ASSOCIATIONS OF THE LIMB FAT TO TRUNK FAT RATIO WITH MARKERS OF CARDIOMETABOLIC RISK IN ELDERLY MEN AND WOMEN

by

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Abstract

Background: It has been reported that the ratio of limb fat to trunk fat (LF/TF) is associated with markers of cardiometabolic risk in elderly men and women. However, it is unknown if LF/TF is associated with cardiometabolic risk beyond that explained by LF and TF independently.

Objective: To determine if LF/TF is associated with markers of cardiometabolic risk in elderly men and women after control for LF and TF. A secondary objective was to examine the independent associations of LF and TF with markers of cardiometabolic risk.

Methods: Subjects included abdominally obese men (n=58) and women (n=78) between 60 and 80 years of age. Regional adiposity was quantified using magnetic resonance imaging. Insulin resistance, fasting glucose, HDL-cholesterol, plasma triglycerides and adiponectin were determined. Regression analyses and partial correlations were used to assess the independent associations between variables.

Results: After control for potential confounders, TF was positively associated with fasting glucose, insulin resistance and plasma triglycerides, and negatively associated with HDL-cholesterol (p<0.05). These associations were strengthened after further control for LF (p<0.05). LF was not associated with any marker of cardiometabolic risk after control for potential confounders (p>0.05). However, after further control for TF, LF was positively associated with HDL-cholesterol and negatively associated with plasma triglycerides (p<0.05). Plasma adiponectin was independently associated with both LF and TF in elderly women (*p*<0.05) but was not independently associated with

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either depot in elderly men ($p>0.05$). LF/TF was not associated with any marker of cardiometabolic risk after control for LF and TF.

Conclusions: These results suggest that it is the absolute, rather than relative amounts of LF and TF which have the greatest influence on cardiometabolic risk in elderly men and women. Further, these results suggest that the associations between plasma adiponectin and regional adiposity are significantly influenced by sex in elderly men and women.

Keywords: Limb Fat, Trunk Fat, Adiponectin, Cardiometabolic Risk, Elderly

Co-Authorship

Writing of the manuscript contained within this dissertation and all statistical analyses were performed by Travis J Saunders. Critical revisions for important intellectual content were provided by Dr. Robert Ross.

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Chapter 1 - General Introduction

Obesity is associated with increased risk for numerous diseases including cancer, type 2 diabetes (T2D) and cardiovascular disease (CVD) (1, 2). It has been estimated that approximately 300 000 Americans die each year due to obesity related diseases (3). However, despite the strong associations between obesity and disease it is now well established that body fat distribution is more closely associated with cardiometabolic risk than general obesity. In particular, it has been reported that an excess accumulation of trunk fat (TF) is a strong and independent predictor of increase morbidity and mortality in both genders (4-6). In contrast, recent evidence suggests that for a given level of TF, increased accumulation of limb fat (LF) is associated with reduced risk of morbidity and mortality (4, 7).

Although the independent and opposing associations of LF and TF with cardiometabolic risk are well-established, it has recently been reported that the ratio of LF to TF (LF/TF) is also strongly associated with metabolic risk factors (8). LF/TF represents a form of interaction between LF and TF, and these results suggest that it may be the relative, rather than absolute amounts of LF and TF which influence cardiometabolic risk. However, the aforementioned study violated important statistical principles which may have influenced the reported associations between LF/TF and cardiometabolic risk. Thus it remains unclear if it is the relative or absolute amounts of LF and TF which influence cardiometabolic risk.

The following review will summarize current knowledge regarding the relationship between body fat distribution and cardiometabolic risk. In the manuscript

contained in Chapter 3 we investigate whether LF/TF is associated with markers of

cardiometabolic risk after control for LF and TF in a cohort of elderly men and women.

Finally, in Chapter 4 we discuss the implications of our findings for both clinicians and

future research.

Chapter 2 - Literature review

> Persons who are naturally very fat are apt to die earlier than
> those who are slender.
>
> -Hippocrates (9)

2.1 General Obesity and Cardiometabolic Risk

It is well-established that obese individuals are at increased risk of chronic disease
including cardiovascular disease (CVD) and type 2 diabetes (T2D) (10, 11). In
Caucasian populations, prospective studies have consistently demonstrated that as body
mass index (BMI) increases from the normal (18.5-24.9 kg/m^2) to overweight (25.0-29.9
kg/m^2) or obese (>30.0 kg/m^2) categories, so too does the risk of morbidity and mortality
(1, 12, 13). For example, Calle et al. (1) examined the association of BMI with death
from all causes over a 14-year period in a sample of more than 1 million Americans.
They report that as BMI increased in Caucasian men and women, so did their risk of all-
cause, cardiovascular disease, and cancer mortality. Further, men and women in the
highest BMI category had more than double the risk of mortality than those who were
normal weight. Although the specific BMI cut-points which represent increased
cardiometabolic may risk differ by ethnicity (14), current evidence suggests that BMI is
also positively associated with increased risk of mortality in Asian (15, 16) and African-
American (17) populations.

Available evidence also suggests that the prevalence of obesity (defined as a BMI
> 30 kg/m^2) in the Canadian population has dramatically increased recent years (18).
Tremblay et al. (18) report that between 1981 and 1996, the rates of overweight increased
19% and 17% in men and women respectively, while the rates of obesity increased by

56% and 50% respectively. Not surprisingly, the number of obesity-related mortalities have also increased sharply in recent years, such that it is estimated that over 4000 Canadians die each year as a direct result of overweight and obesity (19). Further, obesity-related healthcare costs in Canada are reported to exceed $1.8 billion annually (20).

The association of total adiposity with increased risk of chronic disease morbidity and mortality has been known for over 2000 years (9). As discussed above, the increasing prevalence of overweight and obesity in the Canadian population create a significant social and economic burden. However, by their very nature, traditional measures of obesity such as the BMI classify obesity in terms of body weight relative to standing height, and completely ignore individual variations in body fat distribution (11). More than sixty years ago the French physician Jean Vague was the first to suggest that body fat distribution may be a stronger determinant of cardiometabolic risk than body weight alone.

2.2 Trunk Fat and Cardiometabolic Risk

Despite the strong associations between BMI and health risk, Vague noticed that obesity related diseases such as arteriosclerosis and type 2 diabetes were far more prevalent in men and women with excess accumulation of fat in the trunk (21, 22). In contrast, he noticed that individuals who were characterized by excess fat accumulation in the limbs rarely suffered from these conditions. Vague concluded that it was the distribution of fat within the body, rather than the absolute amount of body fat, that resulted in the diseases commonly associated with obesity.

Since Vague's pioneering work in the mid 20th century, researchers have continued to examine the relationship between body fat distribution and health risk. It is now well-established that the accumulation of excess trunk fat (TF) is associated with increased risk of chronic disease in both genders (8, 23-28). For example, TF is positively associated with insulin resistance (8, 25), dyslipidemia (24, 26, 27), resting blood pressure (24, 29) inflammation (28, 29), diabetes and cardiovascular disease (23) in adult men and women. Given these associations, it is not surprising that TF accumulation is also associated with increased risk of mortality in both sexes (5-7, 23). For example, Zhang et al. (6) report that TF is positively associated with all-cause, cardiovascular disease, and cancer related mortality in middle-aged women over a 16-year period. Similar associations have been reported between TF and all-cause (5) and stroke (7) mortality in men.

2.2.1 Adipose Tissue Depots within the Trunk

Modern imaging technologies including computed tomography (CT) and magnetic resonance imaging (MRI) have greatly increased our understanding of TF and its relation to markers of cardiometabolic risk. These technologies make it possible to differentiate adipose tissue (AT) from lean tissue such as muscle and bone, and to accurately quantify the amount of total AT within the trunk, arms and legs (30, 31). Further, CT and MRI are also able to quantify AT accumulation in specific anatomical depots within the trunk and the periphery (30, 31).

The two most prominent AT depots in the trunk are visceral AT (VAT) located within the abdominal cavity and abdominal subcutaneous AT (ASAT) which is located external to the abdominal muscles. A third and smaller volume of fat is also stored as

intermuscular adipose tissue (IMAT) within skeletal muscle. The majority of evidence

suggests that it is the excess VAT which is primarily responsible for the health risk

associated with trunk fat accumulation (32-35). Over 20 years ago, Fujioka et al. (36)

first reported that obese individuals who stored abdominal fat primarily as VAT had

significantly higher triglyceride levels and decreased glucose tolerance in comparison to

those who stored abdominal fat primarily as ASAT. More recent studies have confirmed

these findings, and suggested that that the accumulation of VAT is associated with

increased risk of heart disease (37, 38), diabetes (38, 39), and mortality (40) independent

of other AT depots. Finally, it has also been reported that control for VAT abolishes the

relationship between trunk fat and many metabolic risk factors (27, 41).

Despite numerous reports suggesting that the accumulation of VAT is strongly

and independently associated with increased cardiometabolic risk, limited evidence

suggests that ASAT and IMAT may also be independently associated with increased

health risk (42-47). For example, Goodpaster et al. (44) report than in healthy men and

women ASAT is associated with increased insulin resistance independent of VAT.

Further, the accumulation of IMAT has also been linked to increased cardiometabolic

risk independent of VAT in normal and overweight men and women (42, 45, 46). Thus,

although excess VAT is most consistently reported to be associated with deleterious

health outcomes, available evidence suggests that excess accumulation of any fat depot

within the trunk may be independently associated with increased cardiometabolic risk.

2.3 Limb Fat and Cardiometabolic Risk

Although Vague originally suggested that peripheral fat "does not exercise any

influence on the metabolic disorders" (22), there is now evidence to suggest that fat

accumulation in the limbs may also have an independent and positive influence on cardiometabolic health. For example, Seidell et al. (25) report that for a given waist circumference, a larger hip circumference is associated with increased HDL cholesterol, as well as decreased triglyceride, insulin, and glucose concentrations. Similarly, after control for TF, increased limb fat (LF) is positively associated with HDL-cholesterol (48, 49), as well as decreased IR (48-51), LDL cholesterol, triglycerides (48), fasting glucose (4), brachial artery stiffness and calcification (52, 53), and mortality (7).

2.3.1 Adipose Tissue Depots Within the Limbs

As with TF, LF consists of distinct depots including subcutaneous and intermuscular adipose tissue. In particular, it appears that it is lower body subcutaneous adipose tissue (LBSAT) which is responsible for the independent associations between limb fat and reduced cardiometabolic risk. First and foremost, LBSAT accounts for the vast majority of peripheral fat stores. Further, LBSAT is the only peripheral AT depot which has been associated with reduced metabolic risk after control for VAT and ASAT (41, 43, 46). For example, Snijder et al. report that LBSAT is associated with reduced dyslipidemia in elderly men and women and improvements in glucose metabolism in elderly men independent of both VAT and ASAT (43). This is in contrast to lower body IMAT, which has been reported to be positively associated with cardiometabolic risk (42, 46, 54). For example, a recent study by Yim et al. (46) suggests that lower body IMAT is associated with increased fasting glucose levels independent of VAT and total obesity in middle-aged men and women. Although few studies have directly compared the influence of arm and leg AT depots on cardiometabolic risk, the limited available evidence suggests that the influence of arm AT is negligible (55, 56). Thus, for a given

amount of trunk fat, current evidence suggests that it is LBSAT which mediates the

negative association between limb fat accumulation and reduced cardiometabolic risk.

A.

Subcutaneous AT

Visceral AT

B.

Intermuscular AT

Subcutaneous AT
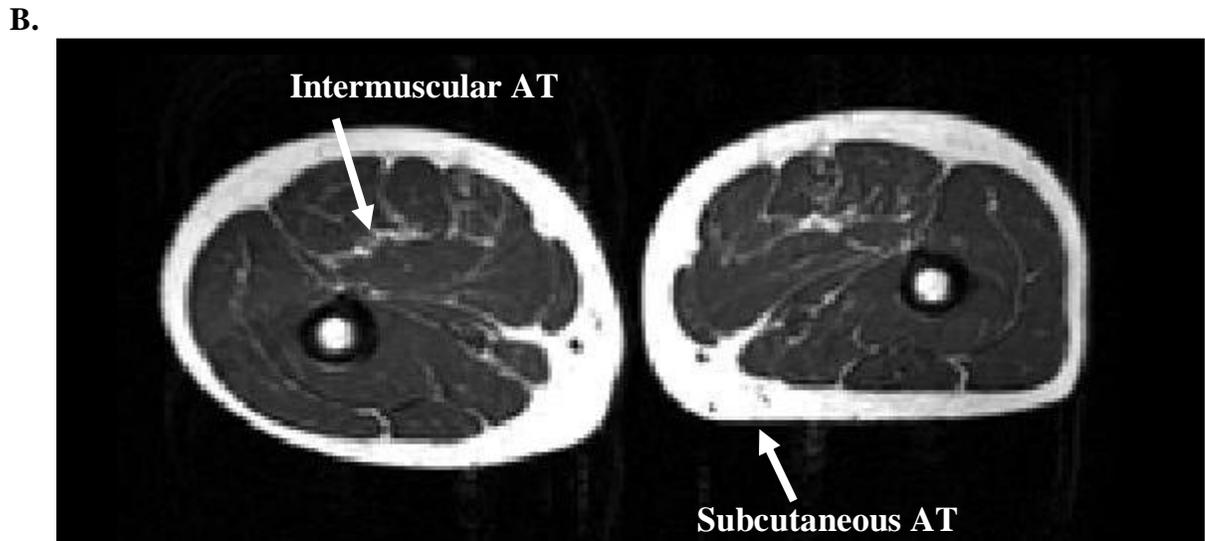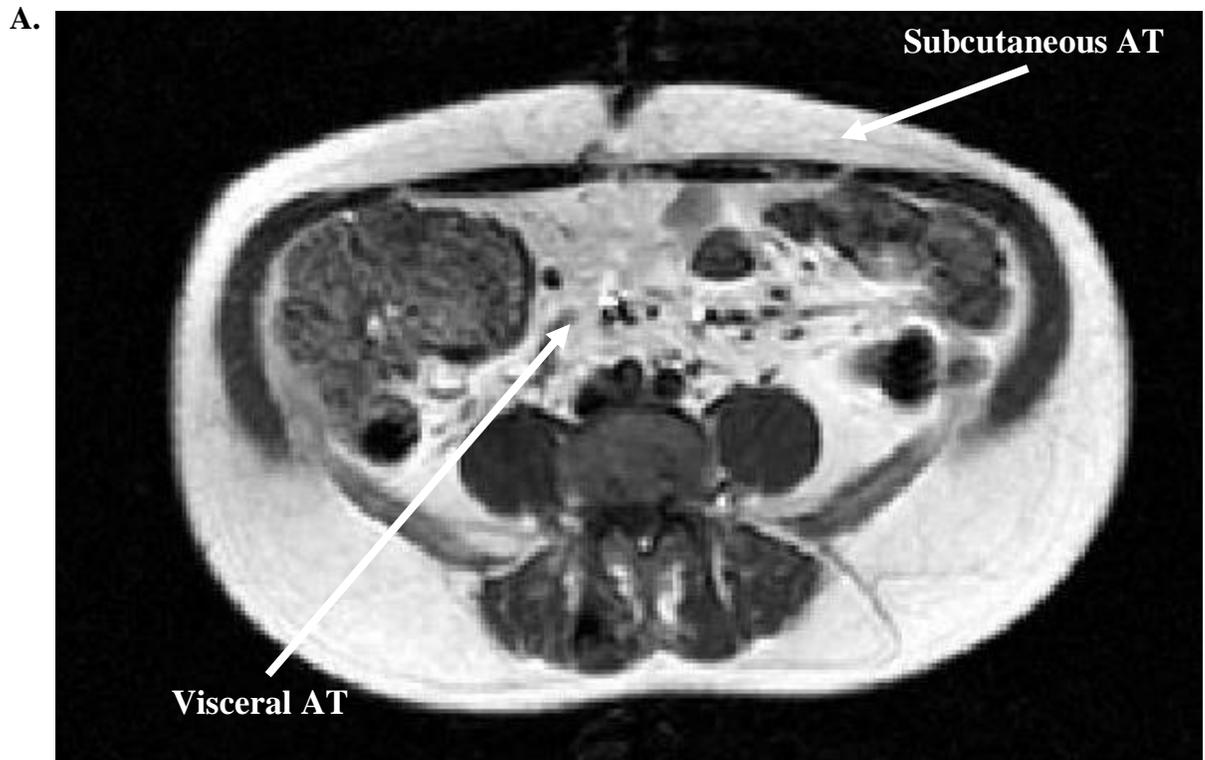
Figure 1. Quantification of Abdominal AT Depots Using Magnetic Resonance Imaging.

A. Image at the intervertebral space L4-L5; B. Image at the mid-thigh, AT = adipose tissue.

2.4 Mechanisms Linking Body Fat Distribution and Cardiometabolic Risk

2.4.1 Substrate Driven Mechanisms

Rather than being linked by any one mechanism, recent evidence suggests that body fat distribution influences cardiometabolic risk through a number of separate pathways (57-59). In comparison to peripheral adipocytes, both visceral and abdominal subcutaneous adipocytes are characterized by increased catecholamine-stimulated lipolysis and decreased sensitivity to the anti-lipolytic effects of insulin (60). As individuals accumulate excess TF these adipocytes enlarge and become increasingly insulin resistant, resulting in further increases in lipolytic activity (57, 61). The high rates of lipolysis in visceral and abdominal subcutaneous adipocytes in individuals with excess TF are thought to result in increased fatty acid release into both the portal and systemic circulation, and eventually ectopic fat deposition in the liver, pancreas and skeletal muscle (57, 58). It is this ectopic fat storage which is thought to underlie many of the deleterious health effects associated with excess TF, including hyperglycemia, insulin resistance and hypertriglyceridemia (57, 58, 61). In particular, it is the deposition of fat within the liver, which can result in hepatic insulin resistance and increased synthesis of very low density lipoproteins, which is thought to be of critical importance to cardiometabolic risk (62).

In the past it has been suggested that fatty infiltration of the liver resulted primarily from the accumulation of excess VAT, rather than ASAT (62). This was due to the fact that while both VAT and ASAT demonstrate high levels of lipolysis in individuals with excess TF, only VAT is directly drained by the portal vein of the liver.

This theory was thus described as the "portal hypothesis". However, current evidence suggests that both VAT and ASAT contribute to fatty infiltration of the liver, as well as skeletal muscle (63-65). Although much of VAT drains directly into the portal vein, less than 20% of the free fatty acids which reach the liver originated in VAT. In contrast, the majority of the free fatty acids which enter the portal vein originate in ASAT, eventually reaching the portal vein through the systemic circulation (63). Similarly, current evidence suggests that the majority of the free fatty acids which reach skeletal muscle come from ASAT, with VAT making a smaller contribution (63). Thus, once again the available evidence suggests that both VAT and ASAT may play important roles in the development of cardiometabolic risk through the delivery of free fatty acids to both central and peripheral tissues.

In contrast to the increased lipolysis which characterizes adipocytes in the trunk, lower body subcutaneous adipocytes are reported to be significantly more sensitive to the anti-lipolytic effects of insulin as well as being less sensitive to catecholamine-stimulated lipolysis (60). Subcutaneous adipocytes are also reported to have a greater capacity for differentiation (66, 67), resulting in increased hyperplasia rather than hypertrophy in times of energy surplus. This hyperplasic growth is thought to result in numerous small, insulin sensitive adipocytes, as opposed to the large, insulin resistant adipocytes that result from the primarily hypertrophic growth that characterizes visceral adipose tissue (68). Taken together, these characteristics may allow LF to act as a metabolic sink, tightly storing large amounts of fatty acids and triglycerides away from muscle and other organs where they could lead to lipotoxicity and increased IR (32). This is in agreement with recent reports that roughly 75% of circulating free fatty acids in obese men and

women originate from adipocytes located within the trunk while only 25% originate in the lower body (69).

2.4.2 Endocrine Mechanisms

Recent evidence has also suggested that adipose tissue is an extremely active endocrine organ, secreting numerous hormones which are collectively known as adipokines (70). One adipokine which has received particular attention is known as adiponectin, a hormone has been shown to have numerous positive effects on cardiometabolic health. Adiponectin has been shown to inhibit gluconeogenesis in the liver and promote glucose uptake and fat oxidation in skeletal muscle (71). Finally, it has also been reported that adiponectin is associated with a decreased risk of both CVD and type 2 diabetes (72, 73).

In contrast to most other adipokines, plasma adiponectin levels are *decreased* in individuals with excess TF (50). It has been suggested that this reduction in plasma adiponectin concentration is likely to represent another mechanism linking the accumulation of TF with increased cardiometabolic risk (57, 58). Recent evidence suggests that the positive health effects of increased LF may also be partially mediated by plasma adiponectin levels. For example, a recent study by Buemann et al. reports that after control for TF, plasma adiponectin levels are positively associated with LF in middle-aged men (48). However, at present it is unclear if the positive associations between LF and plasma adiponectin levels reported by Buemann et al. (48) would remain significant after additional control for VAT and ASAT.
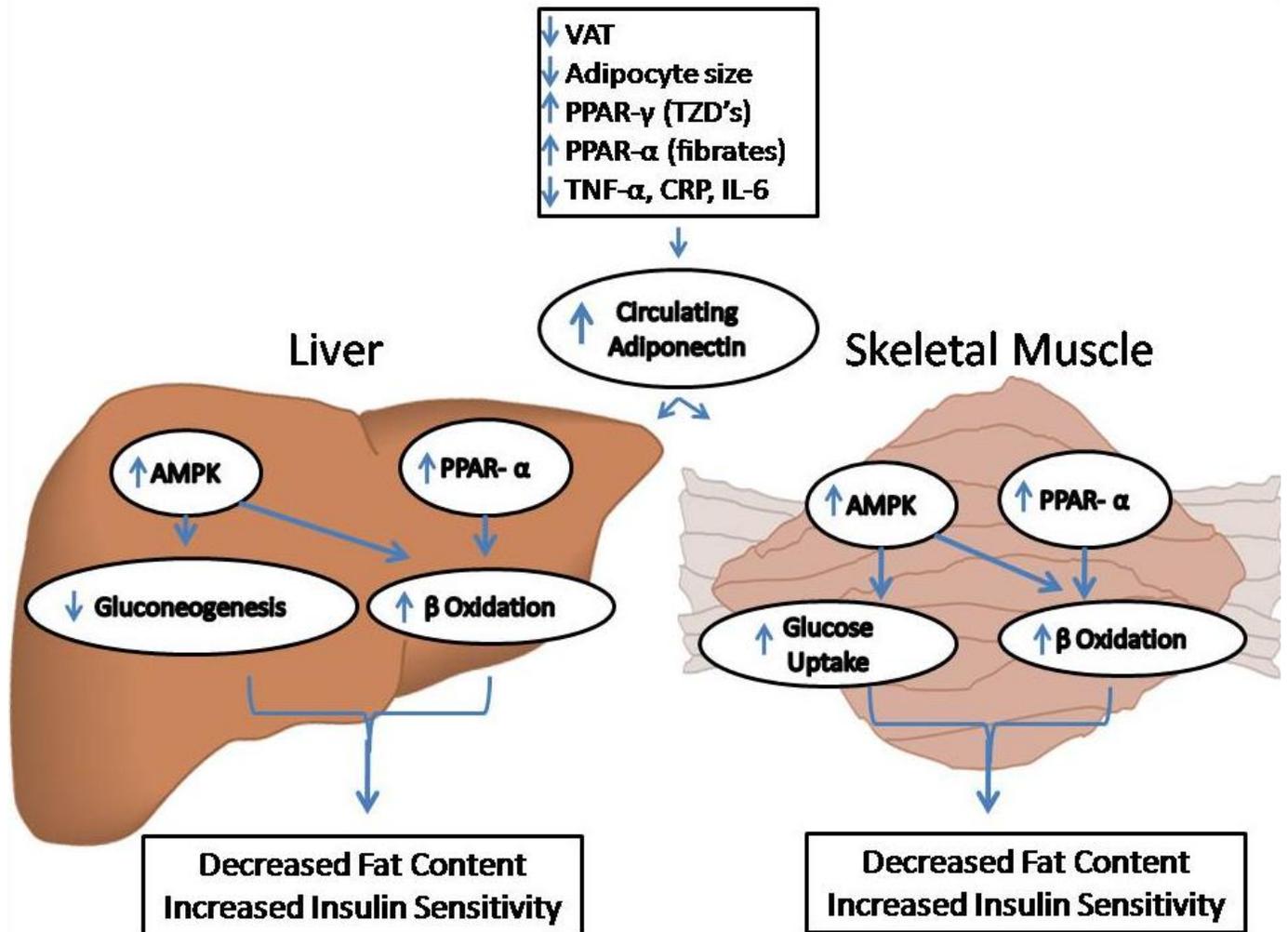
Figure 2. Factors Which Influence Plasma Adiponectin Concentration and Resulting Downstream Effects in Liver and Skeletal Muscle.

VAT = Visceral Adipose Tissue; PPar = Peroxisome Proliferator-Activated Receptor;
TNF = Tumor Necrosis Factor; CRP = C-Reactive Protein; IL = Interleukin;
AMPK = Adenosine Monophoshate-Activated Protein Kinase.

2.5 Body Fat Distribution - Relatives and Absolutes

The independent and opposing associations of TF and LF with markers of cardiometabolic risk are now relatively well-known. Further, several plausible biological mechanisms have been proposed to explain these opposing relationships, as discussed above. However, it is unclear if the ratio of TF and LF (LF/TF) accounts for cardiometabolic risk beyond that explained by LF and TF independently. If LF/TF accounts for cardiometabolic risk after control for LF and TF it would suggest that it is the amount of TF relative to LF, as opposed to their absolute amounts, which influences metabolic risk. This issue was recently investigated by Gavi et al. (8), who examined the association of the limb fat to trunk fat ratio (LF/TF) with metabolic risk in elderly men and women. They report that LF/TF was strongly and negatively associated with insulin resistance and plasma triglycerides, and positively associated with HDL-cholesterol and adiponectin concentrations. Further, in a stepwise regression LF/TF was the single best predictor of insulin resistance in their sample (8). However, the authors failed to control for LF and TF when examining the association between LF/TF and markers of metabolic risk (8). Examining the interaction of two variables (e.g. LF/TF) without controlling for their main effect terms (LF and TF) is a violation of the marginality principle (74), and thus the results of Gavi et al. (8) should not be taken to suggest that LF/TF is a stronger predictor of cardiometabolic risk than the independent effects of LF and TF (75). Thus, it remains unclear if the relative or absolute amounts of LF and TF are more closely associated with markers of cardiometabolic risk.

Briefly, the marginality principle states that in order to include an interaction term such as LF/TF in a regression model, its main effect terms (e.g. LF and TF) must also be included in the model (74). This ensures that linear transformations of the model will not

affect its association with the dependent variable, and that the model is therefore "well-formed"(74). Further, models which violate the marginality principle often result in artificially strong associations between the interaction term and cardiometabolic risk (75). Thus, while it is important to examine whether the interaction of LF and TF is a determinant of cardiometabolic risk, the results reported by Gavi et al. (8) provide little useful information on this topic.

2.6 Body Fat Distribution and Cardiometabolic Risk in the Elderly

The relationships between trunk fat, limb fat, and chronic disease have especially important consequences in the elderly. It has been reported that total fat mass increases at a rate of approximately 7.5% per decade in elderly men and women (76). However, rather than being a uniform increase in adiposity throughout the entire body, this increase in adiposity is concentrated in the trunk, while the amount of adipose tissue in the limbs may actually decrease (77, 78). For example, a longitudinal study by Hughes et al. reports that elderly men and women experience a significant decrease in peripheral subcutaneous adiposity with age, despite an increased waist circumference in women, and an overall increase in body fat percentage in both genders (77). Further, this age-related increase in TF is associated with a disproportionate increase in VAT (79), the depot within the trunk that has been most strongly and consistently associated with increased cardiometabolic risk (32-35). Thus, for a given waist circumference, elderly individuals are reported to have a dramatically increased volume of VAT, as demonstrated in Figure 3 (80). This combination of increased TF (especially VAT) and decreased LF may contribute to the increased prevalence of atherosclerosis, dyslipidemia, hyperinsulinemia, and inflammation observed in the elderly (81, 82). Thus, any

improvement in our understanding of the relationship between body fat distribution and

chronic disease is likely to result in direct clinical benefits which will be especially

relevant to elderly men and women.

60 Year Old Male

Waist Circumference: 106 cm

Visceral AT: 137 cm^2

Thigh Subcutaneous AT: 172 cm^2

80 Year Old Male

Waist Circumference: 106 cm

Visceral Adipose Tissue: 309 cm^2

Thigh Subcutaneous AT: 111 cm^2
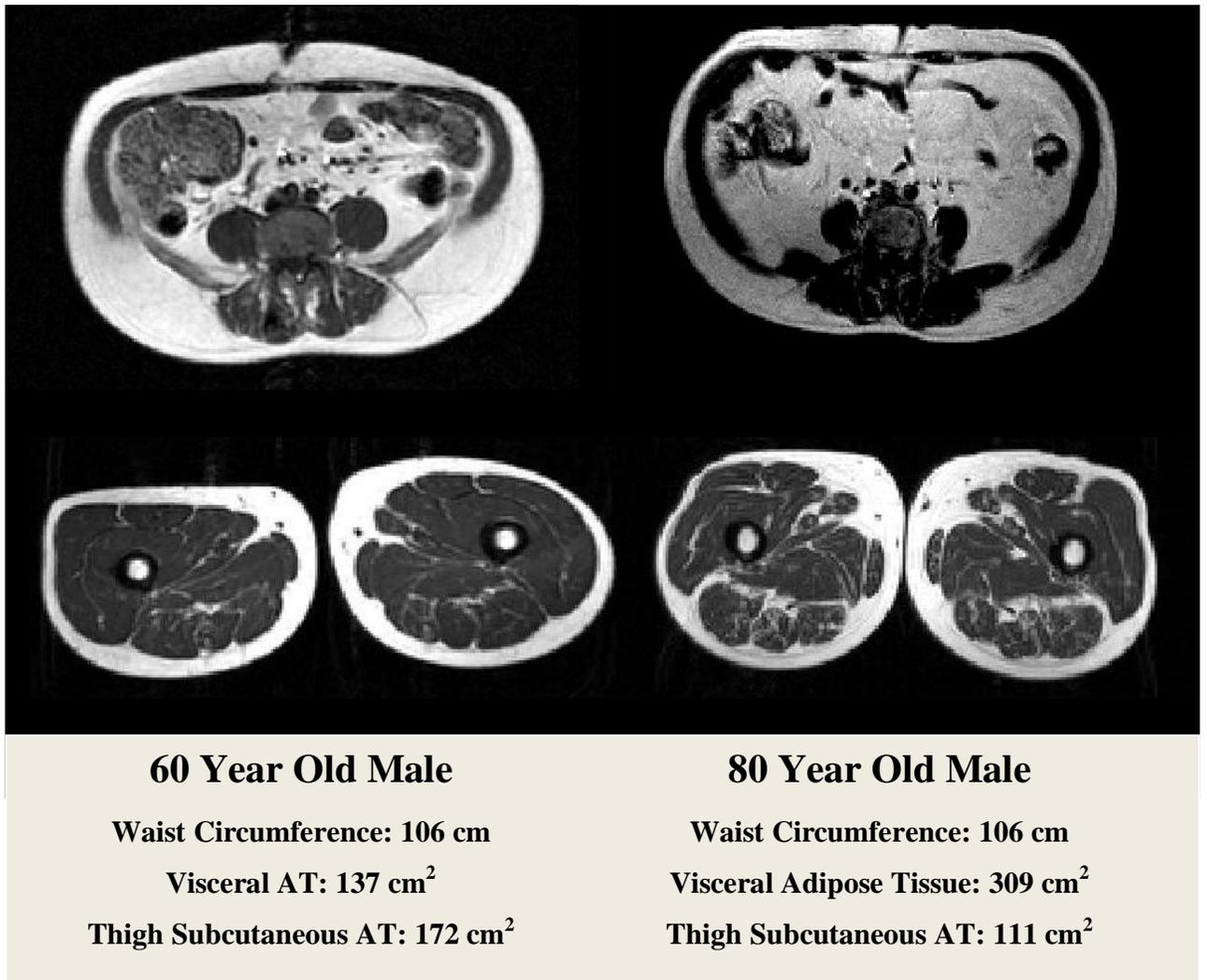
Figure 3. Age-related Changes in Visceral and Thigh Subcutaneous Adipose Tissue.

2.7 Summary

It is well-established that trunk and limb fat have independent and opposing associations with markers of cardiometabolic risk. The accumulation of TF is associated with increased risk of morbidity and mortality in both genders (4, 6). In contrast, after control for TF the accumulation of LF is associated with reduced cardiometabolic risk (4). It is thought that these opposing relationships are at least partially mediated by intrinsic differences of the adipocytes in the two depots (60). Adipocytes in the trunk are characterized by high levels of lipolysis (60) and thus the accumulation of excess TF may result in high levels of circulating free fatty acids and ectopic fat deposition (58). In contrast, peripheral adipocytes are thought to act as a metabolic sink storing lipids away from the ectopic depots where they could result in increased cardiometabolic risk (59). The associations between TF and metabolic risk may also be partially mediated by adiponectin, an adipokine with insulin sensitizing and anti-atherogenic properties which is dramatically decreased in individuals with excess trunk fat (83). In contrast, adiponectin levels are positively associated with LF accumulation after control for TF (48).

At present, is unclear if the interaction of TF and LF accounts for cardiometabolic risk beyond that explained by LF and TF independently. Although this question has recently been investigated (8) the analyses violated important statistical principles which may have influenced the reported associations between LF/TF and cardiometabolic risk. Therefore, the purpose of the present study was to determine if the interaction of LF and TF was associated with markers of cardiometabolic risk in elderly men and women after control for LF and TF.

Chapter 3 - Manuscript

Associations of the Limb Fat to Trunk Fat Ratio with Markers of Cardiometabolic Risk in Elderly Men and Women

3.1 Introduction

Excess trunk fat (TF) is associated with an increased risk of cardiovascular disease (CVD) and type 2 diabetes (T2D) (41, 84, 85), as well as their antecedents - insulin resistance (4, 8), dyslipidemia (27, 86) and systemic inflammation (29). It is thought that the associations between TF and markers of cardiometabolic risk may be partially mediated by adiponectin, an adipokine with insulin sensitizing and anti-atherogenic properties that is dramatically decreased in individuals with excess TF (83). Although the association is weaker, it has been reported that the accumulation of limb fat (LF) is also positively associated with increased cardiometabolic risk (4, 27, 43). However, after control for TF, the associations between LF and markers of cardiometabolic risk are reversed such that for a given amount of TF, increased LF is associated with lower insulin resistance and dyslipidemia (4, 48) and higher plasma adiponectin concentrations (48). Recent reports suggest that the association of increased LF with reduced cardiometabolic risk may persist even after control for visceral adipose tissue (VAT) (41, 43), a depot within the trunk which is independently associated with increased risk of mortality (40).

Although the independent and opposing associations of LF and TF with cardiometabolic risk are well-known, it is unclear if the ratio of LF to TF (LF/TF) accounts for cardiometabolic risk beyond that explained by LF and TF independently. If LF/TF is associated with markers of cardiometabolic risk after control for LF and TF, it would suggest that it is the amount of TF relative to LF, in addition to their absolute amounts, which influences metabolic risk. In this regard, Gavi et al. (8) have recently reported that LF/TF is negatively associated with insulin resistance and plasma

triglycerides, but positively associated with HDL-cholesterol and adiponectin

concentrations in elderly men and women. Further, LF/TF was the single best predictor

of insulin resistance in their sample (8). However, in that study the authors failed to

control for LF and TF when examining the associations between LF/TF and markers of

metabolic risk. Examining the interaction of two variables (e.g. LF/TF) without

controlling for their main effect terms (LF and TF) is a violation of the marginality

principle (74) and hence, confounds interpretation. Thus, it remains unclear if the relative

or absolute amounts of LF and TF are more closely associated with markers of

cardiometabolic risk in elderly men and women.

The primary objective of the present study was to determine if LF/TF was

associated with dyslipidemia, insulin resistance and plasma adiponectin concentrations in

elderly men and women after control for LF and TF.

3.2 Research design and methods

3.2.1 Subjects

Subjects included 78 women and 58 men between the ages of 60 and 80 years who were initially recruited to participate in a study examining the influence of exercise modality on insulin resistance and functional fitness in healthy, abdominally obese elderly men and women (87). This sample allowed us to answer the primary question in a sample similar to that studied by Gavi et al. (8). Further, due to their age (81, 82) and level of abdominal obesity (32), these individuals are at substantially increased risk of both CVD and T2D. It has recently been estimated that 62% of men and 74% of women between the ages of 60 and 69 in the United States are abdominally obese (88). Understanding the relationships between body composition and cardiometabolic risk in this population is thus of significant clinical importance.

Potential participants were excluded from the exercise intervention if they reported a history of heart disease, stroke or diabetes, or were taking glucose lowering or hormone replacement medication. The use of lipid-lowering (e.g. statins) and hypertension (e.g. ACE inhibitors) medications was permitted and recorded. All subjects gave their informed written consent before participation in accordance with the ethical guidelines set by Queen's University.

3.2.2 Anthropometric measurements

Body mass was measured to the nearest 0.1 kg on a calibrated balance. Standing height was measured to the nearest 0.1 cm with the use of a wall-mounted stadiometer. Waist circumference (WC) was measured at the superior border of the iliac crest, and was taken to the nearest 0.1 cm after a normal expiration.

3.2.3 Measurement of regional adiposity by magnetic resonance imaging

Whole-body (45-47 axial images) magnetic resonance imaging data were obtained with a General Electric (Waukesah, WI) 1.5 Tesla magnet using an established protocol (89) (Figure 1). The magnetic resonance imaging data were analyzed with specially designed image analysis software (Tomovision Inc, Montreal, Canada) using established procedures (31, 89). Total adipose tissue and skeletal muscle volumes were determined using all 45-47 images. Trunk fat was derived using the images extending from 5cm below L4-L5 to the humeral head. Visceral adipose tissue was calculated from the 5 images extending from 5 cm below to 15 cm above L4-L5. Limb fat was defined as all subcutaneous adipose tissue (SAT) from the 21 images extending from the top of the femoral head to the toes. We chose to limit our measure of LF to lower body SAT for several reasons. In addition to accounting for the vast majority of LF, lower body SAT is the only peripheral AT depot which is independently associated with reduced cardiometabolic risk (41, 43). We excluded inter-muscular adipose tissue (IMAT) within the limb images because, in contrast to lower body SAT, IMAT is positively associated with cardiometabolic risk independent of visceral adiposity (45, 46). Finally, although magnetic resonance imaging can be used to quantify both lower-body SAT and IMAT, the standard error of the estimate is much greater for IMAT (~23%) in comparison to SAT (~2%) (90).

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Limb Fat (Images 1-21)

Trunk Fat (Images 23-HH)
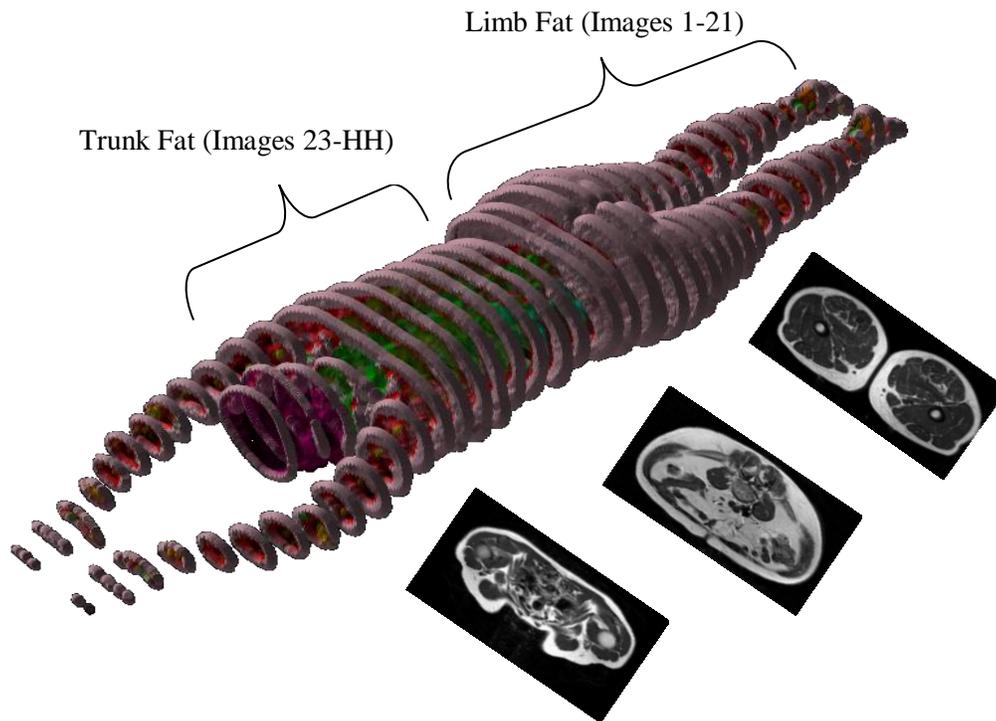
Figure 4. Magnetic Resonance Imaging Protocol.

3.2.4 Insulin sensitivity

Insulin sensitivity was assessed using the hyperinsulinemic euglycemic clamp

technique. Briefly, an antecubital vein was catheterized for infusion of insulin and 20%

glucose. An intravenous catheter was inserted in a retrograde fashion in a hand vein, and

the hand was placed in a heating pad for sampling of arterialized blood. Insulin was

infused at a rate of 40 mU/m2 per minute for 3 hours. Plasma glucose was measured by

using an automated glucose analyzer (YSI 2300 Glucose Analyzer, YSI, Yellow Springs,

Ohio) every 5 minutes in arterialized blood. Glucose disposal rate was calculated by

using the average exogenous glucose infusion rate during the final 30 minutes of euglycemia.

3.2.5 Biochemistry

Fasting blood samples were taken following an overnight, 12-hour fast. HDL-cholesterol and triglyceride levels were measured using enzymatic methods on the Roche Modular analytical system (Roche Diagnostics, Indianapolis, IN, USA). Plasma adiponectin concentrations were determined using an ELISA (B-Bridge International, Inc., San Jose, CA) of whole plasma kept at -80C before use.

3.2.6 Statistical Analyses

To determine if subjects could be collapsed across gender, sex-by-regional adiposity interactions were assessed for all dependent variables (fasting glucose, insulin sensitivity, HDL-cholesterol, plasma triglycerides, and plasma adiponectin concentration). First, sex was dummy-coded as follows: female = 1, male = 2. An interaction term was subsequently created for sex and each measure of regional adiposity (e.g. sex*TF, sex*LF and sex*VAT). Finally, the association of each interaction term with each dependent variable was examined after control for the interaction's main effect terms (e.g. sex and TF for sex*TF). A conservative α-level of 0.10 was used to determine significant associations between the interaction terms and markers of metabolic risk. Lipid lowering and hypertension medications were coded as -1 if participants were taking a medication and 0 if they were not taking said medication.

Baseline differences between men and women for anthropometric and metabolic variables were examined using a Student's *t* test for independent samples. Regression

analyses were performed to determine the univariate and multivariate associations between TF, LF, LF/TF and VAT with markers of cardiometabolic risk, controlling for the following confounders: sex, age and use of lipid or hypertension medications. The associations of LF and TF with markers of cardiometabolic risk were also explored using partial correlation analyses. Stepwise regression analyses were performed to determine the best predictors of each marker of cardiometabolic risk. Normality of distribution was examined for all dependent variables prior to analysis using the Shapiro-Wilk test. Adiponectin, insulin sensitivity, plasma triglycerides and HDL-cholesterol were transformed using a natural log function. All statistical procedures were performed using SPSS 15.0 software (SPSS, Chicago, IL, USA). Statistical significance was accepted at the 0.05 level unless otherwise indicated.

3.3 Results

Subject characteristics are presented in Table 1. Compared to men, women had significantly greater values for total and lower body subcutaneous AT, but lower values for waist circumference, TF and visceral adiposity ($p<0.05$). Women had significantly higher values for insulin sensitivity, adiponectin, and HDL-cholesterol, and lower values for fasting glucose concentration ($p<0.05$). Age, BMI and plasma triglyceride levels were not different between men and women ($p>0.05$).

Since adiponectin was the only marker of metabolic risk which demonstrated significant sex-interactions with either LF or TF (data not shown), analyses including adiponectin as the dependent variable are presented separately for each gender while analyses for all other markers of metabolic risk are presented with men and women combined. After control for age and medications, TF was negatively associated with adiponectin in men (standardized β coefficient = -0.26; $p<0.05$) and women (standardized β coefficient = -0.38; $p<0.05$), while LF was negatively associated with adiponectin in men only (standardized β coefficient = -0.34; $p<0.05$). The relationship between TF and adiponectin remained significant after further control for LF in women (standardized β coefficient = -0.50; $p<0.05$) but not men ($p>0.05$). Statistical control for TF abolished the relationship between LF and adiponectin in men ($p>0.05$), and resulted in a positive association between LF and adiponectin in women (standardized β coefficient = 0.29; $p<0.05$).

Associations of LF and TF with markers of cardiometabolic risk in men and women combined are presented in Table 2. After control for age, sex and medication use, TF was positively associated with fasting glucose (standardized β coefficient = 0.25;

$p<0.05$), insulin resistance (standardized β coefficient = 0.32; $p<0.05$) and plasma triglycerides (standardized β coefficient = 0.18; $p<0.05$), and negatively associated with HDL-cholesterol (standardized β coefficient = -0.18; $p<0.05$). These associations remained significant after further control for LF. After control for age, sex and medication use, LF was not associated with any marker of cardiometabolic risk in the group as a whole. However, after further control for TF, LF was positively associated with HDL-cholesterol (standardized β coefficient = 0.25; $p<0.05$) and negatively associated with plasma triglycerides (standardized β coefficient = -0.40; $p<0.05$).

LF/TF was not associated with any marker of cardiometabolic risk in men and women combined or with adiponectin in either sex after control for TF and LF (See Appendix E). Table 3 displays the variance in individual cardiometabolic risk factors which was explained by models with or without inclusion of LF/TF.

A final analysis was performed to determine if the independent associations of LF with HDL-cholesterol and triglycerides in the group as a whole and with adiponectin in women were mediated by visceral adiposity. When LF and visceral adipose tissue were entered as independent variables in the same model, only visceral adipose tissue was associated with HDL-cholesterol in men and women combined (standardized β coefficient = -0.27) or with adiponectin in women (standardized β coefficient = -0.52). In contrast, LF and visceral adiposity were independently associated with plasma triglyceride levels (respective standardized β coefficients = -0.27 and 0.44) in the group as a whole.

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Table 1. Characteristics of the Study Sample by Sex

	Men	Women
N	58	78
Age (years)	67.7 ± 5.1	67.5 ± 5.1
BMI (kg/m^2)	30.1 ± 2.7	29.7 ± 3.3
Weight (kg)	93.8 ± 10.1	78.7 ± 10.2†
Waist Circumference (cm)	112.4 ± 6.9	103.9 ± 9.0†
Total Adipose Tissue (L)	35.8 ± 7.4	39.1 ± 7.6†
Limb fat (L)	9.8 ± 2.2	14.2 ± 3.5†
Trunk fat (L)	17.1 ± 4.0	15.7 ± 3.7†
Visceral adipose tissue (L)	4.9 ± 1.1	2.7 ± 1.0†
Adiponectin (ug/ml)	5.5 ± 2.6	9.7 ± 4.5†
Fasting glucose (mmol/L)	4.8 ± 0.5	4.6 ± 0.5†
Insulin sensitivity (mg/kg muscle/min)	15.8 ± 6.8	21.3 ± 8.4†
Triglycerides (mmol/L)	1.8 ± 0.8	1.6 ± 0.7
HDL-Cholesterol (mmol/L)	1.2 ± 0.2	1.5 ± 0.3†

Data are means ± SD
† = Significantly different from men, $p \leq 0.05$ (Student's *t* test).

Table 2. Associations between Regional Adipose Tissue and Metabolic Risk Markers

	Fasting Glucose	IR	HDL-C	TG
Model 1				
Trunk fat (L)	0.25 (0.06)†	0.32 (0.12)†	-0.18 (0.04)†	0.18 (0.03)†
Limb fat (L)	-0.01 (0.01)	0.09 (0.01)	0.08 (0.01)	-0.19 (0.03)‡
Model 2				
Trunk fat (L)	0.34 (0.08)†	0.39 (0.12)†	-0.28 (0.07)†	0.34 (0.09)†
Limb fat (L)	-0.21 (0.02)‡	-0.15 (0.01)	0.25 (0.04)†	-0.40 (0.05)†
Model 3				
Limb fat (L)	-0.08 (0.01)	-0.02 (0.01)	0.12 (0.01)	-0.27 (0.05)†

Model 1, controlled for age, sex, and medications
Model 2, controlled as model 1 and for other AT depot
Model 3, controlled for age, sex, medications and visceral adiposity
Data are presented as standardized β coefficient (coefficient of determination)
†$p \leq 0.05$, ‡$p<0.10$
IR, Insulin resistance; TC, Total cholesterol; HDL-C, HDL-cholesterol; TG, Triglycerides

Table 3. R^2 Values for the Prediction of Metabolic Risk Markers with and without Addition of LF/TF to the Prediction Model

	FG	IR	HDL-C	TG
Model 1	0.09†	0.23†	0.30†	0.14†
Model 2	0.14†	0.28†	0.32†	0.16†
Model 3	0.15†	0.28†	0.32†	0.16†

Model 1: LF/TF
Model 2: TF and LF
Model 3: TF, LF and LF/TF
All models are controlled for age, sex and medication use
†$p \leq 0.05$
FG, Fasting glucose; IR, Insulin resistance; HDL-C, HDL-cholesterol; TG, Triglycerides

3.4 Discussion

The primary finding of the present study was that LF/TF was not associated with any marker of cardiometabolic risk in elderly men and women after control for LF and TF. Our results suggest that it is the absolute, rather than relative amounts of LF and TF, which are most closely associated with risk factors for CVD and T2D in elderly men and women. These findings are important for the estimated 34 million elderly Americans with abdominal obesity (88, 91), and reinforce the need to identify and target trunk fat within strategies designed to reduce obesity-related morbidity in older adults.

Our findings are in contrast to those of Gavi et al. (8) who reported that LF/TF was the strongest individual predictor of insulin resistance in elderly men and women. Indeed, our results suggest that after control for LF and TF, LF/TF is not associated with any marker of cardiometabolic risk. Our results also illustrate why it is misleading to examine the associations of a ratio score without controlling for both of its main effect terms. Ratio scores such as LF/TF often contain more information than either of their constituent variables (LF and TF) individually (75). Thus it is not surprising that LF/TF should be a better predictor of insulin resistance than either LF or TF individually, as reported by Gavi et al. (8). In fact, in a stepwise regression analysis we found that LF/TF entered the model before LF or TF when predicting every marker of metabolic risk in the present study (excluding adiponectin in men), and was the single best predictor of insulin resistance, fasting glucose and plasma triglyceride levels (data not shown). However, as mentioned previously, LF/TF was not associated with any marker of metabolic risk after statistical control for LF and TF. These results suggest that researchers and clinicians

will be better served by focusing on the independent effects of TF and LF, rather than LF/TF.

Several previous studies have reported that LF is associated with reduced CVD and T2D risk after control for TF (4, 27, 41, 43, 48, 56). While we could not corroborate the independent associations between LF and markers of carbohydrate metabolism, our results do suggest that independent of TF, LF is associated with lower lipid levels in both genders as well as higher adiponectin levels in women. Further, LF remained associated with plasma triglyceride levels after control for visceral adiposity. This is in agreement with previous results from our group (41) and others (27), which have suggested that LF is associated with triglycerides and HDL-cholesterol, but not insulin resistance, after control for visceral adiposity. In comparison to visceral and abdominal subcutaneous adipocytes, lower body subcutaneous adipocytes are characterized by increased sensitivity to the anti-lipolytic effects of insulin and decreased sensitivity to catecholamine-stimulated lipolysis (60). Subcutaneous adipocytes are also reported to have a greater capacity for differentiation (66, 67), resulting in increased hyperplasia rather than hypertrophy in times of energy surplus. This hyperplasic growth is thought to result in numerous small, insulin sensitive adipocytes, as opposed to the large, insulin resistant adipocytes that result from the primarily hypertrophic growth that characterizes visceral adipose tissue (68). Taken together, these characteristics may allow lower body SAT to act as a metabolic sink, sequestering free fatty acids away from the more lipolytically active visceral adipocytes, as well as other ectopic depots, both of which are thought to lead to increased metabolic risk (32, 59).

After control for visceral adiposity, we found that LF was no longer associated with HDL-cholesterol in men and women combined or with adiponectin levels in elderly women. These results suggest that for a given amount of TF, increased LF may be negatively associated with some markers of metabolic risk merely because it is a proxy for a reduced volume of visceral adipose tissue. This agrees with Motoshima et al. (92), who report that in obese individuals, adiponectin secretion is decreased in visceral, but not subcutaneous adipocytes. This is also consistent with previous reports from our group which suggest that for a given waist circumference, increased thigh and hip circumferences are associated with decreased visceral adiposity but conversely greater subcutaneous adiposity throughout the body(93).

To our knowledge, this is the first study to report a significant sex-interaction between regional AT and plasma adiponectin levels in elderly men and women. After control for age and medication use, both LF and TF were independently associated with adiponectin levels in women, while neither was associated with adiponectin levels in men. These results are novel, and may be due to sex differences in adiponectin clearance by the kidneys. A recent report by Laughlin et al. (94) suggests that the association of adiponectin with blood urea nitrogen (a marker of renal dysfunction) is significantly stronger in elderly men compared to elderly women. Adiponectin and blood urea nitrogen levels increase linearly with age in men, while both are reported to plateau in post menopausal women (94-96). Further, the negative associations of adiponectin with health risk in young men may actually be reversed in elderly men. For example, a report by Wannamethe et al. (97) suggests that adiponectin levels are positively associated with

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CVD and all-cause mortality in elderly men. Taken together, these results suggest that elderly men may experience increases in adiponectin levels due to reduced adiponectin clearance in the kidneys, obscuring the true relationships between adiponectin, regional adiposity, and metabolic risk.

Strengths and limitations of the present study warrant mention. This study was cross-sectional, and thus causality cannot be assumed. In addition, the present study measured total rather than high molecular weight adiponectin levels. In comparison to total adiponectin levels, it has been reported that the high molecular weight form of adiponectin is a superior predictor of cardiometabolic risk (98). Our results are derived from a relatively homogeneous sample of sedentary, abdominally obese and elderly men and women. Although this may limit the generalizability of the results of our study to other groups, the study sample is representative of the majority of elderly American men and women (88). The results of the present study are strengthened by the direct measurement of insulin resistance using the hyperinsulinemic euglycemic clamp technique and measurement of regional adiposity using MRI.

In summary, the results of the present study suggest that LF/TF is not associated with any marker of cardiometabolic risk in elderly men and women after control for LF and TF. Accordingly, our findings reinforce the need to identify and target trunk fat, in particular visceral fat, within strategies designed to reduce obesity-related morbidity in older adults.

Chapter 4 – General Discussion

4.1 Clinical Implications

4.1.1 Clinical Assessment of Trunk Fat

In the present study, the finding with the greatest clinical significance was the strong association between TF, and especially visceral fat, with markers of cardiometabolic risk. This finding agrees with numerous reports (4, 8, 43, 73, 86) and reinforces the importance of clinical interventions aimed at reducing TF and VAT in the elderly. However, if clinicians are to target TF and VAT effectively, they must first quantify it accurately. Imaging methods such as MRI and CT are costly and time consuming, and therefore not practical for the vast majority of clinicians. Fortunately, it has been reported that waist circumference is strongly associated with VAT volume (99, 100), and that changes in VAT are mirrored by changes in WC (101). Further, it has been suggested that measuring WC in tandem with traditional risk factors such as plasma triglycerides can help clinicians dramatically improve their ability to screen and treat patients at high risk for cardiovascular disease (102). For example, Lemieux et al. (102) report that in men with elevated plasma triglycerides, the risk of coronary artery disease is 144% greater in those with a waist circumference above 90 cm compared to those with a waist size below 90 cm. Thus, the measurement of WC in clinical practice to assess TF, VAT and related cardiometabolic risk is strongly recommended.

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In contrast to our findings regarding TF and cardiometabolic risk, our findings concerning LF are of less immediate clinical relevance. Although LF was associated negatively with triglycerides and positively with HDL-cholesterol independent of TF, there are currently no lifestyle modification treatments which would allow an individual to selectively increase LF while holding TF levels constant. Further, a recent study from our lab suggests that although LF is independently associated with reduced cardiometabolic risk, the reduction of LF through negative energy balance does not appear to have any influence on cardiometabolic risk (41). Finally, both the present findings and those of others (41, 43) suggest that TF, and especially VAT, are the strongest predictors of cardiometabolic risk in elderly men and women. Thus, the measurement of LF in the clinical setting does not appear warranted.

4.2 Future Research

At present, the regulation of plasma adiponectin levels in the elderly is not well understood. In young and middle-aged adults, adiponectin levels are strongly and negatively associated with visceral adipose tissue volume (103, 104). However, adiponectin levels are reported to increase with age in both genders (95) despite age-related increases in visceral adiposity (80). It has been suggested that this age-related increase in adiponectin levels may be due to reduced adiponectin clearance by the kidneys (95) but at present the biological mechanisms underlying this proposed method of adiponectin clearance have not been directly investigated. In addition, at present no studies have examined adiponectin mRNA expression or secretion in the elderly, sex differences in these processes, or how they may be influenced by normal aging.

Finally, both the present study and others (73, 97) have suggested that the association of adiponectin with markers of cardiometabolic risk is significantly influenced by both age and gender. In fact, some evidence suggests that adiponectin may be a marker of increased rather than decreased cardiometabolic risk in elderly men (97). However, the precise reasons for this are unclear, and at present remain largely unexamined. Future research is clearly needed in order to improve our understanding of adiponectin regulation, and its relationship to chronic disease risk in elderly men and women.

4.3 How can we reduce obesity in the Canadian population?

As I have been preparing this Thesis, it was inevitable that I would begin to wonder how we as a society can put an end to the current obesity epidemic and all of its associated social and economic costs. One activity which has received increasing attention of late is the area of knowledge "translation". The goal of knowledge translation is to inform the public about obesity, physical inactivity and related health risk, with the assumption that they will use that knowledge to make rational decisions about their health behaviors. However, the recent work of Christakos and Fowler suggests that health behaviors may not result just from our rational decision making processes. Instead, their work suggests that positive and negative health behaviors flow from one person to another like social contagions, eventually "infecting" entire social networks (105, 106).

In an article from 2007, Christakis and Fowler (105) report that our social networks have a dramatic influence on our risk of developing obesity. For example, they report

that if an individual has a friend who develops obesity in a given period of time, their own risk of developing obesity increases by 57%. Similarly, their risk of developing obesity increases 40% if a sibling develops obesity. These findings suggest that we could see an exponential increase in obesity as more and more members of each social network become obese, increasing the risk of obesity in those around them. Perhaps we have already reached this obesity tipping point, setting off a chain reaction that will not end until the vast majority of Canadians are obese.

Although these social networks could be our collective undoing, they may also be our salvation. A subsequent study by Christakis and Fowler (106) suggests that while obesity may be "contagious" among our social networks, so too are positive health choices. For example, they report that if an individuals' spouse quits smoking in a given period of time, that individual becomes 67% more likely to quit smoking themselves. Further, they report that entire groups of interconnected people stopped smoking simultaneously, and that those who continued to smoke became increasingly marginalized within their social networks.

This is not to suggest that we should socially marginalize individuals with obesity or increased cardiometabolic risk. To the contrary, we should place the focus on positive lifestyle choices with the hopes that they may be "contagious" within a social network. Eventually, this could result in those with predominantly unhealthy behaviors becoming increasingly marginalized within their social network. Healthy behaviors like increased physical activity may even become social and cultural norms, just as unhealthy behaviors like smoking were in the past. Thus, rather than merely translating knowledge, we have

to find a way to motivate Canadians towards positive health decisions, with the hope that they will gradually spread throughout the population. This may not "cure" obesity, but it would certainly lead to longer, healthier lives for people of all shapes and sizes.

Summary and Conclusions

The independent and opposing associations of limb and trunk fat with markers of cardiometabolic risk are well-established (4). In addition, previous research suggested that it may be the interaction of LF to TF (LF/TF), rather than their absolute amounts which influence markers of cardiometabolic risk (8). However, using criterion measurements of both body fat distribution and cardiometabolic risk, we have shown that LF/TF is not associated with any marker of cardiometabolic risk in elderly men and women after control for LF and TF. These results suggest that both clinicians and researchers will be better served by focusing on the absolute, rather than relative amounts of LF and TF.

Our results reinforce the importance of trunk fat, and especially visceral adipose tissue, as a marker of increased health risk (40, 41, 43). VAT was the strongest predictor of cardiometabolic risk in the present study, and is thought to play a fundamental role in the etiology of many chronic diseases (80). Although the direct measurement of VAT is unlikely in the clinical setting, the approximation of visceral adiposity using waist circumference should be strongly encouraged.

Finally, the results of the present study suggest that plasma adiponectin levels, and the relationship of adiponectin with cardiometabolic risk, are significantly influenced by gender in the elderly. While it was found that TF and LF were independently associated with plasma adiponectin levels in women, neither TF nor LF were

independently associated with plasma adiponectin in men. At present, the physiological mechanisms underlying these sex-differences remain unclear.

In summary, the results of the present study suggest that there is no interaction between limb and trunk fat when predicting CVD or T2D risk in elderly men and women. This suggests that it is the absolute, as opposed to relative amounts of trunk and limb fat which influence metabolic risk in this population. These results also reinforce the strong associations of trunk and visceral fat accumulation with increased cardiometabolic risk in the elderly. Finally, these results suggest that the associations between adiponectin and regional adiposity in the elderly are strongly influenced by gender.

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Appendix A

Informed 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.**

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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.

BACKGROUND INFORMATION

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 $2^{1/2}$-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 that 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 B

Medical Questionnaire

School of Physical and Health Education

MEDICAL QUESTIONNAIRE FOR RESEARCH STUDY

PREVENTION AND TREATMENT OF ABDOMINAL OBESITY AND RELATED INSULIN RESISTANCE IN ELDERLY MEN AND WOMEN

To the study participant: Please answer all questions in sections 1 and 2 of this form. Have your family doctor fill out section 3.

To the physician: Please fill out section 3 of this form (pages 4-6). Completing this form may not require a medical re-evaluation of your patient. If the results of recent tests are readily available that might prove useful to study personnel while dealing with the participant, please include that information in this questionnaire.

Please note that we will pay all costs for completing this questionnaire. Please bill your patients directly and we will reimburse them accordingly.

SECTION 1: PERSONAL DATA (please print)

Name: _____

Date of Birth: _____

Date: _____

SECTION 2: MEDICAL HISTORY

	Yes	No
A. Has your doctor ever said you have heart trouble?	___	___
B. Do you get pains, pressure or tightness in your chest?	___	___
C. Do you often feel faint or experience dizziness?	___	___
D. Has you doctor ever told you that you have high blood pressure?	___	___
E. Is there a good reason, not mentioned above, why you should avoid exercise?	___	___

F. Do you have, or have you ever had, problems with any of the following?

	Yes	No
i. Heart or blood vessels	___	___
ii. Nerves or brain	___	___
iii. Breathing or lungs	___	___
iv. Hormones, thyroid, or diabetes	___	___
v. Muscles, joints, or bones	___	___
vi. Other (please list)		

_____

G. Please list any serious injuries suffered, or surgeries you have had.

_____

_____

_____

H. If you have had surgery, was any metal (e.g., pins or screws) left in your body?

_____

_____

_____

I. Are you presently taking any medications? If yes, please list.

_____

_____

_____

J. Are you presently undergoing physiotherapy, or any other sort of treatment?
    If yes, please list.

_____

_____

_____

SECTION 3: MEDICAL REFERRAL

Physician: The applicant is considering participation in a research study that is investigating the effects of exercise modality on changes in body composition (e.g., abdominal fat), cardiovascular fitness, glucose tolerance and insulin resistance. As a participant in this study, your patient would undergo a cardiovascular fitness appraisal (see explanation on page 7) and a number of other tests to assess body composition and metabolic health risk. We will forward any test results to you at your patient's request.

Should you have any questions regarding the participation of your patient in this project, please contact Robert Ross Ph.D., School of Physical and Health Education, Queen's University (533-6583).

Review of Systems - please include diagnoses.

a) Cardiovascular _____

b) Respiratory _____

c) Neurological _____

d) Gastrointestinal _____

e) Genitourinary _____

f) Endocrine _____

g) Musculoskeletal _____

h) Skin _____

i) Gynecological _____

II.   Physical Examination

Blood Pressure: _____   Pulse: _____

Cardiovascular: _____

Respiratory: _____

Head and Neck: _____

MSK: _____

Abdomen: _____

12-lead ECG (**not mandatory**):_____

Neurological: _____

III. **Laboratory findings (not mandatory)**   Date of Test(s): _____

Hb _____   WBC _____   Plts _____

Total Cholesterol _____   HDL _____   Chol:HDL ratio _____

LDL _____   Triglycerides _____   Uric Acid _____

TSH _____   Glucose _____   fasting ☐   random ☐

75 g OGTT @ 120 min _____

IV. **Additional abnormalities of which you are aware**
_____

_____

_____

V. **Current medications and doses**
_____

_____

_____

VI. **On the basis of your knowledge and medical evaluation of the applicant, you would recommend (mark the appropriate answer):**

_____ Participation in a fitness appraisal supervised by a physical education graduate, or

_____ Participation in a fitness appraisal only when a physician or paramedic is present, or

_____ Participation in a fitness appraisal is not recommended

Note: An explanation of the fitness appraisal protocol, as well as absolute and relative contraindications to exercise testing, is provided on page 7 of this form.

Physician's Name: _____

Physician's Signature: _____

Date: _____

Phone Number: _____

Address:

_____

_____

_____

Thank you very much for your help. We hope that this study and its results will be beneficial to you and your patient.

Appraisal of Cardiovascular Fitness (VO$_2$max test)

Cardiovascular fitness is assessed using a maximal oxygen uptake (VO$_2$max) test, which is routinely employed within the laboratory of the study investigators. The treadmill walking test begins at a level the study participant can easily accomplish (comfortable walking pace with no incline) and is slowly increased in intensity (by increasing treadmill incline) until the participant reaches volitional fatigue. We may stop the test at any time because of signs of fatigue or the subject may stop the test because of personal feelings of fatigue or discomfort.

The maximal oxygen uptake test involves risks comparable to very strenuous aerobic exercise. Every effort is made to minimize the risk by preliminary medical examination and close observation during the test by physical education graduate students and a physician.

American College of Sports Medicine Contraindications to Exercise Testing

Absolute Contraindications
A recent change in the resting ECG suggesting infarction or other acute cardiac events
Recent complicated myocardial infarction
Unstable angina
Uncontrolled ventricular dysrhythmia
Uncontrolled atrial dysrhythmia that compromises cardiac function
Third-degree A-V block
Acute congestive heart failure
Severe aortic stenosis
Suspected or know dissecting aneurysm
Active or suspected myocarditis or pericarditis
Thrombophlebitis or intracardiac thrombi
Recent systemic or pulmonary embolus
Acute infection
Significant emotion distress (psychosis)

Relative Contraindications
Resting diastolic blood pressure >120 mm Hg or systolic blood pressure >200 mm Hg.
Moderate valvular heart disease
Known electrolyte abnormalities (hypokalemia, hypomagnesemia)
Fixed-rate pacemaker (rarely used)
Frequent of complex ventricular ectopy
Ventricular aneurysm

Cardiomyopathy, including hypertrophic cardiomyopathy

Uncontrolled metabolic disease (e.g., diabetes, thyrotoxicosis, or myxoedema)

Chronic infectious disease (e.g., mononucleosis, hepatitis, AIDS)

Neuromuscular, musculoskeletal, or rheumatoid disorders that are exacerbated by exercise

Advanced or complicated pregnancy

Appendix C

Raw Data

ID	Sex	TF	LF	FG	IS	APN	HDL	TG
1	F	11.93	19.6528691	4.7	21.96	14.398	1.77	0.9
2	F	15.82	16.74199602	4.5	27.69	8.867	1.64	2.6
3	F	18.12	10.69280291	5.2	12.84	11.136	1.81	0.8
4	F	14.52	17.07806068	#NULL!	15.84	9.449	1.25	1.8
5	F	11.66	12.08296276	4.2	28.27	15.374	1.92	0.8
6	F	13.65	14.6974488	4.7	13.56	12.283	2.65	1
7	F	18.1	10.51595117	4.7	24.53	15.484	1.43	1.4
8	F	16.74	14.89922987	4	18.04	11.876	1.35	1.9
9	F	17.68	11.46475066	5.1	10.77	4.947	1.1	3
10	F	11.25	16.08809952	4.4	19.81	9.945	1.77	1
11	F	17.57	16.61168666	4.1	37.46	15.155	1.44	1.1
12	F	15.49	16.52169889	#NULL!	16.71	11.454	1.94	0.9
13	F	18.62	15.77693034	4.6	15.87	6.244	1.91	0.8
15	F	12.7	15.13895941	3.9	21.4	7.465	1.37	2.1
16	F	18.6	17.94751347	3.6	16.7	9.907	1.84	1.9
17	F	20.41	19.57698655	4.6	20.4	4.98	1.36	2.1
18	F	21.56	13.17832748	5.4	19.71	9.926	1.56	1
19	F	14.35	13.22725983	5	8.98	4.61	1.38	2.1
20	F	14.78	17.78965124	4.9	13.46	7.518	1.2	1.4
21	F	10.72	14.00810115	4.1	#NULL!	11.235	1.41	1.2
22	F	13.74	9.701170762	3.8	35.13	13.233	1.4	1.4
23	F	16.19	19.24601366	5.3	12.85	5.889	1.15	3.1
24	F	10.89	20.92230861	4.3	42.18	14.548	1.45	1.3
25	F	19.39	21.47456242	5.4	16.1	7.129	1.53	2.1
26	F	23.55	9.435743977	4	23.33	8.186	1.4	0.9
27	F	13.11	14.3659252	4.3	22.57	7.058	1.89	1.4
28	F	6.96	9.383867374	4.1	42.09	25.73	1.45	0.9
29	F	16.17	19.18647721	4.8	23.13	10.682	1.56	1.3
30	F	18.57	20.58473485	4	21.22	9.236	1.37	1.4
31	F	16.19	11.85744301	5	28.28	13.823	1.91	1.1
33	F	7.6	15.00576339	5.1	32.65	23.361	1.78	0.8
34	F	9.07	14.01465025	4.8	19.57	10.455	1.63	1.9
35	F	10.33	10.4465684	4.3	20.03	8.824	1.42	1
36	F	11.89	24.01006704	5.1	16.03	13.27	1.7	0.8
38	F	18.04	8.47083258	4.5	23.61	7.646	1.73	1.1
39	F	13.41	14.73865822	4	26.85	9.425	1.31	2
40	F	18.53	9.278417289	5.2	13.15	4.848	1.47	1.8

41	F	16.79	15.58062234	4.6	32.88	8.048	1.29	1.6
42	F	16.88	14.99517725	4.1	26.23	14.175	1.68	0.9
43	F	18.71	15.0694445	#NULL!	#NULL!	11.567	1.34	1.6
44	F	15.83	7.069283037	4.9	21.04	9.067	1.59	1.7
45	F	9.84	17.40511773	4	38.58	11.37	0.93	1.7
46	F	15.87	14.94131069	4.2	30.83	11.252	1.55	1.2
47	F	12.69	12.71886554	4.6	32.05	9.482	1.75	1.3
49	F	22.4	12.13180922	5.4	24.49	8.769	1.92	1.2
50	F	11.93	20.06418392	5.3	20.97	19.798	1.45	2.7
51	F	21.25	7.223571201	3.8	12.36	4.916	1.17	1.1
53	F	11.44	10.96382748	4.3	16.18	1.909	1.43	2.2
54	F	14.79	12.20478925	4	19.18	7.171	1.65	1.9
55	F	15.54	13.43260258	4.6	25.74	12.072	1.67	1.4
56	F	16.9	11.96031602	4.9	26.44	10.101	1.21	1.1
57	F	17.2	9.186470364	5.4	11.59	6.59	1.28	2.4
59	F	11.8	18.66049006	4.4	13.49	8.55	1.61	2
60	F	17.99	13.33028839	4.6	12.74	10.352	1.28	1.3
61	F	19.27	14.68996447	5.4	20.32	2.557	1.37	1.9
62	F	17.73	13.08998429	4.4	17.83	4.811	1.27	1.2
64	F	14.64	12.81893231	4.5	31.1	11.27	1.73	0.5
65	F	14.62	12.92702731	#NULL!	#NULL!	12.856	1.55	1.3
66	F	12.7	14.92638505	4.6	20.83	14.971	1.61	2.5
68	F	21.83	12.35813354	5.2	13.87	5.988	1.55	1.2
69	F	17.21	14.59845296	4.6	28.83	6.91	1.18	2.9
70	F	12.85	11.45189338	4	35.2	20.454	1.4	0.9
71	F	17.57	17.03150138	4.3	33	4.006	1.08	1.3
72	F	19.08	15.0731978	#NULL!	6.53	3.906	1	1.6
73	F	18.37	12.64911652	4.4	#NULL!	5.205	0.82	3
74	F	11.95	9.093008795	4.8	13.02	4.45	0.9	2.3
75	F	14.82	13.77504412	5.1	24.14	8.043	1.4	2.3
76	F	21.52	10.22059202	4.7	5.08	6.484	0.95	2.9
77	F	14.71	15.50092767	4.9	14.03	4.106	1.01	3.2
78	F	12.33	18.58364112	5.3	13.97	8.043	1.57	0.8
79	F	16.48	17.30387318	4.6	#NULL!	14.497	1.85	0.5
81	F	18.99	16.53036375	4.6	17.47	4.942	1.17	2.3
82	F	17.74	12.00157368	4.1	30.37	9.604	1.38	0.7
83	F	11.63	14.91797972	4.4	17.64	6.228	1.2	1.2
84	F	22.95	14.81167212	5.2	7.81	8.678	1.23	2.1
85	F	22.83	12.47143697	4.9	14.28	5.304	0.91	2.2

86	F	11.96	10.07075789	5.3	19.16	10.222	1.88	2
87	F	11.38	10.37684536	5.1	24.33	11.894	1.51	0.6
101	M	18.92	9.862275756	4.8	12.11	16.349	1.25	0.8
102	M	15.57	11.40600175	4.3	14.15	10.409	1.31	1.4
103	M	16.15	9.091330305	4.7	21.77	5.99	1.06	1.3
104	M	16.64	12.63028561	5.8	7.5	3.298	1.26	4.4
105	M	24.52	8.159657753	5.6	11.59	5.922	1.16	1.9
106	M	17.3	9.990614663	5.6	22	7.341	1.35	1.1
107	M	17.04	10.65482181	4	10.35	5.671	1.18	0.7
108	M	14.81	12.50157287	4.7	9.64	7.146	1.11	2.5
110	M	18.14	9.185514823	4.6	21.68	4.786	1.2	1.4
111	M	15.01	7.68142204	4.8	12.3	4.802	1.22	2.4
112	M	13.11	11.36211905	4.7	23.11	8.46	0.95	1.7
113	M	20	10.98427312	4.1	10.91	7.914	1.33	1.6
114	M	16.96	7.903647555	4	11.92	3.268	0.83	1.5
115	M	15.64	11.88043575	5	10.16	3.462	1.19	2
116	M	18.06	11.17386346	5.5	7.55	2.122	1.15	2
117	M	17.8	8.185475989	5.2	21.52	4.199	1.68	2.2
118	M	14.94	8.001301791	4.9	11.89	4.435	1.04	1.5
119	M	13.94	7.64285131	3.8	39.45	6.866	1.35	0.7
120	M	15.61	13.75670315	4.1	9.23	3.92	1.31	4.3
121	M	21.47	7.967554371	4.6	5.07	3.372	1.05	1.6
123	M	10.7	13.61330322	3.9	23.55	5.306	1.43	1.6
124	M	23.85	8.919838073	4.8	25.35	8.295	1.09	0.9
125	M	21.99	7.614508748	5.4	#NULL!	6.268	0.77	2.7
126	M	12.81	9.890840206	4.9	14.44	6.374	1.71	1
128	M	22.74	11.26763332	5.9	10.2	5.813	0.94	2.3
129	M	16.31	9.636576329	4.2	15.44	3.396	1.07	2.3
130	M	14.59	9.771533527	4.8	10.79	4.123	0.78	3.1
131	M	16.8	14.39736039	5.4	11.2	4.848	0.87	2.8
132	M	24.03	6.474120826	5.2	13.13	3.151	0.87	1.6
133	M	12.86	7.929818923	4.3	17.19	5.848	1.03	2
134	M	15.76	12.10340498	4.5	16.29	3.543	1.38	1.1
135	M	19.85	8.818667414	4.9	24.72	4.307	1.25	1.3
136	M	20.7	13.36386383	4.6	10.88	5.259	1.07	2.1
137	M	20.5	8.809705622	5	13.29	4.763	0.95	1.4
138	M	16.39	10.01982978	5.2	6.56	7.356	0.65	3.3
139	M	17.22	6.568187986	4.8	19.43	3.543	1.64	1.6
141	M	12.92	10.62749426	4.9	14.8	2.925	0.97	1.5

142	M	11.99	6.234148893	4.5	20.9	4.123	1.42	0.9
143	M	13.77	9.298831518	5.1	13.65	6.658	1.41	1.7
144	M	15.07	9.112124081	4.5	21.64	12.072	1.47	2.6
145	M	16.93	9.682007122	4.1	38.1	4.986	1.05	1.4
146	M	11.73	9.273560655	4.8	20.62	5.03	1.09	1.4
147	M	19.03	8.336771649	5.1	15.93	9.26	1.19	1.8
148	M	12.12	10.51362739	4.2	14.44	4.493	1.35	0.6
149	M	14.51	6.606730401	4.2	15.23	3.835	1.05	1
150	M	13.64	13.97650405	5.1	20.25	4.321	1.1	1.2
151	M	23.58	9.653454488	6.1	6.5	2.557	1.03	3
152	M	18.46	9.655915736	4.8	15.33	3.594	1.07	0.9
153	M	15.12	12.07476461	5.1	19.71	5.661	1.36	0.9
154	M	17.41	8.068953537	5.1	23.74	3.864	1.28	1
155	M	13.6	6.488160206	5	13.35	6.078	0.67	1.8
156	M	11.91	10.11717973	5.2	18.3	12.73	1.33	1.1
157	M	13.4	7.330841907	4.6	13.22	4.206	1.21	2.3
158	M	18.55	15.09158006	5.2	11.67	4.71	1.03	2.4
159	M	33.09	11.20145637	4.4	17.33	3.13	1.2	2.4
160	M	20.23	10.45869762	4.7	7.37	3.906	1.11	1.9
161	M	20.15	6.916116462	4.5	10.66	4.049	1.14	2.6
162	M	13.93		4.4	19.96	5.069	0.91	1.5

Appendix D
Sample of Statistical Analyses Used to Derive the Results of the Manuscript

Sample of participant characteristics

Descriptive Statistics[a]

	N	Minimum	Maximum	Mean	Std. Deviation
Age1	78	60.1	79.2	67.473	5.0747
BMI	78	22.72	38.16	29.7164	3.31188
wk1wt	78	58.5	101.9	78.696	10.2219
ICCM1	78	86.3	124.0	103.896	8.9832
AT1	78	22.55	56.82	39.1056	7.62807
LegSAT	78	7.07	24.01	14.2370	3.51518
TorsoTAT	78	6.96	23.55	15.6531	3.72315
VAT1	78	1.02	5.11	2.7280	1.02366
Adp1	78	1.909	25.730	9.69505	4.469450
Glu	73	3.60	5.40	4.6192	.47570
InsulinSensitivity	73	5.08	42.18	21.3200	8.37001
Trig1	78	.5	3.2	1.573	.6761
HDL1	78	.82	2.65	1.4688	.30760
Valid N (listwise)	70				

a. Sex = F

wk1wt = weight
ICCM1 = waist circumference
AT1 = total adipose tissue volume
LegSAT = leg subcutaneous adipose tissue volume
TorsoTAT = trunk total adipose tissue volume
VAT1 = visceral adipose tissue volume
Adp1 = plasma adiponectin concentration
Glu = fasting glucose concentration
Trig1 = plasma triglyceride concentration
HDL1 = HDL-cholesterol concentration

Example of a natural log transformation to normalize data

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	df	Sig.
Trig1	.100	136	.002	.939	136	.000
LnTrig	.056	136	.200[*]	.989	136	.373

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.
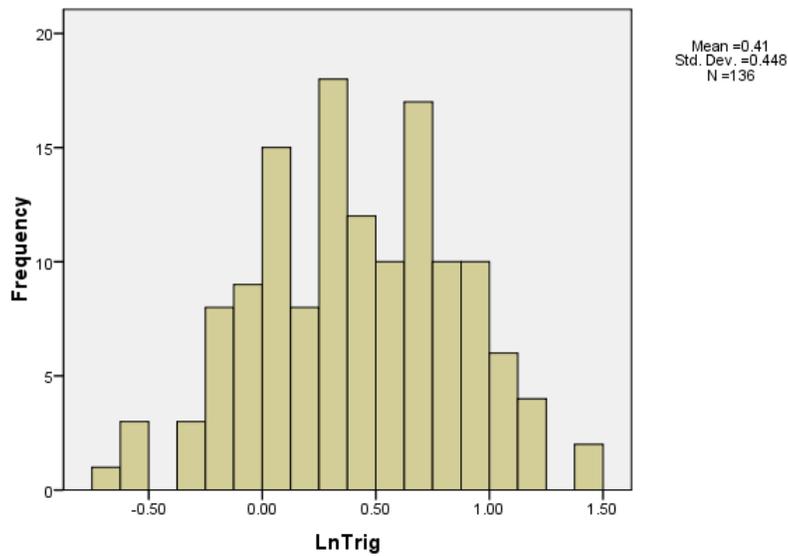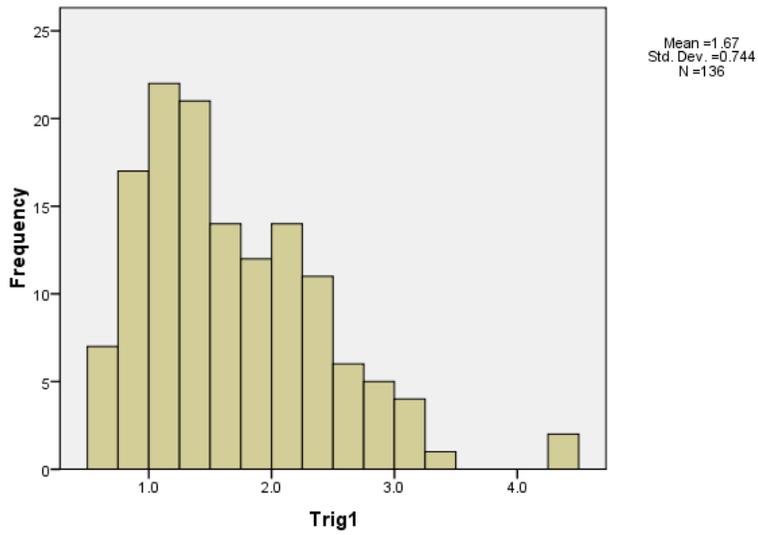
Example test for gender interactions

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	LegSATxsex, LegSAT, Sexnumber[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: LnAdp

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.542[a]	.294	.278	.44294

a. Predictors: (Constant), LegSATxsex, LegSAT, Sexnumber

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	10.798	3	3.599	18.346	.000[a]
	Residual	25.898	132	.196		
	Total	36.696	135			

a. Predictors: (Constant), LegSATxsex, LegSAT, Sexnumber

b. Dependent Variable: LnAdp

Coefficients^a

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.902	.502		3.791	.000
	LegSAT	.073	.039	.523	1.859	.065
	Sexnumber	.120	.344	.114	.347	.729
	LegSATxsex	-.063	.031	-.572	-2.062	.041

a. Dependent Variable: LnAdp

LegSAT = Leg subcutaneous adipose tissue volume
Sexnumber = gender (1 = female, 2 = male)
LegSATxsex = Dummy variable representing interaction of LegSAT and Sexnumber

Example of independent samples –test

Group Statistics

	Sex	N	Mean	Std. Deviation	Std. Error Mean
Age1	F	78	67.473	5.0747	.5746
	M	58	67.709	5.0719	.6660

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Age1	Equal variances assumed	.011	.915	-.268	134	.789	-.2355	.8797	-1.9754	1.5043
	Equal variances not assumed			-.268	122.995	.789	-.2355	.8796	-1.9766	1.5055

Example of multiple regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	TorsoTAT, BPMeds, LipidMeds, Sexnumber, Age1[a]		. Enter

a. All requested variables entered.

b. Dependent Variable: Glu

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.343[a]	.118	.082	.47816

a. Predictors: (Constant), TorsoTAT, BPMeds, LipidMeds, Sexnumber, Age1

ANOVA[b]

Model		Sum of Squares	df	Mean Square
1	Regression	3.778	5	.756
	Residual	28.351	124	.229
	Total	32.129	129	

a. Predictors: (Constant), TorsoTAT, BPMeds, LipidMeds, Sexnumber, Age1

b. Dependent Variable: Glu

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients
		B	Std. Error	Beta
1	(Constant)	3.420	.629	
	Sexnumber	.109	.087	.109
	Age1	.008	.009	.082
	BPMeds	-.113	.094	-.106
	LipidMeds	-.130	.105	-.108
	TorsoTAT	.032	.011	.251

a. Dependent Variable: Glu

Sexnumber = gender (1 = female, 2 = male)
BPmeds = blood pressure medication use
LipidMeds = lipid medication use
TorsoTAT = trunk total adipose tissue volume
Glu = plasma glucose concentration

Example partial correlation grid

Correlations

Control Variables			TorsoTAT	Glu
Sexnumber & BPMeds & LipidMeds & Age1	TorsoTAT	Correlation	1.000	.249
		Significance (2-tailed)	.	.005
		df	0	124
	Glu	Correlation	.249	1.000
		Significance (2-tailed)	.005	.
		df	124	0

Sexnumber = gender (1 = female, 2 = male)
BP meds = blood pressure medication use
LipidMeds = lipid medication use
TorsoTAT = trunk total adipose tissue volume
Glu = plasma glucose concentration

Appendix E

Statistical Output for the Associations of LF/TF with Markers of Cardiometabolic Risk

Regression

Sex = F

Variables Entered/Removed[b,c]

Model	Variables Entered	Variables Removed	Method
1	LegSATtoTF, BPMeds, LipidMeds, Age1, LegSAT, TorsoTAT[a]	.	Enter

a. All requested variables entered.

b. Sex = F

c. Dependent Variable: LnAdp

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.553[a]	.306	.247	.41439

a. Predictors: (Constant), LegSATtoTF, BPMeds, LipidMeds, Age1, LegSAT, TorsoTAT

b. Sex = F

ANOVA[b,c]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	5.303	6	.884	5.147	.000[a]
	Residual	12.021	70	.172		
	Total	17.324	76			

a. Predictors: (Constant), LegSATtoTF, BPMeds, LipidMeds, Age1, LegSAT, TorsoTAT

b. Sex = F

c. Dependent Variable: LnAdp

Coefficients[a,b]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.304	.856		1.524	.132
	Age1	.006	.010	.064	.591	.557
	BPMeds	.057	.115	.052	.501	.618
	LipidMeds	.298	.126	.249	2.372	.020
	LegSAT	-.027	.041	-.201	-.671	.504
	TorsoTAT	.001	.040	.010	.034	.973
	LegSATtoTF	.939	.528	.578	1.778	.080

a. Sex = F

b. Dependent Variable: LnAdp

Sex = M

Variables Entered/Removed[b,c]

Model	Variables Entered	Variables Removed	Method
1	LegSATtoTF, Age1, LipidMeds, BPMeds, LegSAT, TorsoTAT[a]		.Enter

a. All requested variables entered.

b. Sex = M

c. Dependent Variable: LnAdp

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.515[a]	.266	.179	.36341

a. Predictors: (Constant), LegSATtoTF, Age1, LipidMeds, BPMeds, LegSAT, TorsoTAT

b. Sex = M

ANOVA[b,c]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2.436	6	.406	3.074	.012[a]
	Residual	6.736	51	.132		
	Total	9.171	57			

a. Predictors: (Constant), LegSATtoTF, Age1, LipidMeds, BPMeds, LegSAT, TorsoTAT

b. Sex = M

c. Dependent Variable: LnAdp

Coefficients[a,b]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.779	1.393		.559	.579
	Age1	.018	.010	.234	1.880	.066
	BPMeds	.001	.105	.002	.012	.990
	LipidMeds	.350	.117	.376	2.999	.004
	LegSAT	-.091	.119	-.491	-.766	.447
	TorsoTAT	.015	.066	.150	.227	.822
	LegSATtoTF	.534	1.909	.135	.280	.781

a. Sex = M

b. Dependent Variable: LnAdp

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Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Sexnumber, Age1, LipidMeds, TorsoTAT, BPMeds, LegSAT, LegSATtoTF[a]		. Enter

a. All requested variables entered.

b. Dependent Variable: Glu

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.381[a]	.145	.096	.47444

a. Predictors: (Constant), Sexnumber, Age1, LipidMeds, TorsoTAT, BPMeds, LegSAT, LegSATtoTF

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	4.668	7	.667	2.963	.007[a]
	Residual	27.461	122	.225		
	Total	32.129	129			

a. Predictors: (Constant), Sexnumber, Age1, LipidMeds, TorsoTAT, BPMeds, LegSAT, LegSATtoTF

b. Dependent Variable: Glu

Coefficients^a

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	3.467	.713		4.866	.000
	Age1	.008	.009	.078	.887	.377
	BPMeds	-.113	.093	-.106	-1.208	.230
	LipidMeds	-.129	.104	-.106	-1.231	.221
	LegSAT	-.057	.034	-.431	-1.693	.093
	TorsoTAT	.066	.026	.520	2.479	.015
	LegSATtoTF	.430	.429	.256	1.002	.318
	Sexnumber	-.037	.117	-.037	-.313	.755

a. Dependent Variable: Glu

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Sexnumber, Age1, LipidMeds, TorsoTAT, BPMeds, LegSATtoTF, LegSAT[a]		. Enter

a. All requested variables entered.

b. Dependent Variable: LnInsulinSensitivity

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.532[a]	.283	.241	.39149

a. Predictors: (Constant), Sexnumber, Age1, LipidMeds, TorsoTAT, BPMeds, LegSATtoTF, LegSAT

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	7.314	7	1.045	6.818	.000[a]
	Residual	18.545	121	.153		
	Total	25.860	128			

a. Predictors: (Constant), Sexnumber, Age1, LipidMeds, TorsoTAT, BPMeds, LegSATtoTF, LegSAT

b. Dependent Variable: LnInsulinSensitivity

Coefficients^a

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	3.095	.586		5.280	.000
	Age1	.004	.007	.046	.559	.577
	BPMeds	.181	.079	.187	2.303	.023
	LipidMeds	.183	.086	.168	2.120	.036
	LegSAT	-.004	.029	-.033	-.141	.888
	TorsoTAT	-.027	.022	-.237	-1.216	.226
	LegSATtoTF	.322	.364	.205	.885	.378
	Sexnumber	-.134	.097	-.149	-1.383	.169

a. Dependent Variable: LnInsulinSensitivity

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Sexnumber, Age1, LipidMeds, TorsoTAT, BPMeds, LegSATtoTF, LegSAT[a]		. Enter

a. All requested variables entered.

b. Dependent Variable: LnTrig

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.404[a]	.164	.117	.42026

a. Predictors: (Constant), Sexnumber, Age1, LipidMeds, TorsoTAT, BPMeds, LegSATtoTF, LegSAT

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	4.387	7	.627	3.548	.002[a]
	Residual	22.430	127	.177		
	Total	26.817	134			

a. Predictors: (Constant), Sexnumber, Age1, LipidMeds, TorsoTAT, BPMeds, LegSATtoTF, LegSAT

b. Dependent Variable: LnTrig

Coefficients^a

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.326	.618		2.147	.034
	Age1	-.013	.008	-.149	-1.754	.082
	BPMeds	.021	.081	.022	.257	.798
	LipidMeds	-.164	.091	-.151	-1.811	.072
	LegSAT	-.058	.030	-.481	-1.932	.056
	TorsoTAT	.047	.023	.412	2.018	.046
	LegSATtoTF	.145	.379	.095	.383	.702
	Sexnumber	-.150	.103	-.166	-1.457	.148

a. Dependent Variable: LnTrig

Regression

Variables Entered/Removed^b

Model	Variables Entered	Variables Removed	Method
1	Sexnumber, Age1, LipidMeds, TorsoTAT, BPMeds, LegSATtoTF, LegSAT^a		. Enter

a. All requested variables entered.

b. Dependent Variable: LnHDL

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.566^a	.320	.283	.20232

a. Predictors: (Constant), Sexnumber, Age1, LipidMeds, TorsoTAT, BPMeds, LegSATtoTF, LegSAT

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2.447	7	.350	8.541	.000^a
	Residual	5.199	127	.041		
	Total	7.646	134			

a. Predictors: (Constant), Sexnumber, Age1, LipidMeds, TorsoTAT, BPMeds, LegSATtoTF, LegSAT

b. Dependent Variable: LnHDL

Coefficients^a

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.290	.297		.977	.331
	Age1	.005	.004	.096	1.253	.212
	BPMeds	-.006	.039	-.012	-.161	.872
	LipidMeds	.070	.044	.120	1.601	.112
	LegSAT	.019	.014	.301	1.343	.182
	TorsoTAT	-.020	.011	-.324	-1.760	.081
	LegSATtoTF	-.051	.182	-.063	-.281	.779
	Sexnumber	-.141	.049	-.294	-2.858	.005

a. Dependent Variable: LnHDL