.05). Multiple mediation revealed that after adjustment for change in total AT, abdominal AT remained a significant mediator with an effect ratio of 0.79 (p < 0.05), whereas total AT was not significant when
adjusted for abdominal AT ($p > 0.05$). Abdominal subcutaneous AT was a significant mediator after adjustment for visceral AT ($p < 0.05$), but visceral AT was not when adjusted for abdominal subcutaneous AT ($p > 0.05$). The effect ratio for changes in WC and BMI combined (0.63) was greater ($p < 0.05$) than either WC (0.39) or BMI alone (0.24).

**Conclusion:** CRF did not mediate the exercise-induced change in insulin sensitivity in older adults. Alternatively, abdominal adiposity was a strong mediator independent of corresponding changes in total adiposity. The combined use of WC and BMI should be considered when assessing the efficacy of exercise treatment strategies on glucose management in older adults.

**Key words:** exercise, insulin sensitivity, obesity, cardiorespiratory fitness, mediation
3.2.0 Introduction

Aging is associated with a reduction in insulin sensitivity (63), a strong predictor of morbidity and mortality (76, 118). Aging is also characterized by an increase in abdominal adipose tissue (AT), in particular visceral AT (76), which is inversely related to insulin sensitivity (24). There is also an age-related decline in cardiorespiratory fitness (CRF) (52), and CRF is associated with insulin sensitivity in older adults (50, 96).

There is little argument that, in older adults, chronic exercise is associated with improvement in insulin sensitivity (126) and CRF (96), as well as a reduction in abdominal obesity (18). Unclear is whether the change in CRF and/or abdominal obesity mediate the exercise-associated change in insulin sensitivity. Limited evidence suggests that exercise-induced improvement in insulin sensitivity is associated with reductions in abdominal obesity (96, 139). Whereas the findings from some short-term intervention studies suggest that changes in CRF are associated with exercise-induced change in insulin sensitivity (96, 154), others do not (103, 139, 147). Absent from the literature are data from randomized controlled trials that consider whether changes in abdominal obesity and CRF independently mediate the exercise-induced improvement in insulin sensitivity in older adults.

The purpose of this study was to identify whether and to what extent change in CRF and abdominal obesity mediate the association between exercise and insulin sensitivity in older adults. Mediation analysis was used to decompose the total effect of exercise on insulin sensitivity into its direct and indirect (via the mediator) effect.
3.3.0 Methods

Participants

In the original investigation 136 obese elderly individuals aged 60-80 years were recruited to participate in an exercise trial (18). This secondary analysis includes the 80 subjects who participated in three arms of the trial: a control group, an aerobic exercise group, or a combination of both aerobic and resistance exercise group. Recruited participants were previously sedentary, abdominally obese (≥102 cm waist circumference for males, ≥ 88 cm for females), and weight stable (± 2kg) for 6 months before study entry. The protocol used in the original investigation was approved by the Queen's University Health Sciences Research Ethics Board. All participants gave informed consent prior to participation.

Dietary and Exercise Interventions

Eligible participants were randomized to a control group, or two different exercise modalities. Both exercise groups were required to participate in supervised exercise for approximately 150 minutes per week for 6 months. Those in the aerobic exercise group were asked to exercise 5 times per week for 30 minutes each session (18). The combined exercise group was asked to perform 30 minutes of aerobic exercise, 3 times a week, and 3 sessions of resistance exercise (8 exercises) per week. All participants were instructed by a nutritionist on how to maintain a healthy diet throughout the intervention. All participants attended 9 individual 1-hour seminars in which the nutritionist taught healthy food selection and preparation (18).

Anthropometric Measurements
Anthropometric measurements were taken at baseline and at the end of the intervention. Body weight was measured with participants dressed in standard T-shirts and shorts. Waist circumference (WC) was obtained in a standing position using the mean of 2 measures obtained at the superior edge of the iliac crest.

**Measurement of Total and Regional Fat and Skeletal Muscle Mass**

Total AT, abdominal AT, abdominal subcutaneous AT, and visceral AT were measured by magnetic resonance imaging using established procedures (118). Visceral and abdominal subcutaneous AT depots were calculated using 5 images extending from 5 cm below to 15 cm above the L4-5 intervertebral space.

**Measurement of Insulin Resistance**

Insulin resistance was assessed using a 3-hour hyperinsulinemic-euglycemic clamp protocol (18). Post-intervention insulin resistance was assessed 36-48 hours after the final exercise session.

**Cardiorespiratory Fitness Measurement and Exercise Energy Expenditure**

Cardiorespiratory fitness (measured as oxygen consumption per unit of time [peak VO$_2$]) was determined using results of a maximal treadmill test combined with standard open-circuit spirometry techniques (SensorMedics Corp, Yorba Linda, California). Oxygen cost of aerobic exercise was determined using the relationship between heart rate and oxygen consumption obtained from the graded exercise test results (peak VO$_2$). Oxygen consumption during resistance exercise was estimated to be 45% of maximal oxygen uptake. Energy expenditure for both exercise modalities was determined by multiplying oxygen consumption by 5.04 kcal/L.
Statistical Analysis

With the exception of a greater reduction of total and visceral AT in men than women in the aerobic exercise group only, and aerobic exercise group did not significantly change skeletal muscle mass post-intervention, men and women in both exercise modalities did not differ significantly in their response to exercise for any variable. Therefore, we collapsed across exercise modality and sex for all statistical procedures. All mediation analyses are controlled for age and baseline characteristics.

Changes in adiposity, CRF, and anthropometric variables between exercise and control groups were compared with an independent t-test (Table 1). Mediation analysis using the product-of-coefficient method originally described by Baron and Kenny (4) were used to assess whether an indirect effect of exercise on insulin sensitivity was attributed to a mediator. Mediation analysis uses a series of regression analyses that delineate the effects of exercise on insulin sensitivity through possible mediators, including CRF, adiposity and anthropometric variables (Table 2). All regression analyses were adjusted for age and baseline variables.

First, we assessed the total effect of exercise on insulin sensitivity (c-path) (Figure 1). Second, we assessed the association of exercise with our proposed mediator (CRF, adiposity, or anthropometric variable) (a-path). Third, we assessed the association between our proposed mediator and the insulin sensitivity variable (b-path). The product of a and b paths (a*b) represent the mediating or indirect effect. Finally, we assessed the association of exercise and insulin sensitivity after adjusting for the effects of the mediator (c’-path). This type of analysis permits determination of the indirect effects of exercise via the mediator in addition to the direct effect of exercise on insulin sensitivity independent of its indirect effects via the mediator.
Simple mediation was employed, wherein proposed mediators were entered into the mediation model separately to determine each variable’s indirect effects (Table 2). Multiple mediation analysis was subsequently used to examine whether combinations of different adipose tissue, CRF, and anthropometric variables mediated the effects of exercise on change in insulin sensitivity. This also allowed us to determine if independent mediator variables remained significant mediators after adjusting for the effects of each other.

To determine the relative magnitude of the effects, effect ratios were calculated as the ratio of the indirect effect of exercise on insulin sensitivity through the mediator (path $a*b$) to the total effect of exercise on insulin sensitivity (path $c$), i.e., the proportion of the total effect attributable to the indirect effect ($a*b/c$). For example, if abdominal AT and total AT had effect ratios of 0.51 and 0.48, respectively, abdominal AT would be considered a stronger mediator than total AT. Associations were significant at a p-value less than 0.05. Statistical procedures were performed using SPSS (IBM SPSS Statistics for Windows Version 21.0. Armonk, NY: IBM Corp.) and a mediation analysis custom dialog (INDIRECT) provided by Preacher and Hayes (105) which allowed for computation of total, direct and indirect effects of exercise on insulin sensitivity.
3.4.0 Results

Participant baseline characteristics and post-intervention changes are shown in Table 1. Significant improvement in insulin sensitivity was observed in the exercise group compared to control \((p < 0.05)\). Significant reductions were observed for all anthropometric and adiposity variables in response to exercise compared to control group \((p < 0.05)\). A significant increase in CRF was also observed for exercise compared to control \((p < 0.05)\). There were no significant between-group differences for exercise energy expenditure or adherence to the recommended calorie intake targets \((18)\).

Mediation analysis results are shown in Table 2. Exercise was significantly associated with change in insulin sensitivity \((\text{path } c, p < 0.05)\). In simple mediation, exercise was significantly associated with change in CRF \((\text{path } a, p < 0.05)\), however, CRF was not related to change in insulin sensitivity \((\text{path } b, p > 0.05)\); thus CRF was not a mediator of the association between exercise and insulin sensitivity \((95\% \text{ CI: -1.52, 1.37})\). The direct effect of exercise on insulin sensitivity remained significant \((\text{path } c’, p < 0.05)\) after adjusting for CRF.

All AT variables in addition to WC and BMI were mediators of the association between exercise and insulin sensitivity \((\text{path } a*b, p < 0.05)\). The effect ratios were 0.51, 0.43, and 0.35 for abdominal, visceral, and abdominal subcutaneous AT respectively. After controlling for each adiposity and anthropometric variable separately, the direct effect of exercise on insulin sensitivity was no longer significant \((\text{path } c’, p > 0.05)\).

Multiple mediation was used to explore whether abdominal obesity is a mediator of the association between exercise and insulin sensitivity after controlling for other variables \((\text{Table 2})\). After controlling for total AT, abdominal AT remained a significant mediator \((0.40, 6.34)\).
with an effect ratio of 0.79. However, after adjusting for abdominal AT, total AT did not remain a significant mediator (-3.68, 1.60). After controlling for BMI, WC was not a significant mediator (-0.44, 3.45); similarly, BMI was not a significant mediator (-0.78, 2.36) after control for WC. However, the effect ratio for changes in WC and BMI combined (63%) was greater than that observed for either WC (49%) or BMI (41%) alone.

Abdominal subcutaneous AT remained a significant mediator after adjusting for visceral AT (0.13, 2.55), however, visceral AT was not significant after adjusting for abdominal subcutaneous AT (-0.53, 2.55). Abdominal subcutaneous AT did not remain a significant mediator after adjusting for total AT (-1.07, 2.39) or BMI (-0.37, 2.49). After adjustment for abdominal subcutaneous AT, total AT (-1.00, 3.67) and BMI (-1.07, 2.39) did not significantly mediate the association.
3.5.0 Discussion

The primary finding of this study is that changes in CRF as determined by VO₂peak may not be associated with the exercise-induced change in insulin sensitivity in older adults whereas change in abdominal AT was significantly associated independent of total AT and/or CRF. That change in WC and BMI combined explained a greater variance of the exercise-induced reduction in insulin resistance compared to either WC or BMI alone, supports the recommendation that both of these simple tools be used to determine the efficacy of strategies designed to improve glucose management in older adults.

To our knowledge this is the first study to examine the independent effects of exercise-induced change in abdominal obesity and CRF on insulin sensitivity in older adults. Our observation that reduction in abdominal adiposity mediates exercise-induced change in insulin sensitivity substantively extends an earlier report by Stewart et al. (139) wherein an exercise-induced reduction in abdominal adiposity was associated with change in insulin sensitivity in older adults. In that study no attempt was made to determine whether the association between changes in abdominal obesity and insulin sensitivity remained independent of corresponding changes in CRF.

Our finding that reduction in abdominal adiposity mediates the association between exercise and insulin sensitivity independent of total adiposity is consistent with a large body of cross-sectional evidence confirming a strong independent association between abdominal obesity and insulin sensitivity (70, 96). The mechanisms that link abdominal adipose tissues with cardiometabolic risk factors including insulin resistance are numerous and continue to be the source of intense investigation (144). Whether the mechanisms be of a substrate and/or cytokine
origin, the importance of the exercise-induced decrease in abdominal adiposity is underscored by the fact that the absolute reduction in abdominal AT in our study was approximately one-third that of total adiposity (Table 1). Indeed, the reduction in abdominal AT accounted for 79% of the change in insulin resistance after adjusting for the reduction in total AT. Thus the findings from this report and others (96, 139) reinforce the importance of reducing abdominal obesity for improving the management of insulin sensitivity in older adults.

Our observation that reductions in abdominal subcutaneous AT mediated the exercise-induced reduction in insulin sensitivity independent of change in visceral AT whereas the reverse was not true was an unexpected finding. We (57) and others (24, 40) have previously observed that changes in visceral AT are associated with change in insulin resistance independent of abdominal subcutaneous AT. However, our finding in this study is consistent with others (17) and suggests that exercise-induced change in abdominal subcutaneous AT is a mechanism by which exercise improves insulin sensitivity in older adults.

Despite our finding that change in MRI-measured abdominal adiposity mediated the change in insulin resistance independent of change in total adiposity, the combination of WC and BMI was better than WC alone to follow exercise-induced improvement in insulin sensitivity. Although it may be argued that changes in BMI are confounded by simultaneous changes in adiposity and skeletal muscle, our findings clearly demonstrate that changes in MRI-measured skeletal muscle were not associated with change in insulin sensitivity (Table 2). Thus, from a clinical perspective, although both measurements alone benefit the determination of identifying exercise-induced change in insulin sensitivity, our findings suggest the use of both WC and BMI to follow the efficacy of exercise-induced improvement in insulin sensitivity. The underlying mechanisms supporting this observation remain unclear.
Our observations suggest that CRF is a characteristic that improves consequent to increases in physical activity, but does not mediate or provide the mechanism by which exercise improves insulin resistance. While some prior cross-sectional and small-sample intervention studies observe a significant association between CRF and insulin sensitivity (96, 154), others do not (103, 139, 147). It is not clear the extent to which improvements in CRF are mechanistically linked to improvements in insulin resistance (39). Since improvement in CRF is a central adaptation driven by improvement in cardiac output (5), it follows that change in insulin sensitivity, a peripheral adaptation in skeletal muscle glucose uptake (20), is not associated with changes in CRF. This finding does not negate the importance of measuring CRF in practice as it is established that CRF is an independent predictor of morbidity and mortality (80). Our finding only suggests that the change in CRF that occurs in response to exercise does not inform clinicians regarding the individual’s ability to manage blood glucose in the short term.

It is apparent from our findings that the exercise-induced improvement in insulin sensitivity (direct effect) was abolished after accounting for change in any adiposity and/or anthropometric (WC, BMI) variable. While these findings reinforce the importance of exercise-induced changes in adiposity for improving insulin resistance, it is important to note that the measurement of insulin resistance post-intervention was conducted 36-48 hours after the participant’s last exercise session. The purpose in doing so was to attenuate the well-established acute effects of chronic exercise on insulin sensitivity (146). Accordingly, had we measured insulin resistance within 12 to 24 hours of the last exercise session it is possible that the direct effects of exercise on insulin resistance mediated in part by acute changes in muscle metabolism (22) favoring enhanced glucose uptake may have persisted despite control for changes in adiposity.
Limitations to our study include the homogeneity of the participants which limits the generalizability of our findings. However, given that the worldwide prevalence of aging and related obesity is already high and increasing, our findings are relevant for a large segment of the older adult population with the high-risk form of obesity. Although our analysis included criterion measures of adipose tissue distribution as potential mediators, other body composition variables including liver fat (46, 101) and intramuscular lipid (41) known to be associated with insulin sensitivity were not measured. The strengths of our study include rigorously controlled and supervised exercise protocols and the use of criterion methods to measure insulin sensitivity and whole-body adiposity. The use of mediation analysis allowed us to decompose the total effect of exercise on insulin resistance into the direct and indirect (via the mediator) effects. This type of analysis addresses the limitations of commonly employed traditional regression analysis, which fails to identify whether or not a covariate is a true mediator, described as a variable associated with both the exposure (exercise) and the outcome (insulin resistance).

In summary, our findings suggest that change in abdominal AT have a significant influence on the beneficial effects of exercise on insulin resistance in older adults, but CRF may not. Further, WC and BMI combined are simple tools that mediate the exercise-induced improvement in insulin sensitivity better than either measure alone. Health care providers are encouraged to obtain these anthropometric measures in order to determine the efficacy of exercise combined with a healthful diet as a strategy to improve insulin sensitivity in older adults.
Figure 1: Overall mediation model
Table 1. Participant baseline characteristics and post-intervention changes

<table>
<thead>
<tr>
<th></th>
<th>Exercise Men</th>
<th>Control Men</th>
<th>Exercise Women</th>
<th>Control Women</th>
<th>Exercise Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N = 26)</td>
<td>(N = 8)</td>
<td>(N = 33)</td>
<td>(N = 13)</td>
<td>(N = 59)</td>
<td>(N = 21)</td>
</tr>
<tr>
<td>Age, years</td>
<td>67.6 ± 5.51</td>
<td>67.3 ± 9.79</td>
<td>66.68 ± 5.35</td>
<td>66.36 ± 4.06</td>
<td>67.26 ± 5.64</td>
<td>67.07 ± 3.94</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30.60 ± 3.17</td>
<td>-1.05 ± 0.88</td>
<td>30.17 ± 1.94</td>
<td>0.14 ± 0.32</td>
<td>29.48 ± 3.19</td>
<td>-0.9 ± 0.85</td>
</tr>
<tr>
<td>WC, cm</td>
<td>112.32 ± 8.81</td>
<td>-4.96 ± 3.08</td>
<td>111.62 ± 5.76</td>
<td>0.20 ± 2.31</td>
<td>99.06 ± 3.93</td>
<td>-3.57 ± 3.22</td>
</tr>
<tr>
<td>Body Weight, kg</td>
<td>94.87 ± 12.92</td>
<td>-3.01 ± 2.24</td>
<td>92.16 ± 9.79</td>
<td>0.31 ± 0.72</td>
<td>78.93 ± 10.49</td>
<td>-2.23 ± 2.01</td>
</tr>
<tr>
<td>VAT, kg</td>
<td>4.56 ± 1.02</td>
<td>-0.58 ± 0.42</td>
<td>4.23 ± 1.29</td>
<td>0.002 ± 0.23</td>
<td>2.43 ± 0.85</td>
<td>-0.25 ± 0.21</td>
</tr>
<tr>
<td>AbAT, kg</td>
<td>8.97 ± 2.48</td>
<td>-1.08 ± 0.68</td>
<td>8.78 ± 1.54</td>
<td>-0.008 ± 0.32</td>
<td>7.59 ± 1.84</td>
<td>-0.58 ± 0.58</td>
</tr>
<tr>
<td>TAT, kg</td>
<td>34.00 ± 8.66</td>
<td>-4.09 ± 2.19</td>
<td>33.51 ± 6.21</td>
<td>-0.46 ± 1.10</td>
<td>34.95 ± 7.1</td>
<td>-2.55 ± 2.1</td>
</tr>
<tr>
<td>ASAT, kg</td>
<td>4.41 ± 1.92</td>
<td>-0.49 ± 0.38</td>
<td>4.56 ± 0.96</td>
<td>-0.08 ± 0.18</td>
<td>5.16 ± 1.38</td>
<td>-0.33 ± 0.42</td>
</tr>
<tr>
<td>CRF, L/min</td>
<td>2.60 ± 0.48</td>
<td>0.27 ± 0.34</td>
<td>2.72 ± 0.35</td>
<td>-0.17 ± 0.30</td>
<td>1.82 ± 0.31</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Insulin Sensitivity, M/I</td>
<td>14.58 ± 7.05</td>
<td>4.12 ± 5.13</td>
<td>20.00 ± 8.08</td>
<td>-1.24 ± 5.36</td>
<td>22.85 ± 8.68</td>
<td>4.24 ± 5.29</td>
</tr>
<tr>
<td>Skeletal Muscle, kg</td>
<td>30.06 ± 3.64</td>
<td>0.26 ± 1.18</td>
<td>28.91 ± 3.78</td>
<td>0.12 ± 0.82</td>
<td>19.50 ± 2.98</td>
<td>0.39 ± 0.65</td>
</tr>
</tbody>
</table>

Means ± standard deviation (SD) at baseline measurements, for all participants who were randomized to an exercise group. BMI = body mass index, VAT = visceral adipose tissue, CRF = cardiorespiratory fitness, AbAT = abdominal adipose tissue, TAT = total adipose tissue, ASAT = abdominal subcutaneous adipose tissue, WC = waist circumference. Insulin sensitivity was the rate of glucose uptake per unit of insulin per kg of muscle mass per minute x 100. All post-intervention changes were significantly different from control groups ($p < 0.05$).
Table 2. Simple and multiple mediation analysis of the association between exercise and insulin sensitivity

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Effect (path C)</th>
<th>Direct Effect (path C')</th>
<th>Indirect Effect (via mediators (a*b)</th>
<th>Effect Ratio (Indirect: Total Effect)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B(SE)</td>
<td>p-value</td>
<td>B(SE)</td>
<td>p-value</td>
</tr>
<tr>
<td>Simple Mediation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRF (L/min)</td>
<td>3.66(1.25)</td>
<td>0.0045</td>
<td>3.53(1.46)</td>
<td>0.0181</td>
</tr>
<tr>
<td>VAT (kg)</td>
<td>3.78(1.21)</td>
<td>0.0024</td>
<td>2.16(1.32)</td>
<td>0.1060</td>
</tr>
<tr>
<td>ASAT (kg)</td>
<td>3.52(1.26)</td>
<td>0.0068</td>
<td>2.29(1.33)</td>
<td>0.0897</td>
</tr>
<tr>
<td>AbAT (kg)</td>
<td>3.43(1.22)</td>
<td>0.0066</td>
<td>1.68(1.34)</td>
<td>0.2146</td>
</tr>
<tr>
<td>TAT (kg)</td>
<td>3.57(1.26)</td>
<td>0.0059</td>
<td>1.86(1.41)</td>
<td>0.1902</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>3.32(1.23)</td>
<td>0.0084</td>
<td>1.70(1.42)</td>
<td>0.2353</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>3.53(1.25)</td>
<td>0.0059</td>
<td>2.07(1.42)</td>
<td>0.1488</td>
</tr>
<tr>
<td>SM (kg)</td>
<td>3.59(1.24)</td>
<td>0.0049</td>
<td>3.80(1.25)</td>
<td>0.0033</td>
</tr>
<tr>
<td>Multiple Mediation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.66(1.22)</td>
<td>0.0038</td>
<td>1.78(1.33)</td>
<td>0.1840</td>
</tr>
<tr>
<td>VAT (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASAT (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.78(1.22)</td>
<td>0.0029</td>
<td>1.70(1.36)</td>
<td>0.2158</td>
</tr>
<tr>
<td>VAT (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.53(1.27)</td>
<td>0.0070</td>
<td>1.91(1.42)</td>
<td>0.1844</td>
</tr>
<tr>
<td>ASAT (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.52(1.21)</td>
<td>0.0048</td>
<td>1.52(1.32)</td>
<td>0.2479</td>
</tr>
<tr>
<td>AbAT (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.75(1.22)</td>
<td>0.0030</td>
<td>1.76(1.39)</td>
<td>0.2089</td>
</tr>
<tr>
<td>VAT (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.54(1.26)</td>
<td>0.0065</td>
<td>2.10(1.44)</td>
<td>0.1489</td>
</tr>
<tr>
<td>ASAT (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>VAT (kg)</td>
<td>WC (cm)</td>
<td>ASAT (kg)</td>
</tr>
<tr>
<td>---------------</td>
<td>---------</td>
<td>----------</td>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td></td>
<td>3.65(1.25)</td>
<td>0.0046</td>
<td>1.56(1.45)</td>
<td>0.2847</td>
</tr>
<tr>
<td></td>
<td>3.34(1.24)</td>
<td>0.0087</td>
<td>1.27(1.42)</td>
<td>0.3800</td>
</tr>
<tr>
<td></td>
<td>3.50(1.25)</td>
<td>0.0064</td>
<td>1.62(1.43)</td>
<td>0.2619</td>
</tr>
<tr>
<td></td>
<td>3.31(1.24)</td>
<td>0.0089</td>
<td>1.23(1.47)</td>
<td>0.4070</td>
</tr>
<tr>
<td></td>
<td>3.51(1.24)</td>
<td>0.0061</td>
<td>2.15(1.50)</td>
<td>0.1569</td>
</tr>
<tr>
<td></td>
<td>3.33(1.24)</td>
<td>0.0087</td>
<td>1.86(1.50)</td>
<td>0.2188</td>
</tr>
<tr>
<td></td>
<td>3.41(1.23)</td>
<td>0.0069</td>
<td>1.51(1.39)</td>
<td>0.2820</td>
</tr>
<tr>
<td></td>
<td>3.78(1.22)</td>
<td>0.0027</td>
<td>2.15(1.38)</td>
<td>0.1238</td>
</tr>
<tr>
<td></td>
<td>3.41(1.25)</td>
<td>0.0080</td>
<td>2.12(1.36)</td>
<td>0.1226</td>
</tr>
</tbody>
</table>

CRF = cardiorespiratory fitness, VAT = visceral adipose tissue, ASAT = abdominal subcutaneous adipose tissue, AbAT = abdominal adipose tissue, TAT = total adipose tissue, WC = waist circumference, BMI = body mass index, SM = skeletal muscle. Effect ratio = indirect effect / total effect. * = Significant (p < 0.05). Effect ratio = indirect effect / total effect.
Chapter 4: General discussion

4.1.0 Further interpretation

To our knowledge this is the first study to examine the independent effects of abdominal obesity and CRF in mediating the exercise-induced change in insulin sensitivity in older adults. While several investigations have been conducted in the past to assess this association (96, 139, 147, 154), they did not identify the independent effect.

The finding that abdominal obesity mediates exercise-induced change in insulin sensitivity is consistent with the existing literature (96, 103, 120, 139). That CRF may not be a mediator of the association between exercise and insulin sensitivity is consistent with some studies (103, 147), but not all (96, 154). Perhaps differences in study design may produce these results. O’Leary et al. (96) and Yassine et al. (154) measured insulin resistance 16-18 hours after the last exercise session, so there may be an overestimation of the contribution of CRF to changes in insulin sensitivity. Their sample size is also small, which may statistically influence the results. However, our findings agree with both O’Leary et al. and Yassine et al. on abdominal obesity mediating exercise-induced change in insulin sensitivity in older adults. The mechanisms that link abdominal obesity to insulin resistance are still unclear (144).

The fact that abdominal subcutaneous fat is a mediator independent of visceral fat, but the reverse is not true, is rather surprising. Many investigators have reported that change in visceral AT is associated with change in insulin resistance independent of abdominal subcutaneous AT (40, 57). However, our study is consistent with others (17). Since both have been reported to be associated with insulin resistance, it may be that abdominal obesity as a whole should be taken into consideration for the contribution of some mechanistic function to insulin resistance in older adults.
4.2.0 Clinical implications and future research

Our findings have two main clinical implications. CRF does not statistically mediate the association between exercise and insulin sensitivity. However, a longer intervention study or a larger sample size may have shown a significant mediation from CRF. Although a change in CRF via VO\textsubscript{2}peak may not inform clinicians of exercise-induced change in plasma glucose tolerance, it can predict improvements in other risk factors, and therefore we do not negate its importance for reducing risks associated with morbidity and mortality. For example, several investigations have reported that a low CRF is associated with incidental hypertension, which is a risk factor associated with morbidity and mortality (14). High CRF and sufficient participation in physical activity could prevent 34% of new hypertension cases (14, 84, 130). Lee et al. reported that high CRF may attenuate risks associated with metabolic risk when controlling for abdominal adiposity variables (83). Low CRF is also associated with incidental T2D (82, 128). Additionally, it remains well established that low CRF is a strong and independent predictor of CVD and all-cause mortality (152).

Assessing CRF clinically is rather difficult, considering the equipment and expertise required to conduct a true measure of VO\textsubscript{2}max. However, recent publications from Jackson and group have established reliable alternative measures of CRF, without the performance of a graded VO\textsubscript{2}max test (53). These methods should be used in clinic to determine an individual’s risk for morbidity in relation to their CRF.

Our findings support that WC and BMI should be used as simple clinical measures to identify the efficacy of exercise-induced change in insulin sensitivity. As reported by Janssen et al. (58), both measures predict AT distribution better than either variable alone. It is also suggested that WC is more reflective of body fat distribution, while BMI reflects more on lean
muscle mass in older adults (60). These findings would emphasize the advantage of assessing both in clinical practice to better identify the efficacy of exercise-induced change in insulin sensitivity. Anthropometric measurements can also be combined with other measures, for example, WC and fasting plasma triglyceride concentrations as proposed by Lemieux et al. to identify risks associated with a 'hypertriglyceridemic waist' (85). Beyond simple anthropometric measurements, if clinicians are given direct measures of abdominal AT, there is a necessity to establish the quantity of abdominal obesity that defines an individual as having increased risk of obesity-related disease, since there is currently no defined criteria (132).

Since older adults generally have more abdominal fat mass (77), in particular visceral fat (76), and a decline in CRF (52), this study has importance for the elderly population. Indeed, our findings show that abdominal obesity mediates exercise-induced change in insulin sensitivity, which further extends our understanding of treatment strategies to attenuate risks associated with insulin resistance in older adults. Our results also suggest that healthcare providers should use WC and BMI measurements to determine the efficacy of exercise combined with a healthful diet as a strategy to improve insulin sensitivity in older adults. We should continue to promote exercise as a treatment strategy to improve CRF and reduce abdominal obesity, which would attenuate risks associated with morbidity and mortality, independent of age (70).

Although it is rather surprising that abdominal subcutaneous AT is a mediator when controlled for visceral AT, it has been suggested that abdominal subcutaneous AT contributes a greater amount of FFA than visceral AT postprandially (43). However, since FFA is not the only mechanism by which insulin resistance occurs, there must be other factors to consider for the association between changes in abdominal subcutaneous AT and insulin resistance in older
adults (127, 144). More investigations are needed, especially since older adults have reduction in abdominal subcutaneous AT and increase in visceral AT (76).

There should be an awareness of proper anthropometric measurements used by practitioners. Different guidelines have been proposed for different age and ethnic groups (26). There are also different protocols established, depending on measurement sites (116). These factors may not be well known to some practitioners and could potentially result in individuals not receiving a diagnosis for their obese condition (87). However, since obesity is increasing globally (2, 54), it is necessary for physicians to be educated on properly acquiring anthropometric measurements so they are able to develop strategies for identifying and assisting in reducing obesity-related health risks of their patients. Primary care professionals are also encouraged to have an understanding of how to prescribe exercise to a patient, such that we can shift our focus from treatment after onset to prevention of disease associated with insulin resistance (100).

4.3.0 Limitations

To our knowledge, this is the first study to assess whether CRF and/or abdominal obesity independently mediate the association between exercise and change in insulin sensitivity in older adults. Limitations to our study include the homogeneous sample size of Caucasian older adults, therefore affecting the generalizability of our findings. However, given that our global population is increasing in prevalence of aging and obesity, our findings are relevant for a large cohort of the older adult population.
As a secondary analysis, we were restricted to variables already measured. Other factors such as different abdominal subcutaneous AT (64), muscle adaptations (90), liver fat (46) and cytokine secretions (65) warrant inclusion in subsequent studies using this type of analysis. We did not measure intra-myocellular triglyceride concentrations, so it does not allow us to investigate whether exercise-induced change in insulin sensitivity is due to changes in muscle lipids (41). Although the quantity of skeletal muscle mass does not mediate exercise-induced change in insulin sensitivity, it does not mean that the quality or change in muscle on a biochemical level does not mediate the change in insulin sensitivity.

No attempt in cytokine measurements were made, so we cannot determine whether exercise-induced change in insulin sensitivity was from reduction of pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-6 (144, 145). Exercise-induced changes in liver fat were not measured, which is important because liver fat has been reported to be associated with risks that predict CVD and T2D (71). Although we recognize that the acute response to exercise may affect glucose metabolism from hours to several days, the use of our clamp technique to measure glucose infusion is recorded 36-48 hours after the last exercise bout. There may still have been some residual acute responses from the last exercise session (42, 113).
Summary and conclusions

We have shown that CRF does not statistically mediate the association between exercise and insulin sensitivity in older adults. Obesity, in particular abdominal obesity, as well as anthropometric variables are significant mediators of exercise-induced change in insulin sensitivity. Abdominal AT independently explains 51% of the total effect of exercise-induced change in insulin sensitivity. This suggests that there are other variables influenced by chronic exercise that may explain part of the association on changes in insulin sensitivity. Our findings also support the pragmatic use of combining both WC and BMI as simple tools to determine the effectiveness of exercise-induced changes in insulin sensitivity in older adults.

While CRF does not mediate the association between exercise and insulin sensitivity in older adults, improvement in CRF is still crucial for reduction of risks associated with all-cause mortality (152). That CRF remains an important predictor of CVD and risks associated with all-cause mortality should be acknowledged and considered by primary health professionals when assessing treatment strategies for reducing morbidity and mortality in the elderly (9, 152). Physicians are encouraged to promote physical activity to maintain a high CRF, as well as reducing abdominal obesity in order to attenuate several risks associated with morbidity and mortality in older adults.
References


### Appendix A, Table 1. Description of exercise interventions

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Control</th>
<th>Aerobic Exercise</th>
<th>Combined Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of weekly visits</td>
<td>1</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Type of Exercise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic</td>
<td>None</td>
<td>30 minutes of moderate-intensity treadmill exercise (measured heart rate to ensure 60-75% VO2peak range obtained from GXT)</td>
<td>30 minutes of moderate intensity treadmill exercise (measured heart rate to ensure 60-75% VO2peak range obtained from GXT)</td>
</tr>
<tr>
<td>Resistance</td>
<td>None</td>
<td>None</td>
<td>1 set of 9 exercises, each to volitional fatigue; chest press, shoulder raise, shoulder flexion, leg extension, leg flexion, triceps extension, bicep curl, abdominal crunches, modified push-ups; approximate duration each session, 20 min</td>
</tr>
<tr>
<td>Weekly exercise duration, min</td>
<td>None</td>
<td>150</td>
<td>150</td>
</tr>
</tbody>
</table>

Abbreviations: GXT, graded exercise test; VO₂, oxygen consumption per unit of time. Weekly exercise time durations and energy expenditure were supervised.
### Appendix A, Table 3. Simple and multiple mediation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Effect (path C)</th>
<th>Direct Effect (path C')</th>
<th>Indirect Effect (via mediators (a*b))</th>
<th>Effect Ratio (Indirect:Total Effect)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Simple Mediation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRF (L/min)</td>
<td>3.66(1.25)</td>
<td>0.0045</td>
<td>3.53(1.46)</td>
<td>0.0181</td>
</tr>
<tr>
<td>VAT (kg)</td>
<td>3.78(1.21)</td>
<td>0.0024</td>
<td>2.16(1.32)</td>
<td>0.1060</td>
</tr>
<tr>
<td>ASAT (kg)</td>
<td>3.52(1.26)</td>
<td>0.0068</td>
<td>2.29(1.33)</td>
<td>0.0897</td>
</tr>
<tr>
<td>AbAT (kg)</td>
<td>3.43(1.22)</td>
<td>0.0066</td>
<td>1.68(1.34)</td>
<td>0.2146</td>
</tr>
<tr>
<td>TAT (kg)</td>
<td>3.57(1.26)</td>
<td>0.0059</td>
<td>1.86(1.41)</td>
<td>0.1902</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>3.32(1.23)</td>
<td>0.0084</td>
<td>1.70(1.42)</td>
<td>0.2353</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>3.53(1.25)</td>
<td>0.0059</td>
<td>2.07(1.42)</td>
<td>0.1488</td>
</tr>
<tr>
<td>SM (kg)</td>
<td>3.59(1.24)</td>
<td>0.0049</td>
<td>3.80(1.25)</td>
<td>0.0033</td>
</tr>
<tr>
<td><strong>Multiple Mediation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.66(1.22)</td>
<td>0.0038</td>
<td>1.78(1.33)</td>
<td>0.1840</td>
</tr>
<tr>
<td>VAT (kg)</td>
<td>3.78(1.22)</td>
<td>0.0029</td>
<td>1.70(1.36)</td>
<td>0.2158</td>
</tr>
<tr>
<td>ASAT (kg)</td>
<td>3.33(1.23)</td>
<td>0.0086</td>
<td>1.08(1.45)</td>
<td>0.4590</td>
</tr>
<tr>
<td>TAT (kg)</td>
<td>3.53(1.25)</td>
<td>0.0061</td>
<td>2.02(1.58)</td>
<td>0.2057</td>
</tr>
<tr>
<td>AbAT (kg)</td>
<td>3.52(1.21)</td>
<td>0.0048</td>
<td>1.54(1.32)</td>
<td>0.2479</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>3.66(1.22)</td>
<td>0.0038</td>
<td>1.78(1.33)</td>
<td>0.1840</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variable</td>
<td>Total</td>
<td>AbAT (kg)</td>
<td>ASAT (kg)</td>
<td>VAT (kg)</td>
</tr>
<tr>
<td>------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>----------</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>TAT (kg)</td>
<td>ASAT (kg)</td>
<td>WC (cm)</td>
</tr>
<tr>
<td>--------------------</td>
<td>----------------</td>
<td>----------</td>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>3.53(1.27)</td>
<td>0.0070</td>
<td>1.91(1.42)</td>
<td>0.1844</td>
</tr>
<tr>
<td></td>
<td>0.97(1.16)</td>
<td>-1.00(3.67)</td>
<td>0.65(0.84)</td>
<td>-1.07(2.39)</td>
</tr>
<tr>
<td></td>
<td>1.91(1.42)</td>
<td>0.0070</td>
<td>1.72(1.42)</td>
<td>0.1844</td>
</tr>
<tr>
<td></td>
<td>0.97(1.16)</td>
<td>-1.00(3.67)</td>
<td>0.65(0.84)</td>
<td>-1.07(2.39)</td>
</tr>
<tr>
<td></td>
<td>1.62(0.72)*</td>
<td>0.0070</td>
<td>1.72(1.42)</td>
<td>0.1844</td>
</tr>
<tr>
<td></td>
<td>0.97(1.16)</td>
<td>-1.00(3.67)</td>
<td>0.65(0.84)</td>
<td>-1.07(2.39)</td>
</tr>
<tr>
<td></td>
<td>1.62(0.72)*</td>
<td>0.0070</td>
<td>1.72(1.42)</td>
<td>0.1844</td>
</tr>
<tr>
<td></td>
<td>0.97(1.16)</td>
<td>-1.00(3.67)</td>
<td>0.65(0.84)</td>
<td>-1.07(2.39)</td>
</tr>
<tr>
<td></td>
<td>1.62(0.72)*</td>
<td>0.0070</td>
<td>1.72(1.42)</td>
<td>0.1844</td>
</tr>
</tbody>
</table>

**Simple**: Simple mediator variables of the association between exercise and insulin sensitivity. CRF = cardiorespiratory fitness, VAT = visceral adipose tissue, ASAT = abdominal subcutaneous adipose tissue, AbAT = abdominal adipose tissue, TAT = total adipose tissue, WC = waist circumference, BMI = body mass index, SM = skeletal muscle. Effect ratio = indirect effect / total effect. * = Significant mediation ($p < 0.05$).
Multiple: A multiple mediation analysis of the association between exercise and insulin sensitivity with several proposed mediator variables. Effect ratio = indirect effect / total effect. * = Significant mediation ($p < 0.05$).

Several multiple mediator models were not included in the manuscript. The ones included in the manuscript were related to the primary question of whether CRF and/or abdominal obesity were mediators of exercise-induced change in insulin sensitivity.
Appendix A, Figure 1  Multiple mediation model
Appendix B, Hyperinsulinemic-euglycemic clamp measurements

Milestone time measurements

T – 60 minutes  YSI automated glucose analyzer check
                   Glucometer check
T – 60 minutes  Subject arrives, changes into lounge clothes, voids and rests for 30 minutes
T – 30 minutes  Prepare lines, solutions, and supplies
T – 30 minutes  Assess height, weight, body surface area (BSA)
                   Assess vital signs
                   IV insertion (antecubital and hand or anticubital left and right)
                   Blood collection for fasting laboratory tests
T 0 minute       Start infusion
T 10 minutes     Glucose samples in small purple-top tubes. Place on ice
T 30 minutes     Insulin samples in large purple-top tubes. Place on ice
T 60-90 minutes  Blood gas samples collected
T 90-120 minutes Blood gases repeated if necessary
T 120            Bathroom deadline
T 145            Glucose samples taken every 5 minutes
T 150-180        Clamped, depending on the individual
T 180            End of the clamp
                   Removal of the IV sampling site
                   Lunch for the subject
T 210-225        Final glucose measurement
                   Removal of the IV infusion site
The hyperinsulinemic-euglycemic clamp measurements were taken after a 12-14 hour overnight fast, and 36-48 hours after last exercise session. This procedure serves the purpose of measuring glucose infusion rate, per unit of insulin. Insulin was infused at a rate of 40 mU/m² per minute for 3 hours. This is an approximate value, depending on the body surface area of the subject. Glucose disposal rate (insulin sensitivity) was calculated by using the average exogenous infusion rate during the final 30 minutes of euglycemia.

Once the liver is 'clamped' for hepatic glucose production, exogenous glucose is the only source to provide plasma glucose. Exogenous glucose infusion rate aimed to maintain plasma glucose concentration at 5.1 mmol/L. Blood samples are collected every 5 minutes to ensure the plasma glucose is maintained. The normal insulin sensitive non-obese glucose disposal rate can range between 4.7 to 8.7 mg/kg per minute. If the glucose disposal rate is < 4.7 mg/kg body weight per minute to maintain 5.1 mmol/L during the last 30 minutes of clamp time, the participant is considered to be insulin resistant. Glucose disposal > 8.7 mg/kg per min considers the subject to be very insulin sensitive (143).

The clamp was measured 36-48 hours after the last exercise session. The purpose of this was to attenuate the measurement of insulin sensitivity to have very little or no residual effects from the last bout of exercise, and therefore no acute response (146). This delay in clamp procedure allowed Davidson et al. to determine whether any exercise-induced change in CRF and/or abdominal obesity mediate the change in insulin sensitivity was primarily from the exercise-induced changes in mediator variables.
Appendix C, Maximal treadmill test

To test CRF of the participants, a graded treadmill test was conducted. A constant speed was set on the treadmill, and this varied depending on the ability of the individual. The test starts at 0 graded incline for 3 minutes, and at every 2 minutes thereafter a 1% increase in incline was placed on the treadmill. This 1% increase occurred every 2 minutes until volitional fatigue from the participant ended the test. To measure oxygen uptake, an open-circuit spirometry $V_{\text{max}}$ analyzer was used to assess the participant’s gas exchange. A VO$_{2\text{peak}}$ was reached if the participant's sustained their plateau 40 seconds before or after their highest oxygen uptake (VO$_{2\text{peak}}$); no greater than 0.05 L/min difference. Respiratory quotient greater than 1.1 and a maximal heart rate greater than predicted were also taken into consideration. CRF treadmill tests were conducted in Hotel Dieu Hospital, in Kingston, Ontario, Canada.
Appendix D, Dietary intake

During the exercise trial, participants were instructed by nutritionists to follow Canada's recommended food guide. They were encouraged to eat healthily and maintain baseline caloric intake. Healthy eating included 7 servings of fruits and vegetables, 7 servings of grain products, 3 servings of dairy and alternatives, and 3 servings of meat and alternatives every day for men. For women, it was suggested that older adults take 7 servings of fruits and vegetables, 6 servings of grain products, 3 servings of dairy and alternatives, and 2 servings of meat and alternatives every day. Portion sizes can be found here (http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/index-eng.php).
Appendix E, Anthropometric measurements

Body mass index was determined by the weight of the individuals in kilograms, divided by their height in meters squared. Waist circumference was determined by measuring the superior edge of the iliac crest, which is a National Institute of Health (NIH) protocol. The participants were asked to stand during both measurements. During measurements, participants wore standard T-shirts and shorts. Threshold measurements for WC were followed under Health Canada guidelines (26). The identification of measurement sites was based off anatomic skeletal sites. There are several different locations to measure WC, differences in WC measurement protocol have no substantial influence on the association between WC, all-cause mortality, CVD and T2D (116). Since there are no substantial differences between measurement methods, the NIH protocol may be simpler and more easily adopted.

Note: Following the WHO protocol, the measure is taken midway between the highest point of the iliac crest and the bottom of the ribcage. Following the NIH protocol, the measure is taken at the highest point of the iliac crest.

Appendix E, Figure 1. For WC measurements, the study conducted by Davidson et al. based WC anthropometric measurements from the National Institute of Health (99).
Appendix F, SPSS mediation analysis

As shown in Appendix F, Figure 1, baseline values and age were adjusted for in the mediation analysis. This occurred for all analyses. The independent variable was either the treatment variable, which was categorized by control group ‘0’, or exercise group ‘1’. Therefore, they were established as dichotomous numerical variables. The dependent variable was always the change in peripheral insulin sensitivity from baseline.

A bootstrap procedure was used to explore model uncertainty of distribution. The Sobel test was not used because it is useful for large samples, and assumes normal distribution. Bootstrapping in mediation is non-parametric, and does not assume normality or a symmetrical sample set. This method is valuable in controlling for Type I error (105). Bootstrapping was used to determine confidence intervals. Bootstrap resampling was set to 2000, as there is no consensus for bootstrapping values, but between 1000-5000 is recommended (105). Bias-corrected was selected to adjust for bias in the bootstrap distribution.

Confidence intervals were set at 95%, which uses the 2.5\textsuperscript{th} and 97.5\textsuperscript{th} percentiles of the bootstrap values as the lower and upper bounds of the interval, respectively (104, 105). If the bootstrap estimate of the computed data does not include zero, we can conclude that the indirect effect is indeed significantly different from zero at $p < 0.05$. Upper and lower bounds will likely not be equidistant from zero, therefore implying the non-parametric distribution of data. If the confidence intervals were placed at 90%, it would give a narrower confidence interval, but results are less likely to contain the true value. If it was at 99%, it is more likely that the result will contain the true value, but will produce a wider interval range. 95\% confidence interval is a standard procedure for mediation analysis.
Appendix F, Figure 1. SPSS custom dialog produced by Preacher and Hayes. This dialog allowed for mediation analysis, therefore permitting the analysis of whether CRF and/or obesity mediate exercise-induced change in insulin sensitivity in older adults. MKGSMD = Skeletal muscle insulin sensitivity difference, MKGSM1 = Baseline skeletal muscle insulin sensitivity, AbATD = Abdominal adipose tissue difference, AbAT1 = Baseline abdominal adipose tissue. The treatment (exercise) was a dichotomous variable.
Appendix F, Figure 2. Simple mediation output on SPSS. AbAT = Abdominal adipose tissue. The total effect between exercise (IV) and insulin sensitivity (DV) were significant. When adding the mediator into the model, in this case AbAT, the association between exercise and insulin sensitivity was no longer significant. AbAT is a mediator of the association between exercise and insulin sensitivity.
Appendix F, Figure 3. Multiple mediation output on SPSS. AbATD = Abdominal adipose tissue difference, AbAT1 = Baseline total adipose tissue, ATwtD = Total adipose tissue difference, ATwt1 = Baseline total adipose tissue.
Appendix G. Full statistical score

Abdominal obesity, anthropometric measurements, or CRF are the mediators (M)
Exercise is the independent variable (X)
Insulin sensitivity is the dependent variables (Y)

c is the total effect, and would be if X --> Y by itself

$c'$ is the direct effect, if a mediator was added to the pathway

a is the relation of X --> M
b is the relation of M --> Y
ab is the indirect effect

Exercise is a dichotomous variable, and all exercise groups were categorized as ‘1’, whereas control groups were categorized as ‘0’.

$P_M = ab/c$, represents the proportion of total effect which was mediated. If $P_M > 1$, then Y is affected more through the indirect effect rather than the total effect. If $P_M$ is negative, there may be a suppressive effect from M.

Simple mediation:

<table>
<thead>
<tr>
<th>Visceral AT as the Mediator:</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>$c'$</th>
<th>c-c'=ab</th>
<th>$P_M$</th>
<th>CI</th>
<th>Mediator?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin sensitivity</td>
<td>-0.39* (SE 0.08)</td>
<td>-4.13* (SE 1.59)</td>
<td>3.78* (SE 1.21)</td>
<td>2.16 (SE 1.32)</td>
<td>1.62* (SE 0.72)</td>
<td>0.43</td>
<td>0.42, 3.3</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Abdominal AT as the Mediator</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>$c'$</th>
<th>c-c'=ab</th>
<th>$P_M$</th>
<th>CI</th>
<th>Mediator?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin sensitivity</td>
<td>-0.75* (SE 0.16)</td>
<td>-2.34* (SE 0.86)</td>
<td>3.43* (SE 1.23)</td>
<td>1.68 (SE 1.34)</td>
<td>1.75* (SE 0.64)</td>
<td>0.51</td>
<td>0.69, 3.25</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Abdominal Subcutaneous AT as the Mediator</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>$c'$</th>
<th>c-c'=ab</th>
<th>$P_M$</th>
<th>CI</th>
<th>Mediator?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin sensitivity</td>
<td>-0.35* (SE 0.1)</td>
<td>-3.47* (SE 1.46)</td>
<td>3.52* (SE 1.26)</td>
<td>2.29 (SE 1.33)</td>
<td>1.23* (SE 0.5)</td>
<td>0.35</td>
<td>0.49, 2.53</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total AT as the Mediator</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>$c'$</th>
<th>c-c'=ab</th>
<th>$P_M$</th>
<th>CI</th>
<th>Mediator?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin sensitivity</td>
<td>-2.61* (SE 0.52)</td>
<td>-0.65* (SE 0.27)</td>
<td>3.57* (SE 1.26)</td>
<td>1.86 (SE 1.41)</td>
<td>1.71* (SE 0.66)</td>
<td>0.48</td>
<td>0.59, 3.24</td>
<td>Yes</td>
</tr>
<tr>
<td>Mediator?</td>
<td>CRF as the Mediator</td>
<td>WC as the Mediator</td>
<td>BMI as the Mediator</td>
<td>SM as the Mediator</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Insulin sensitivity</strong></td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>c'</td>
<td>c-c'=ab</td>
<td>P&lt;sub&gt;M&lt;/sub&gt;</td>
<td>CI</td>
<td>Mediator?</td>
</tr>
<tr>
<td>CRF as the Mediator</td>
<td>0.34* (SE 0.07)</td>
<td>0.37 (SE 2.15)</td>
<td>3.66* (SE 1.25)</td>
<td>3.53* (SE 1.46)</td>
<td>0.13 (SE 0.7)</td>
<td>0.03</td>
<td>-1.52, 1.37</td>
<td>No</td>
</tr>
<tr>
<td>WC as the Mediator</td>
<td>-4.11* (SE 0.75)</td>
<td>-0.39* (SE 0.18)</td>
<td>3.32* (SE 1.23)</td>
<td>1.7 (SE 1.42)</td>
<td>1.62* (SE 0.72)</td>
<td>0.49</td>
<td>0.37, 3.32</td>
<td>Yes</td>
</tr>
<tr>
<td>BMI as the Mediator</td>
<td>-1* (SE 0.2)</td>
<td>-1.46* (SE 0.72)</td>
<td>3.53* (SE 1.25)</td>
<td>2.07 (SE 1.42)</td>
<td>1.46* (SE 0.64)</td>
<td>0.41</td>
<td>0.24, 2.8</td>
<td>Yes</td>
</tr>
<tr>
<td>SM as the Mediator</td>
<td>0.31 (SE 0.22)</td>
<td>-0.68 (SE 0.64)</td>
<td>3.59* (SE 1.24)</td>
<td>3.80 (SE 1.25)</td>
<td>-0.21 (SE 0.26)</td>
<td>-</td>
<td>-1.01, 0.13</td>
<td>No</td>
</tr>
</tbody>
</table>
### Multiple mediation:

Visceral AT and CRF are the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.8*</td>
<td>1.84</td>
<td>1.96*</td>
<td>0.52</td>
<td>0.36, 4.03</td>
<td>Yes</td>
</tr>
<tr>
<td>VAT</td>
<td>-0.4*</td>
<td>-4.02*</td>
<td>1.6*</td>
<td>0.42</td>
<td>0.28, 3.43</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRF</td>
<td>0.34*</td>
<td>1.07</td>
<td>0.36</td>
<td>0.09</td>
<td>-0.88, 1.53</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total AT and CRF are the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.55*</td>
<td>1.74</td>
<td>1.8*</td>
<td>0.51</td>
<td>0.01, 3.62</td>
<td>Yes</td>
</tr>
<tr>
<td>TAT</td>
<td>-2.63*</td>
<td>-0.77*</td>
<td>2.02*</td>
<td>0.57</td>
<td>0.7, 3.79</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRF</td>
<td>0.34*</td>
<td>-0.63</td>
<td>-0.22</td>
<td>-0.06</td>
<td>-1.64, 1.09</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abdominal AT and CRF are the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.43*</td>
<td>1.53</td>
<td>1.9*</td>
<td>0.55</td>
<td>0.19, 3.64</td>
<td>Yes</td>
</tr>
<tr>
<td>AbAT</td>
<td>-0.75*</td>
<td>-2.6*</td>
<td>1.95*</td>
<td>0.57</td>
<td>0.68, 3.47</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRF</td>
<td>0.35*</td>
<td>-0.12</td>
<td>-0.04</td>
<td>-0.01</td>
<td>-1.33, 1.28</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abdominal subcutaneous AT and CRF are the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.46*</td>
<td>2.18</td>
<td>1.28</td>
<td>0.37</td>
<td>-0.29, 2.9</td>
<td>No</td>
</tr>
<tr>
<td>ASAT</td>
<td>-0.36*</td>
<td>-3.93*</td>
<td>1.42*</td>
<td>0.41</td>
<td>0.5, 2.76</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRF</td>
<td>0.34*</td>
<td>-0.39</td>
<td>-0.13</td>
<td>-0.04</td>
<td>-1.59, 1.08</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

WC and CRF as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.31*</td>
<td>1.25</td>
<td>2.06*</td>
<td>0.62</td>
<td>0.21, 4.15</td>
<td>Yes</td>
</tr>
<tr>
<td>WC</td>
<td>-4.13*</td>
<td>-0.4*</td>
<td>1.64*</td>
<td>0.5</td>
<td>0.45, 3.48</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRF</td>
<td>0.36*</td>
<td>1.15</td>
<td>0.41</td>
<td>0.12</td>
<td>-0.94, 1.82</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Visceral AT and Abdominal Subcutaneous AT as the Mediators

<table>
<thead>
<tr>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td>3.66* (SE 1.22)</td>
<td>1.77 (SE 1.33)</td>
<td>1.88* (SE 0.73)</td>
<td>0.51</td>
<td>0.59, 3.43</td>
<td>Yes</td>
</tr>
<tr>
<td>VAT</td>
<td>-0.37* (SE 0.08)</td>
<td>-2.28 (SE 1.88)</td>
<td>0.85 (SE 0.77)</td>
<td>0.23</td>
<td>-0.53, 2.55</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>ASAT</td>
<td>0.35* (SE 0.1)</td>
<td>-2.96 (SE 1.63)</td>
<td>1.03* (SE 0.58)</td>
<td>0.28</td>
<td>0.13, 2.55</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

### Visceral AT and WC as the Mediators

<table>
<thead>
<tr>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td>3.65* (SE 1.25)</td>
<td>1.56 (SE 1.45)</td>
<td>2.09* (SE 0.81)</td>
<td>0.57</td>
<td>0.56, 3.7</td>
<td>Yes</td>
</tr>
<tr>
<td>VAT</td>
<td>-0.37* (SE 0.09)</td>
<td>-3.22 (SE 1.82)</td>
<td>1.18 (SE 0.76)</td>
<td>0.32</td>
<td>-0.1, 2.97</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>WC</td>
<td>-4.17* (SE 0.77)</td>
<td>-0.22 (SE 0.2)</td>
<td>0.9 (SE 0.8)</td>
<td>0.25</td>
<td>-0.56, 2.7</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

### Abdominal Subcutaneous AT and WC as the Mediators

<table>
<thead>
<tr>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td>3.34* (SE 1.24)</td>
<td>1.27 (SE 1.42)</td>
<td>2.07* (SE 0.78)</td>
<td>0.62</td>
<td>0.69, 3.8</td>
<td>Yes</td>
</tr>
<tr>
<td>ASAT</td>
<td>-0.36* (SE 0.1)</td>
<td>-2.84 (SE 1.53)</td>
<td>1.01* (SE 0.57)</td>
<td>0.3</td>
<td>0.06, 2.4</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>WC</td>
<td>-4.02* (SE 0.75)</td>
<td>-0.26 (SE 0.20)</td>
<td>1.05 (SE 0.79)</td>
<td>0.31</td>
<td>-0.36, 2.82</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

### Visceral AT and Total AT as the Mediators

<table>
<thead>
<tr>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td>3.78* (SE 1.22)</td>
<td>1.70 (SE 1.36)</td>
<td>2.08* (SE 0.79)</td>
<td>0.55</td>
<td>0.63, 3.70</td>
<td>Yes</td>
</tr>
<tr>
<td>VAT</td>
<td>-0.38* (SE 0.08)</td>
<td>-1.81 (SE 2.36)</td>
<td>0.68 (SE 0.97)</td>
<td>0.18</td>
<td>-1.24, 2.77</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>TAT</td>
<td>-2.56* (SE 0.52)</td>
<td>-0.54 (SE 0.38)</td>
<td>1.40 (SE 0.92)</td>
<td>0.37</td>
<td>-0.25, 3.47</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

### Visceral AT and Abdominal AT as the Mediators

<table>
<thead>
<tr>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td>3.66* (SE 1.22)</td>
<td>1.78 (SE 1.33)</td>
<td>1.88* (SE 0.74)</td>
<td>0.51</td>
<td>0.60, 3.55</td>
<td>Yes</td>
</tr>
<tr>
<td>VAT</td>
<td>-0.37* (SE 0.08)</td>
<td>0.68 (SE 3.07)</td>
<td>-0.25 (SE 1.18)</td>
<td>-0.06</td>
<td>-2.54, 2.17</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>AbAT</td>
<td>-0.72* (SE 0.16)</td>
<td>-2.96 (SE 1.63)</td>
<td>2.13* (SE 1.16)</td>
<td>0.58</td>
<td>0.04, 4.77</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>
### Visceral AT and BMI as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.75*</td>
<td>1.76</td>
<td>1.99*</td>
<td>0.53</td>
<td>0.52, 3.44</td>
<td>Yes</td>
</tr>
<tr>
<td>VAT</td>
<td>-0.37*</td>
<td>-3.14</td>
<td>0.81</td>
<td></td>
<td></td>
<td>0.22</td>
<td>-0.75, 2.54</td>
<td>No</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.99*</td>
<td>-0.82</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### WC and Total AT as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.34*</td>
<td>1</td>
<td>2.34*</td>
<td>0.7</td>
<td>0.91, 4.25</td>
<td>Yes</td>
</tr>
<tr>
<td>TAT</td>
<td>-2.64*</td>
<td>-0.54</td>
<td>1.44</td>
<td></td>
<td></td>
<td>0.43</td>
<td>-0.08, 3.1</td>
<td>No</td>
</tr>
<tr>
<td>WC</td>
<td>-4.07*</td>
<td>-0.22</td>
<td>0.91</td>
<td></td>
<td></td>
<td>0.27</td>
<td>-0.62, 2.87</td>
<td>No</td>
</tr>
</tbody>
</table>

### BMI and CRF as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.53*</td>
<td>2.02</td>
<td>1.51</td>
<td>0.43</td>
<td>-0.29, 3.15</td>
<td>No</td>
</tr>
<tr>
<td>BMI</td>
<td>-1*</td>
<td>-1.49*</td>
<td>1.49*</td>
<td></td>
<td></td>
<td>0.42</td>
<td>0.35, 3.06</td>
<td>Yes</td>
</tr>
<tr>
<td>CRF</td>
<td>0.34*</td>
<td>0.06</td>
<td>0.02</td>
<td></td>
<td></td>
<td>0.006</td>
<td>-1.38, 1.25</td>
<td>No</td>
</tr>
</tbody>
</table>

### Abdominal AT and BMI as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.44*</td>
<td>1.52</td>
<td>1.92*</td>
<td>0.56</td>
<td>0.60, 3.38</td>
<td>Yes</td>
</tr>
<tr>
<td>AbAT</td>
<td>-0.74*</td>
<td>-2.44</td>
<td>1.81*</td>
<td></td>
<td></td>
<td>0.53</td>
<td>0.11, 3.96</td>
<td>Yes</td>
</tr>
<tr>
<td>BMI</td>
<td>-1.01*</td>
<td>-0.10</td>
<td>0.10</td>
<td></td>
<td></td>
<td>0.03</td>
<td>-1.91, 2.01</td>
<td>No</td>
</tr>
</tbody>
</table>

### Abdominal AT and WC as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.33*</td>
<td>1.08</td>
<td>2.25*</td>
<td>0.68</td>
<td>0.90, 4.10</td>
<td>Yes</td>
</tr>
<tr>
<td>AbAT</td>
<td>-0.75*</td>
<td>-1.96*</td>
<td>1.48*</td>
<td></td>
<td></td>
<td>0.44</td>
<td>0.14, 3.11</td>
<td>Yes</td>
</tr>
<tr>
<td>WC</td>
<td>-4.12*</td>
<td>-0.19</td>
<td>0.77</td>
<td></td>
<td></td>
<td>0.23</td>
<td>-0.62, 2.76</td>
<td>No</td>
</tr>
</tbody>
</table>
### Abdominal AT and Abdominal Subcutaneous AT as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.66* (SE 1.22)</td>
<td>1.78 (SE 1.33)</td>
<td>1.88* (SE 0.72)</td>
<td>0.51</td>
<td>0.62, 3.62</td>
<td>Yes</td>
</tr>
<tr>
<td>AbAT</td>
<td>-0.72* (SE 0.16)</td>
<td>-2.28 (SE 1.88)</td>
<td></td>
<td>1.65 (SE 1.45)</td>
<td></td>
<td>0.45</td>
<td>-0.87, 5.07</td>
<td>No</td>
</tr>
<tr>
<td>ASAT</td>
<td>-0.35* (SE 0.10)</td>
<td>-0.68 (SE 3.07)</td>
<td></td>
<td>0.24 (SE 1.04)</td>
<td></td>
<td>0.06</td>
<td>-1.93, 2.23</td>
<td>No</td>
</tr>
</tbody>
</table>

### Abdominal AT and Total AT as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.52* (SE 1.21)</td>
<td>1.54 (SE 1.32)</td>
<td>1.98* (SE 0.81)</td>
<td>0.56</td>
<td>0.54, 3.78</td>
<td>Yes</td>
</tr>
<tr>
<td>AbAT</td>
<td>-0.74* (SE 0.16)</td>
<td>-3.78* (SE 1.84)</td>
<td></td>
<td>2.78* (SE 1.46)</td>
<td></td>
<td>0.79</td>
<td>0.40, 6.34</td>
<td>Yes</td>
</tr>
<tr>
<td>TAT</td>
<td>-2.61* (SE 0.52)</td>
<td>0.31 (SE 0.54)</td>
<td></td>
<td>-0.80 (SE 1.37)</td>
<td></td>
<td>-0.23</td>
<td>-3.92, 1.60</td>
<td>No</td>
</tr>
</tbody>
</table>

### Total AT and WC as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.34* (SE 1.23)</td>
<td>1.00 (SE 1.44)</td>
<td>2.34* (SE 0.81)</td>
<td>0.70</td>
<td>0.92, 4.14</td>
<td>Yes</td>
</tr>
<tr>
<td>TAT</td>
<td>-2.64* (SE 0.52)</td>
<td>-0.54 (SE 0.31)</td>
<td></td>
<td>1.44 (SE 0.81)</td>
<td></td>
<td>0.43</td>
<td>-0.13, 3.16</td>
<td>No</td>
</tr>
<tr>
<td>WC</td>
<td>-4.07* (SE 0.74)</td>
<td>-0.22 (SE 0.22)</td>
<td></td>
<td>0.91 (SE 0.85)</td>
<td></td>
<td>0.28</td>
<td>-0.70, 2.77</td>
<td>No</td>
</tr>
</tbody>
</table>

### Total AT and BMI as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.58* (SE 1.25)</td>
<td>1.91 (SE 1.44)</td>
<td>1.67* (SE 0.73)</td>
<td>0.47</td>
<td>0.32, 3.28</td>
<td>Yes</td>
</tr>
<tr>
<td>TAT</td>
<td>-2.61* (SE 0.52)</td>
<td>-0.55 (SE 0.47)</td>
<td></td>
<td>1.43 (SE 1.06)</td>
<td></td>
<td>0.40</td>
<td>-0.74, 3.64</td>
<td>No</td>
</tr>
<tr>
<td>BMI</td>
<td>-1.01* (SE 0.20)</td>
<td>-0.24 (SE 1.26)</td>
<td></td>
<td>0.24 (SE 1.06)</td>
<td></td>
<td>0.07</td>
<td>-1.70, 2.52</td>
<td>No</td>
</tr>
</tbody>
</table>

### Total AT and Abdominal Subcutaneous AT as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.53* (SE 1.27)</td>
<td>1.91 (SE 1.42)</td>
<td>1.62* (SE 0.72)</td>
<td>0.46</td>
<td>0.40, 3.32</td>
<td>Yes</td>
</tr>
<tr>
<td>TAT</td>
<td>-2.54* (SE 0.52)</td>
<td>-0.38 (SE 0.48)</td>
<td></td>
<td>0.97 (SE 1.15)</td>
<td></td>
<td>0.27</td>
<td>-1.00, 3.67</td>
<td>No</td>
</tr>
<tr>
<td>ASAT</td>
<td>-0.36* (SE 0.10)</td>
<td>-1.82 (SE 2.56)</td>
<td></td>
<td>0.65 (SE 0.84)</td>
<td></td>
<td>0.18</td>
<td>-1.07, 2.39</td>
<td>No</td>
</tr>
</tbody>
</table>
### WC and BMI as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c’</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.31*</td>
<td>1.23</td>
<td>2.09*</td>
<td>0.63</td>
<td>0.68, 4.16</td>
<td>Yes</td>
</tr>
<tr>
<td>WC</td>
<td>-4.13*</td>
<td>-0.32</td>
<td>1.31</td>
<td>0.39</td>
<td>-0.44, 3.45</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-1.00*</td>
<td>-0.78</td>
<td>0.78</td>
<td>0.24</td>
<td>-0.78, 2.36</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### SM and CRF as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c’</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.57*</td>
<td>3.16*</td>
<td>0.41</td>
<td>0.11</td>
<td>-1.13, 2.10</td>
<td>No</td>
</tr>
<tr>
<td>SM</td>
<td>0.28</td>
<td>-1.02</td>
<td>-0.29</td>
<td>-0.08</td>
<td>-1.18, 0.06</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRF</td>
<td>0.36*</td>
<td>1.97</td>
<td>0.70</td>
<td>0.20</td>
<td>-0.79, 2.41</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### SM and TAT as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c’</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.50*</td>
<td>1.62</td>
<td>1.88*</td>
<td>0.54</td>
<td>0.58, 3.46</td>
<td>Yes</td>
</tr>
<tr>
<td>SM</td>
<td>0.32</td>
<td>-2.44</td>
<td>-0.11</td>
<td>-0.03</td>
<td>-0.81, 0.26</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT</td>
<td>-2.64*</td>
<td>-0.10*</td>
<td>1.99</td>
<td>0.57</td>
<td>0.76, 3.56</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### SM and BMI as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c’</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.51*</td>
<td>2.14</td>
<td>1.36</td>
<td>0.39</td>
<td>-0.23, 2.98</td>
<td>No</td>
</tr>
<tr>
<td>SM</td>
<td>0.32</td>
<td>-0.22</td>
<td>-0.07</td>
<td>-0.02</td>
<td>-0.83, 0.32</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-1.00*</td>
<td>-1.42</td>
<td>1.43*</td>
<td>0.41</td>
<td>0.05, 2.93</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### SM and WC as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c’</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.33*</td>
<td>1.86</td>
<td>1.47</td>
<td>0.44</td>
<td>-0.13, 3.39</td>
<td>No</td>
</tr>
<tr>
<td>SM</td>
<td>0.34</td>
<td>-0.27</td>
<td>-0.09</td>
<td>-0.03</td>
<td>-0.89, 0.34</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WC</td>
<td>-4.15*</td>
<td>-0.38</td>
<td>1.56*</td>
<td>0.47</td>
<td>0.22, 3.26</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SM and AbAT as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.41* (SE 1.23)</td>
<td>1.51 (SE 1.39)</td>
<td>1.90* (SE 0.78)</td>
<td>0.56</td>
<td>0.44, 3.52</td>
<td>Yes</td>
</tr>
<tr>
<td>SM</td>
<td>0.33 (SE 0.22)</td>
<td>-0.12 (SE 0.63)</td>
<td></td>
<td></td>
<td></td>
<td>-0.04 (SE 0.26)</td>
<td>-0.02</td>
<td>-0.76, 0.41</td>
</tr>
<tr>
<td>AbAT</td>
<td>-0.75* (SE 0.16)</td>
<td>-2.57* (SE 0.90)</td>
<td></td>
<td></td>
<td></td>
<td>1.94* (SE 0.71)</td>
<td>0.57</td>
<td>0.69, 3.50</td>
</tr>
</tbody>
</table>

SM and ASAT as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.41* (SE 1.25)</td>
<td>2.12 (SE 1.36)</td>
<td>1.28* (SE 0.65)</td>
<td>0.38</td>
<td>0.18, 2.85</td>
<td>Yes</td>
</tr>
<tr>
<td>SM</td>
<td>0.31 (SE 0.22)</td>
<td>-0.24 (SE 0.64)</td>
<td></td>
<td></td>
<td></td>
<td>-0.07 (SE 0.25)</td>
<td>-0.02</td>
<td>-0.85, 0.28</td>
</tr>
<tr>
<td>ASAT</td>
<td>-0.36* (SE 0.1)</td>
<td>-3.76* (SE 1.50)</td>
<td></td>
<td></td>
<td></td>
<td>1.36* (SE 0.55)</td>
<td>0.40</td>
<td>0.50, 2.84</td>
</tr>
</tbody>
</table>

SM and VAT as the Mediators

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>c'</th>
<th>c-c'=ab</th>
<th>P_M</th>
<th>CI</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3.78* (SE 1.22)</td>
<td>2.15 (SE 1.38)</td>
<td>1.63* (SE 0.83)</td>
<td>0.43</td>
<td>0.14, 3.36</td>
<td>Yes</td>
</tr>
<tr>
<td>SM</td>
<td>0.27 (SE 0.22)</td>
<td>-0.15 (SE 0.64)</td>
<td></td>
<td></td>
<td></td>
<td>-0.04 (SE 0.22)</td>
<td>-0.01</td>
<td>-0.74, 0.26</td>
</tr>
<tr>
<td>VAT</td>
<td>-0.40* (SE 0.08)</td>
<td>-4.16* (SE 1.65)</td>
<td></td>
<td></td>
<td></td>
<td>1.67* (SE 0.79)</td>
<td>0.44</td>
<td>0.23, 3.34</td>
</tr>
</tbody>
</table>

**Appendix G, Table 1.** Full mediation data score.

For all models, * = Significant \( p < 0.05 \), or 95% confidence interval did not encompass zero.
Appendix H, MRI analysis

Adipose tissue was examined with the use of MRI at Kingston General Hospital in Ontario, Canada. Data acquisition was T1 weighted, and therefore have a short repeat and echo time. Repeat times often range from 300-2000 msec, while echo times range from 30-150 msec. Our acquisitions had repeat times set at 210 msec, and echo times at 17 msec.

MRI images were segmented and categorized for different AT with Slice-O-Matic. This is a research tool designed to diversify different tissues. Field of view was 480mm x 360mm, with a resolution of 256 x 256 produced voxels at 1.87mm x 1.4mm x 10mm. MRI images were taken every 5 cm transverse slices. To calculate the mass of different tissues from pixel to volume, specific tissues were identified in comparison with anatomy textbooks. Assuming constant density, fat mass was multiplied by 0.92 to produce AT mass in kilograms (115). Muscle was multiplied by 1.04 to produce SM mass in kilograms.
Appendix H, Figure 1. MRI segments are T1 weighted for this investigation.
Appendix H, Figure 2. Segmentation of different abdominal tissues with the Slice-O-Matic software.
Appendix I, Participant consent

CONSENT TO VOLUNTEER FOR PARTICIPATION IN A STUDY

TITLE: Prevention and treatment of abdominal obesity and related insulin resistance in elderly men and women

PRINCIPAL INVESTIGATOR: Robert M.J. Ross, Ph.D.
Queen’s University
School of Physical and Health Education/
Medicine, Division of Endocrinology and Metabolism
Kingston, Ontario, K7L 3N6
533-6583

CO-INVESTIGATORS:

Chris Boesch, M.D., Ph.D.
University of Berne
Switzerland

Robert Hudson, M.D., Ph.D., FRCPC
Kingston General Hospital
Medicine, Division of Endocrinology and Metabolism
Kingston, Ontario, K7L 3N6
533-2973
BACKGROUND INFORMATION

You are invited to participate in a research study on the influence of aging and exercise on abdominal fat and insulin resistance. 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.

Many elderly people have problems keeping their blood glucose (sugar) levels normal, a condition referred to as “insulin resistance” by scientists and medical doctors. Although the reasons associating aging with insulin resistance are unclear, the increase in fat in the abdominal region is at least in part responsible for the increased insulin resistance. A reduction in physical activity with aging may also contribute to an increase in insulin resistance.

Recent studies have shown a relationship between muscle lipid (fat) content and insulin resistance in young healthy individuals and persons with diabetes. This is important as the amount of fat within the muscle increases with aging. Thus, muscle fat may partially explain why insulin resistance increases with age. However, at this time it is unknown whether or not muscle fat content is related to insulin resistance with aging.

Although muscle fat content is associated with insulin resistance, we also know that muscle fat content is increased in individuals who exercise on a regular basis. This is important because individuals who exercise on a regular basis are very insulin sensitive (opposite to insulin resistance). Thus, it appears that an increase in muscle fat content does not always suggest a person will become insulin resistant. An important question to ask then is whether the muscle fat in insulin resistant (e.g., sedentary elderly) and insulin sensitive (e.g., physically active elderly) individuals follows the same pattern. We will also be looking at whether the normal insulin sensitivity seen in physically active elderly persons is due to changes in skeletal muscle fat content.

Exercise is thought to be a good thing to do for reducing both abdominal fat and insulin resistance. However, whether aerobic (e.g., walking) or resistance-type exercise (e.g., weight lifting) is best for reducing either is unknown. Further, whether the two forms of exercise combined (aerobic and resistance exercise) is better than either one alone for reducing abdominal fat and insulin resistance is unknown. This is especially true for older persons.

Therefore, you are invited to participate in a study to assess the relationships between exercise, abdominal fat, muscle fat, and insulin resistance. We hope that the results of this study will provide a better understanding of the reasons for the age-related increase in insulin resistance. In addition, we hope to determine whether regular exercise, and more importantly what type of exercise, can prevent the insulin resistance common to the aging process. As insulin resistance is a major predictor of diabetes and cardiovascular disease, these results may have important implications for developing ways to prevent and treat diabetes and cardiovascular disease in elderly persons.
EXPLANATION OF PROCEDURES

Pre-participation screening

You will be required to have a medical exam prior to participation in this study. The examination will be performed by your family physician. In addition to the medical examination, you will have a fasting blood test to measure your blood sugar levels. This procedure is explained in further detail on page five (5) of this form.

Study Protocol

The study will be approximately 7 months in duration. The exercise part of the study will last 6 months. The 6-month exercise period will begin and end with a 3-week weight maintenance period - thus about 7 months in total. 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) Aerobic (walking) exercise, (3) Resistance exercise, (4) Aerobic and Resistance exercise.

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

Aerobic Exercise Group: As a participant in the aerobic exercise group you will be asked to perform walking type exercise on a motorized treadmill for 30 minutes, 5 times per week, at about 65% 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.

Resistance Exercise Group: As a participant in this group you will be asked to perform a series of 10 exercises, 3 times per week, for the duration of the 6 month treatment period. Eight of the exercises will be performed using Nautilus strength training equipment and 2 using your own body weight (e.g., sit-ups). All resistance exercise sessions will be supervised by a qualified undergraduate or graduate level student and performed within Dr. Ross’ laboratory at Queen’s.
**Aerobic and Resistance Exercise Program Combined:** As a participant in this group you will perform an exercise regimen that combines resistance and aerobic exercise. The resistance exercise program will be the same as that described for the resistance exercise only group. In addition, the aerobic exercise will be performed at the same intensity (~65% of your cardiovascular fitness for 30 minutes) on 3 days of the week. In general, the aerobic exercise (e.g., brisk walking) would be performed on the days when resistance exercise is not performed. Thus if you are a participant in this group you will exercise 6 days of the week.

**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 Physical Education building 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 we will ask you to maintain this caloric intake. In other words, 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 imaging or creating pictures of body structures or organs. MRI gives 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 employ x-rays or other forms of radiation. Instead, a large magnet, a radio transmitter/receiver and a computer are used to gather chemical information from the body, and to produce images or pictures of internal anatomy. No harmful effects have been associated with MRI under existing conditions of use. It is important that you fill out the enclosed MRI questionnaire to determine if there is any reason why you should not have the MRI exam.

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 35 minutes and will be performed by appointment at Kingston General Hospital.

Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) works the same way that MRI does. You will be positioned in the magnet as in the MRI test. The difference is that MRS does not provide pictures of the body. Instead, the radio signal emitted from the body is used to provide information about where the fat is positioned within your muscle. In other words, MRI provides pictures of muscle and fat, which tell us about the quantity of muscle and fat. Information obtained using MRS tells us something about the quality of muscle. As with MRI, the MRS procedure will be performed at Kingston General Hospital and will take about 30 minutes to complete.

Computerized Tomography (CT)

Computerized tomography or CT is another method that will be used to obtain information about the quality of your muscle and your liver. Unlike MRI, CT provides pictures of the muscle and liver that can be used to determine the amount of fat in your liver and muscle. Specifically, two CT images of your mid-thigh will be obtained to measure the amount of fat in the muscle; one CT image of the liver will be obtained to determine the amount of fat in the liver. You will be asked to lie on an exam table while the CT scan is being performed. The entire CT procedure will take about 10 minutes.
**Dual Energy X-Ray Absorptiometry (DEXA)**

DEXA measures whole body fat, bone quality, and skeletal muscle. For this test you lie on your back on a table. The scanner moves above you measuring the transmission of X-rays from a source under the table. During this procedure you are asked to lie still for approximately 20 minutes. The radiation exposure involved with this test is approximately equivalent to one percent of a chest X-ray. This measurement will take place by appointment and be performed within the Department of Radiology at Kingston General Hospital.

**Anthropometry (Skinfolds and Circumferences)**

Many circumference measurements will be taken at numerous sites on your body. These measures can be used to derive estimates of body composition. In addition, through the use of skinfold calipers, skinfold thickness will be measured at 8 different sites on your body. The anthropometric measurements require about one hour to complete and will be obtained at the School of Physical and Health Education, Queen’s University.

**Bioelectrical Impedance**

This is a very simple and safe procedure requiring no more than 5 minutes to complete. While you lie on your back, 2 electrodes will be placed on the surface of your right hand and foot. Two of the electrodes will introduce an alternating current that you can’t feel into the body, while the other 2 record the resistance. The results are used to determine body composition. The bioelectrical impedance measurements will be obtained at the School of Physical and Health Education, Queen’s University.
Assessment of Cardiovascular Fitness

We will measure your cardiovascular fitness (endurance) using a treadmill procedure. 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. Your fitness test will be conducted by a graduate student in the presence of a paramedic. You will perform the exercise test 3 times: once at the beginning, once after one month, and once at the end of the exercise training period.

Assessment of Muscular Strength

Improvement in muscular strength will be assessed before and at weeks 8, 16 and 24 using a 3-repetition maximum (3RM) test. The 3RM is defined as the maximal resistance that could be moved through the full range of motion for 3 repetitions.

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 Hotel Dieu Hospital. To determine your ability to manage blood sugar you will be asked to perform two (2) separate tests. The first test, an Oral Glucose Tolerance Test or OGTT, will be performed after an overnight fast. You will be asked to arrive at the hospital at about 8am after an overnight fast (no eating after 7pm the night before). The first step of this test will involve a venipuncture with a needle and the removal of about 30 ml (3 tablespoons) of blood from a vein in your arm. 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 from a vein in your arm for the purpose of measuring the amount of sugar in the blood.
The second test will also be performed after an overnight fast, and, after having not exercised on the prior 3 days. This procedure will also be performed at Hotel Dieu Hospital. Having arrived at the hospital you will be asked to lie comfortably on a bed for about 4 hours. Catheters (needles) will be placed in a vein on the top of one hand and in a vein in both arms. A catheter in one arm vein will be used to give glucose (sugar) and insulin at a rate designed to keep blood sugar level normal for 3 hours. Every 5 minutes during this procedure a small amount of blood will be taken from a vein in your hand to measure blood sugar to ensure that it remains normal. A physician will monitor this procedure at all times.

The purpose of these tests is to determine your ability to maintain normal blood sugar levels (insulin sensitivity). Reduced sensitivity to insulin is a complication of aging and may be associated with diabetes mellitus, high blood pressure, and other health problems. These tests should not have any lasting side effects.

**Summary of Appointments and Time Requirements**

For the testing you will be required to make one 2-hour appointment at the Queen’s University Physical Education Centre to complete the cardiovascular fitness, anthropometry, and bioelectrical impedance tests. We will arrange four appointments for you at Kingston General Hospital and Hotel Dieu Hospital. One 2½-hour appointment for the oral glucose tolerance test (Hotel Dieu Hospital); one 5-hour appointment for the insulin sensitivity and blood lipid/cholesterol tests (Hotel Dieu Hospital); one 2-hour appointment to complete the MRI, MRS, (Kingston General Hospital) and CT tests (Hotel Dieu Hospital); and one 30-minute appointment for the DEXA test. All of these appointments will be scheduled at a time that is convenient for you. Further, each of these tests will be performed twice, once at the beginning and once at the end of the treatment period.
**Risks and Benefits**

You will gain no direct benefit through participation in this study. Participation may involve some risks. The known risks are:

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

2) Computerized Tomography (CT). Participation in this research study will involve a small radiation exposure (1.0 RAD or 2.0 RAD) from the CT scan to a small region of your thighs and chest (liver). For comparison, a radiation worker is allowed 5 RADS of whole body exposure per year.

3) The effective dose (the term used to describe your exposure to radiation) that you will receive during the Dual Energy X-ray Absorptiometry (DEXA) exam is approximately 5 microsieverts. The average background radiation that you are exposed to on a daily basis is estimated to be approximately 10 microsieverts. Thus the DEXA exam adds approximately half the daily background radiation.

For CT and DEXA, there is no known minimum level of radiation exposure that is recognized as being totally free of the risk of causing genetic defects or cancer. However, the risk associated with the amount of radiation exposure you will receive from these procedures is considered to be very low and comparable to other everyday risks.

4) MRI or MRS has certain conditions which 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 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.

5) The risk of receiving insulin (as in the test at Hotel Dieu Hospital in which your sensitivity to insulin is measured) is the development of hypoglycemia (blood sugar which is too low). Because we give you glucose (sugar) throughout the test, and, your blood sugar levels are measured every 5 to 10 minutes, the likelihood of your having a low blood sugar is very low. The symptoms of low blood sugar include increased sweating, fast heart rate, feeling shaky and/or hungry. In very rare cases when your blood sugar levels fall to low, seizures or death may occur.
6) 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 present. If you develop chest pain the test will be stopped immediately.

You should inform the investigators if you have participated in any other research study during the previous year. This will help to ensure that you have not been exposed to a procedure in another study that may influence your ability or eligibility to participate in this one. You should understand that this study is a research study and may not be of direct benefit to you. If requested, a report will be generated for your medical record, which will include any information important for your medical care.
CONFIDENTIALITY

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

In the event you are injured as a result of taking study medications or 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 will not be provided to you for participation in this study.
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 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 (533-6583), Dr. Robert Hudson (533-2973), Dr. Janice Deakin, Head, School of Physical and Health Education (533-6601), Dr. Donald Brunet, Head, Department of Medicine (533-6327), or Dr. Albert Clark, Chair of the Ethics Review Board at Queen’s (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.

______________________________  ______________________________
Date:                           Volunteer’s Signature

______________________________
Witness’ Signature
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 J, Ethics approval

QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD-DELEGATED REVIEW
September 27, 2013

Mr. Gifferd Ko
School of Kinesiology and Health Studies
Queen’s University

Dear Mr. Ko

Study Title: PHE-137-13 Do Cardiorespiratory Fitness and Abdominal Obesity Mediate the Exercise-Induced Change in Insulin Sensitivity in Older Adults?
File # 6010945
Co-Investigators: Dr. R. Ross

I am writing to acknowledge receipt of your recent ethics submission. We have examined the protocol for your project (as stated above) and consider it to be ethically acceptable. This approval is valid for one year from the date of the Chair’s signature below. This approval will be reported to the Research Ethics Board. Please attend carefully to the following listing of ethics requirements you must fulfill over the course of your study:

**Reporting of Amendments:** If there are any changes to your study (e.g. consent, protocol, study procedures, etc.), you must submit an amendment to the Research Ethics Board for approval. Please use event form: HSREB Multi-Use Amendment/Full Board Renewal Form associated with your post review file # 6010945 in your Researcher Portal (https://eservices.queensu.ca/romeo_researcher/)

**Reporting of Serious Adverse Events:** Any unexpected serious adverse event occurring locally must be reported within 2 working days or earlier if required by the study sponsor. All other serious adverse events must be reported within 15 days after becoming aware of the information. Serious Adverse Event forms are located with your post review file 6010945 in your Researcher Portal (https://eservices.queensu.ca/romeo_researcher/)

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

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

Yours sincerely,

Albert Z. Clark.

Chair, Health Sciences Research Ethics Board

September 27, 2013

Investigators please note that if your trial is registered by the sponsor, you must take responsibility to ensure that the registration information is accurate and complete
QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD

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

Federalwide Assurance Number: #FWA00004184, #IRB00001173

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

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

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

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

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

Dr. M. Evans, Community Member

Ms. J. Hudacin, Community Member

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

Mr. D. McNaughton, Community Member

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

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

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

Ms. K. Weisbaum, LL.B. and Adjunct Instructor, Department of Family Medicine (Bioethics)