INVESTIGATING THE HETEROGENEITY IN REGIONAL BODY COMPOSITION, METABOLIC RISK AND RESPONSE TO WEIGHT LOSS AMONG OBESE MEN AND WOMEN

by

PETER M. JANISZEWSKI

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Abstract

While the excess accumulation of abdominal subcutaneous and visceral adipose tissue (abdominal SAT and VAT, respectively), is independently associated with increased health risk, the relative amount of lower body SAT is associated with an improved health profile. In contrast to the established metabolic benefit of reducing VAT and abdominal SAT, the first study in this thesis investigated whether loss of lower-body SAT during diet and/or exercise induced weight-reduction resulted in a deterioration of metabolic profile in a sample of obese men (n = 58) and women (n = 49). After control for potential confounders, reductions in VAT, abdominal and lower body SAT were all associated with improvements in certain metabolic risk factors. However, only reductions in VAT and abdominal SAT remained associated with improvement in risk factors independent of changes in other AT depots.

Recently, it was suggested that a large breast size among women may predict type-II diabetes risk independent of body mass index (BMI) and waist circumference. While the mechanism is unknown, a large breast size may predict health risk by indicating fat deposition in ectopic depots such as VAT or inter-muscular AT (IMAT). The second study investigated the independent associations between breast volume with metabolic risk and regional fat distribution in 92 overweight or obese premenopausal women. Although breast volume was not significantly associated with metabolic risk, after control for age, BMI and waist circumference level, women with the highest breast volume had approximately 1.1 and 1.3 kg more VAT and IMAT, respectively, by comparison to women with the smallest breast volume.

In contrast to the metabolic benefits of weight loss among metabolically-abnormal obese (MAO) individuals, weight loss among metabolically healthy obese (MHO) individuals may be unnecessary or even harmful. In the third study, 63 MHO and 43 MAO men and women
participated in an exercise or diet weight-loss intervention. In response to similar weight loss, MHO and MAO men and women showed significant improvements in insulin sensitivity. However, significant improvements in other metabolic variables (triglycerides, fasting glucose, HDL-cholesterol, and total cholesterol) were only observed among MAO, but not MHO subjects.
Co-Authorship

The co-authors of the manuscripts contained within this thesis are Dr. Robert Ross (Chapter 3-5), Dr. Jennifer L. Kuk (Chapter 3), and Mr. Travis J. Saunders (Chapter 4). Peter M. Janiszewski was responsible for conception of the studies (in collaboration with co-authors), all statistical data analysis, and writing of the manuscripts contained within this thesis. Critical revisions for intellectual content were provided by Dr. Ross, as well as Dr. Kuk and Mr. Saunders.
Acknowledgements

Where do I begin? It all started with a captivating cover of a National Geographic magazine at the Oshawa Public Library back in 2003. This led to an undergraduate thesis, under the supervision of Dr. Earl Noble entitled “Obesity: A Condition of Great Proportions and Great Misconceptions.” Dr. Troy Gregory, who taught me innumerable things during the last year of my undergraduate degree and whose advice still guides me to this day, was a key behind-the-scenes contributor to that thesis. At the end of that work, seeing my keen interest in obesity, Dr. Noble suggested I contact some fellow at Queen’s university by the name of Bob Ross. The rest is history.

Over the past six years, I have had the pleasure to work and play with many individuals who have significantly enriched my graduate school experience. While an individual thank you to everyone who appropriately deserves thanks would add substantial volume to this acknowledgement, a number of people deserve special mention.

During my time in the Ross lab, many graduate students have come and gone, and I am thankful to all of them for the help and more importantly the friendship they provided during my tenure. Lance, Jen, and So Jung made me feel welcome when I first arrived back in 2004. Having Kate go through all the trials and tribulations in parallel with me made the MSc process that much more enjoyable. Jennifer was then, and long after her departure continues to be my other supervisor, and for all her selfless assistance I am forever grateful. After becoming the senior student, Travis, Ashlee, Andrew, and most recently, Morgan and Kaitlyn have joined our lab, and each and every one of them have been absolutely wonderful to work with.

I owe a special thanks to my good friend, Travis, who has helped bring me into the digital age. Travis’ idea to start a no-nonsense blog dealing with all matters of obesity, exercise,
and health has given me a wonderful new means by which to satisfy my incessant need to write about science. This endeavor has given rise to various opportunities, and has become one of the most enjoyable aspects of the past year, and more broadly, of my graduate experience. One day we’ll strike gold with some off-the-wall idea we discussed via one of our extended Gmail chats. Apart from being a great collaborator on the blog, Travis has been a fantastic friend who has provided me with invaluable advice during the most trying of times – often via online chats. Having my close friend there at my defense and the celebration afterwards really made the finale of my graduate experience memorable. I can’t wait to cheer you on during your defense!

Special thanks to all the lovely ladies who have worked in the Ross laboratory over the years. Each and every one of you have at one point or another provided crucial assistance on matters for which I have embarrassingly little aptitude (i.e. using a fax machine, or even a phone). Melinda, Shelley, Amanda, Alison, Paula, and most recently Jenn – you are the most hard-working and yet the nicest people I have ever met. I wish you nothing but the best in your future endeavors.

Before I even set foot on Queen’s campus I already knew Angie Maltby was the most helpful person on the planet, not to mention a mean drummer! I cannot stress enough how many times Angie has helped me out, usually at the very last minute, almost always dealing with some emergency. To this day, she continues to be helpful and available despite the significant growth of our department. If anyone in our department deserves a raise, it is Angie!

Thanks to my rotating gym buddies over the years for ensuring that I practice what I preach, and take time to inject some physical activity into my days. Ryan, Ian, Travis, and Lance – at one point or another you were my spotter on bench-press or a competitor in squash. Thanks sincerely for the much needed catharsis.
Thanks to my close friends Ian and Katie and their son, Teddy who have on many occasions given me a much-needed mini-vacation from the academic bubble in the form of dinners, movie nights, game nights, etc. Despite the sometimes poor movie choices (e.g. Duplicity) I always felt at home and among family when relaxing at your place.

Dr. Bob Ross, in the acknowledgements section of my Master’s thesis, I promised to teach you “a thing or two about a thing or two (obviously not science related)” during my PhD. I hope I have accomplished that goal. You are now in a very elite group of individuals who know about Joe Satriani, and the fact you can now play one of his songs just may be the highlight of my PhD accomplishments. Also, after adding more and more Beatles into my music library over the past few years, I can see you may have been right during our Nirvana versus Beatles debate. Thanks sincerely for all the time you’ve invested in my growth as a researcher and the opportunities that you made available. You have taught me more than you will ever know.

Thanks to my mom and dad for all the unwavering support throughout these past 6 years – you can finally breathe a sigh of relief as it is all over. Nevertheless, be warned that despite the decade of post-secondary education, and the Dr. designation, I just may be reviving my dreams of opening up that fast-food Pierogi and Sausage franchise in Poland I jokingly alluded to back in 2006. You have worked so hard, made tremendous sacrifices, and risked everything to ensure a better future for me. I will never be able to thank you enough for any of this, but I hope that you can see it was all worth it in the end.

And finally, I must thank the one person who provided the most critical piece of my life puzzle over the past 6 years: Mr. David Hasselhoff. Seriously though, Marinka, you have been the most extraordinary counterpart a man could ask for. You were the first person I met when I arrived in Kingston in September 2004, and the moment I saw you I knew my life would never
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Dr. Peter Janiszewski

April 29, 2010
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>adipose tissue</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>DEXA</td>
<td>dual-energy x-ray absorptiometry</td>
</tr>
<tr>
<td>FFA</td>
<td>free fatty acids</td>
</tr>
<tr>
<td>HDL</td>
<td>high-density lipoprotein</td>
</tr>
<tr>
<td>IMAT</td>
<td>inter-muscular adipose tissue</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>L4-L5</td>
<td>Intervertebral space between the fourth and fifth lumbar vertebraeas</td>
</tr>
<tr>
<td>MAO</td>
<td>metabolically abnormal obese</td>
</tr>
<tr>
<td>MHO</td>
<td>metabolically healthy obese</td>
</tr>
<tr>
<td>MONW</td>
<td>metabolically-obese, normal weight</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>OGTT</td>
<td>oral glucose tolerance test</td>
</tr>
<tr>
<td>SAT</td>
<td>subcutaneous adipose tissue</td>
</tr>
<tr>
<td>VAT</td>
<td>visceral adipose tissue</td>
</tr>
<tr>
<td>WHR</td>
<td>waist-to-hip ratio</td>
</tr>
<tr>
<td>VO$_2$max</td>
<td>maximal oxygen consumption</td>
</tr>
</tbody>
</table>
Chapter 1: General Introduction

Although obesity has been a rare condition in the past (1), trends over the past 2 decades indicate a drastic increase in prevalence in most regions of the world (2-4). It is well established that obesity substantially increases the risk of type-2 diabetes (5), hypertension (5), cardiovascular disease (6) and all-cause mortality (7). Nevertheless, it is becoming increasingly apparent that the mere presence of excess body fat does not always indicate an elevated disease risk; nor does its absence guarantee a lack thereof (8). Thus, an ongoing challenge in the obesity field is that of proper clinical classification of health risk, specifically, the identification and treatment prioritization of individuals at highest risk of developing disease and early mortality.

Since the seminal observations of Jean Vague (9), it has become accepted that the location of excess adiposity is a strong determinant of obesity-related health risk. For example, abdominal obesity, and specifically the excess accumulation of visceral adipose tissue (VAT) has emerged as a powerful predictor of type-2 diabetes (10), cardiovascular disease (11), and mortality (12) independent of other fat depots. In accordance with the cross-sectional evidence, the reduction of abdominal obesity and specifically VAT through diet and/or exercise is associated with substantial improvements in obesity-related cardiometabolic risk factors (13, 14). On the other hand, the accumulation of AT in the subcutaneous depot of the lower-body may actually be protective against cardiometabolic risk (15, 16). To that effect, several studies report that after control for abdominal AT and/or VAT, greater levels of lower-body SAT are associated with reduced risk of glucose intolerance, insulin resistance, dyslipidemia, and arterial stiffness (17-23). However, it remains largely unknown whether loss of lower-body SAT during
weight-reduction could lead to deterioration in cardiometabolic profile, and thus increase the risk of type-2 diabetes and cardiovascular disease.

More recently, it has been suggested that women’s breast size may be yet another indicator of obesity-related health risk. In a prospective epidemiological study, a large bra cup size was shown to predict the risk of type-2 diabetes among premenopausal women independent of anthropometric markers of total and abdominal obesity (BMI and waist circumference) (24). However, due to a number of limitations inherent to this study, it remains unclear whether breast size as assessed directly (using criterion measures) is associated with cardiometabolic risk among women independent of already established measures of anthropometry and body composition. In fact, limited evidence suggests that a large breast size may predict cardiometabolic risk by simply acting as a surrogate for excess fat deposition in ectopic depots such as VAT. These issues must be addressed before clinical assessment of breast size for cardiometabolic risk screening in women is advocated (25).

While clearly there exists marked heterogeneity among obese individuals in body fat distribution, cardiometabolic profile, and risk of chronic disease and mortality, leading health authorities currently recommend weight reduction as the primary treatment strategy for all obese patients (26-28). According to these recommendations, every patient exceeding the BMI threshold for obesity, regardless of health status, should be counseled to lose 5-10% of their body weight. However, it is estimated that 30% of obese individuals have no known history of disease and present with a normal cardiometabolic profile (29-31). Termed metabolically healthy obese (MHO), such individuals are insulin sensitive, and exhibit normal blood pressure, blood lipid levels (32, 33) despite their excess adiposity, and are at no greater risk for developing type-2 diabetes or cardiovascular disease than normal weight subjects (30). Accordingly, various
authors have argued that weight reduction among MHO individuals may be unnecessary given their rather normal metabolic profile (34-36). Instead, weight maintenance has been proposed as the clinical strategy of choice among MHO individuals (37).

Further support for the contention that weight loss among otherwise healthy obese subjects may be uncalled for came recently when it was reported in a small study that weight reduction by caloric restriction among MHO women may not only be unnecessary, but may actually increase health risk (38). The notion that weight reduction among a third of obese patients may be needless or counterproductive calls to question the uniform treatments currently recommended for all obese patients (34, 35). Given the potential implications for clinical practice and public health, further inquiry in this area is clearly warranted. Specifically, it remains unclear whether exercise- and diet-induced weight reduction among MHO men and women improves or deteriorates cardiometabolic status.
Chapter 2: Literature Review

2.1.0.0.0 The Obesity Pandemic

As evidenced by the Venus figurines found throughout Europe, human obesity has been documented for at least 25,000 years, although it remained a rare condition for most of human history (1). However, trends over the past 2 decades indicate a drastic increase in obesity prevalence in most regions of the world (2-4). In fact, the prevalence of adult obesity, as defined by a body-mass index (BMI) in excess of 30 kg/m$^2$, has escalated in many countries including Canada (39) and the United States (40) to such a degree that a normal BMI (between 18.5 and 24.9 kg/m$^2$) is no longer the norm, as only a minority of the population fall into this category. Recent estimates in Canada suggest that $4.3$ billion (2.2% of total) of yearly health care expenditures are attributable to obesity (41), while in the United States these figures are truly staggering at $75$ billion per year (approximately 6% of total) (42).

Longitudinal results from the World Health Organization (WHO) Multinational Monitoring of Trends and Determinants of Cardiovascular Disease (MONICA) project, suggest that an increased obesity prevalence is not endemic only to North America (3). Indeed, during a 10-year period from the mid 1980’s to the mid 1990’s, the mean population BMI increased in 16 of the 21 countries assessed including China, Australia, New Zealand and most of Western Europe. Further, while obesity and chronic diseases have historically been a problem exclusive to affluent, developed countries, as of recent years, there has been a growing recognition of an emerging epidemic of obesity in the developing societies of Africa, Asia, and South America (43-45). In many transitioning societies, the emerging epidemic of obesity and associated chronic disease exists alongside the remaining widespread problems of malnutrition and infectious diseases, leading to what has been termed the double burden of disease (44). In fact, it is fairly
common among developing countries to find underweight and overweight persons living in the same household (46), with obese mothers often caring for undernourished children (44).

It is now apparent that most countries of the world, even those which just recently struggled with under-nutrition, and some that continue to do so (44), are experiencing increases in the prevalence of obesity. Current estimates from the International Obesity Task Force suggest that at least 1.1 billion people across the globe are overweight and 312 million of them are obese (47). Thus, the obesity epidemic is more appropriately the obesity pandemic, that is, an epidemic of obesity that has spread throughout most of the civilized world. While the exact causes of the obesity pandemic are still debated, a number of potential factors have been suggested and include increased sedentary lifestyle – particularly a decrease in occupational physical activity (48), increased dietary caloric intake and alterations in diet composition (3, 49), smoking cessation (50), and others (51).

2.2.0.0 Obesity and Health Risk

By the time of Hippocrates, obesity was already recognized as a condition with deleterious health consequences. Indeed, Hippocratic writings state that “Sudden death is more common in those who are naturally fat than in the lean” (52). Since that time numerous studies have reported that overweight and obesity are associated with risk of type-2 diabetes (5), hypertension (5), cardiovascular disease (CVD) (6) and all-cause mortality (7).

Although Adolphe Quetelet first developed the simple ratio of weight to height squared in an effort to portray a constant relationship between weight and height across a population (53), the Quetelet Index, more commonly known today as the body mass index (BMI), was subsequently shown to predict total adiposity with a moderate degree of accuracy (54). Since then, BMI has become the most common, indirect measurement of obesity in large population
studies and in clinical settings. Evidence-based guidelines have been developed to define overweight and obese using BMI cutpoints (27). These guidelines identified a BMI between 25.0-29.9 kg/m$^2$ as overweight, and a BMI greater than or equal to 30.0 kg/m$^2$ as obese. The relationship between increasing BMI and health risk is well portrayed in the seminal work of Calle and colleagues (7) who prospectively studied over one million US men and women for a period of 14 years, and found a graded increase in mortality risk with BMIs greater than 25.0 kg/m$^2$ - a relationship that was independent of gender, race, age and other confounders.

2.3.0.0.0 Obesity: A Heterogeneous Condition

Although the positive association between obesity and disease and mortality is well documented (5, 7, 55), there exist different phenotypes or subtypes of obesity that appear to deviate from this seemingly straightforward relationship. Indeed, the mere presence of excess body fat does not always indicate an elevated disease risk; nor does its absence guarantee a lack thereof (8). That is, individuals within a given BMI category or even with similar amounts of excess body fat can present with vastly different metabolic profiles, and experience varying risk of disease and mortality. Since better understanding of the heterogeneity among obese individuals could impact on clinical treatment decisions (8, 56), it has been repeatedly suggested that obese individuals be characterized beyond simply having a high BMI or excess fat mass. Thus, proper clinical classification of health risk, specifically, the identification and treatment prioritization of individuals at highest risk of developing disease and early mortality, remains an ongoing challenge in the obesity field. Over the years a number of strategies have been developed in an attempt to distinguish high-risk from low-risk phenotypes of obesity. These classification systems and their potential implications are described below.

2.4.0.0.0 Android (Abdominal) Versus Gynoid (Peripheral) Obesity
In the late 1940’s Jean Vague was the first to note the heterogeneity of health profiles among obese individuals, when he observed that the location of excess body weight was an important indicator of obesity-related health risk (9). Through anthropometric measurements Vague defined two unique obesity phenotypes; gynoid obesity, represented by predominantly lower body fat accumulation, and android obesity, of predominantly upper body fat accumulation (Figure 1). While gynoid obesity was found to be a fairly benign condition, android obesity, on the other hand, was associated with premature artheroscleoris, diabetes, gout, and uric calculous disease. This seminal work of Jean Vague has been considerably expanded upon in subsequent years by large studies using anthropometric markers of body fat distribution, and more recently those using sophisticated imaging studies to quantify regional body fat distribution. While a detailed description of the various methodologies used in the quantification of total and regional distribution of body fat is beyond the scope of this review, Table 1 provides a brief overview of the most common methods employed in the field. For further information on methods of body composition measurement, the reader is encouraged to seek out a number of excellent reviews on the topic (57, 58).

2.4.1.0 Evidence from Anthropometric Studies

2.4.1.1 Abdominal Obesity: The High-Risk Phenotype

In the early 1980’s the laboratories of Bjorntorp et al. from Sweden and Kissebah et al. from the US expanded on Vague’s work and showed that for a given degree of obesity, the regional distribution of fat plays an important role in the development of metabolic dysregulation (59, 60). More specifically, they noted that upper body (60) or abdominal obesity (59) was a stronger correlate of fasting insulin, blood glucose and triglyceride levels as well as blood pressure and glucose tolerance than was peripheral obesity. Furthermore, long-term
Figure 1. Android or Abdominal Obesity vs. Gynoid or Peripheral Obesity

Adapted from Jean Vague, 1956

High-risk

Low-risk
### Table 1. Popular methods for quantifying total and/or regional body fat

<table>
<thead>
<tr>
<th>Method</th>
<th>Total body fat measurement capability</th>
<th>Regional body fat measurement capability</th>
<th>Applicability in clinical practice</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Mass Index</td>
<td>Moderate</td>
<td>Low</td>
<td>High</td>
<td>Simple, fast, inexpensive</td>
<td>Does not distinguish muscular from fat</td>
</tr>
<tr>
<td>Waist Circumference</td>
<td>Low</td>
<td>Moderate</td>
<td>High</td>
<td>Simple, fast, characterizes BMI</td>
<td>Does not distinguish visceral from subcutaneous AT</td>
</tr>
<tr>
<td>Hip or Thigh Circumference</td>
<td>Low</td>
<td>Moderate</td>
<td>High</td>
<td>Simple, fast, characterizes BMI</td>
<td>Does not distinguish muscular, subcutaneous, and intermuscular AT</td>
</tr>
<tr>
<td>Skinfolds</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Direct measure of regional subcutaneous AT</td>
<td>Limited use with increasing obesity, no measure of visceral AT</td>
</tr>
<tr>
<td>Bioelectrical Impedance</td>
<td>Moderate</td>
<td>Low</td>
<td>High</td>
<td>Inexpensive, fast, fairly easy</td>
<td>Questionable reliability, does not distinguish AT depots</td>
</tr>
<tr>
<td>Dual-Energy X-Ray Absorptiometry</td>
<td>High</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Accurate and repeatable</td>
<td>Radiation exposure (minimal), does not distinguish AT depots</td>
</tr>
<tr>
<td>Magnetic Resonance Imaging</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>“Gold standard”; very accurate and repeatable; distinguishes different AT depots; non-hazardous</td>
<td>Expensive, data acquisition and analysis time-consuming</td>
</tr>
<tr>
<td>Computed Tomography</td>
<td>Moderate</td>
<td>High</td>
<td>Low</td>
<td>Very accurate and repeatable; distinguishes different AT depots</td>
<td>Radiation exposure; expensive, data analysis time-consuming</td>
</tr>
</tbody>
</table>
longitudinal studies of large cohorts of Swedish men (61) and women (62) demonstrated that abdominal obesity as assessed by the waist-to-hip ratio (WHR) predicted myocardial infarction, stroke, and death independent of age and total obesity (BMI). More recent investigations including a combined population of over 250,000 participants from over 60 countries continue to support the notion that abdominal obesity phenotype, rather than total obesity, is the best predictor of health risk (63-65). For example, the International Day for Evaluation of Abdominal Obesity (IDEA) Study, used data on an astounding 168,000 men and women from 63 countries to examine the relationship between total and abdominal obesity with risk of cardiovascular disease and type-2 diabetes (66). While both overall (BMI) and abdominal obesity independently predicted cardiovascular disease and type-2 diabetes risk in this study, abdominal obesity was a better predictor of both outcomes (OR [95% CI] ranging from 1.90 [1.80 - 2.02] to 3.94 [3.66 - 4.24]), displaying a graded relationship with cardiovascular disease and diabetes at all levels of BMI, even in lean subjects.

While most of the earlier studies used the ratio of waist to hip circumference, the majority of recent studies utilize waist circumference alone as the index of abdominal obesity. Waist circumference has been chosen over WHR as the abdominal measurement of choice due to its closer association with VAT amount (discussed later) and cardiovascular risk (67). Based on this evidence the National Institutes of Health published sex specific waist circumference cut-points to define abdominal obesity (Table 2) (27). Subsequently, it was reported that within each BMI strata, those with abdominal obesity (waist circumference > 102 and 88cm in men and women, respectively) exhibited greater risk of hypertension, type-2 diabetes and dyslipidemia, than those of normal waist circumference values (68). In other words, the measurement of abdominal obesity and total obesity in combination is thought to provide the best assessment of health risk (68-70).
Table 2. Classification of obesity-related disease risk by BMI and waist circumference

<table>
<thead>
<tr>
<th>BMI (kg/m²)</th>
<th>Obesity Class</th>
<th>Men ≤ 102cm</th>
<th>Women ≤ 88cm</th>
<th>Men &gt; 102cm</th>
<th>Women &gt; 88cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;18.5</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Normal</td>
<td>18.5-24.9</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Overweight</td>
<td>25.0-29.9</td>
<td>Increased</td>
<td>High</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>30.0-34.9</td>
<td>I</td>
<td>High</td>
<td>Very High</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35.0-39.9</td>
<td>II</td>
<td>Very High</td>
<td>Very High</td>
<td></td>
</tr>
<tr>
<td>Extreme Obesity</td>
<td>&gt;40.0</td>
<td>III</td>
<td>Extremely High</td>
<td>Extremely High</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from National Institutes of Health; National Heart, Lung, and Blood Institute.
However, a recent consensus statement from the American Diabetes Association, the Obesity Society, and the American Society for Nutrition criticized the clinical utility of waist circumference measurement, suggesting that it is unclear whether waist circumference predicts health risk beyond that explained by BMI and commonly evaluated cardiometabolic risk factors (71). In response, we performed a study using data on 5882 adults from the 1999-2004 National Health and Nutrition Examination Surveys (NHANES), and reported that waist circumference predicted the likelihood of diabetes (OR [95%CI] = 5.03 [2.87–8.83] for high vs low waist circumference) beyond that explained by common cardiometabolic risk factors (i.e. blood pressure, triglycerides, etc.) and BMI (72), thereby reinforcing the unique utility of waist circumference in the clinical identification of high-risk, abdominally obese patients.

2.4.1.2.0 Gynoid Obesity: The Benign Phenotype

Although most studies following up on Jean Vague’s seminal observations focused on the incremental health risk associated with an expanding waistline (or the abdominal obesity phenotype), a number of studies over the years have specifically considered the health consequences of an expanding hip or thigh circumference, in line with Vague’s description of the gynoid or lower-body obesity phenotype (73-81). Interestingly, as summarized in Table 3, in contrast to the positive association between waist circumference and health risk, these studies report that for a given waist circumference or BMI, a larger hip or thigh circumference is actually associated with a lower risk of metabolic dysregulation, cardiovascular disease, type-2 diabetes, and mortality. As a caveat, it must be appreciated that a large hip or thigh circumference is inversely associated with health risk only after statistical consideration of waist circumference or BMI; in simple correlation analyses, a larger hip or thigh circumference is positively associated with health risk, much as waist circumference and BMI (80, 81).
Table 3. Independent inverse association between hip/thigh circumference and health risk (after control for waist circumference or BMI)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample</th>
<th>Dyslipidemia</th>
<th>Glucose/Insulin Dynamics</th>
<th>CVD</th>
<th>T2D</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seidell et al. 1997</td>
<td>66 men, 95 women</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>✓</td>
<td>NA</td>
</tr>
<tr>
<td>Seidell et al. 2001</td>
<td>313 men, 382 women</td>
<td>✓</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Lissner et al. 2001</td>
<td>1405 women</td>
<td>NA</td>
<td>NA</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Snijder et al. 2003</td>
<td>1099 men, 1281 women</td>
<td>NA</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Snijder et al. 2004</td>
<td>3854 men, 4340 women</td>
<td>✓</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Snijder et al. 2004</td>
<td>3818 men, 4582 women</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>✓</td>
<td>NA</td>
</tr>
<tr>
<td>Yusuf et al. 2005</td>
<td>20 310 men, 6788 men</td>
<td>NA</td>
<td>NA</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Rocha et al. 2008</td>
<td>140 women</td>
<td>X</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Heitmann et al. 2009</td>
<td>1436 men, 1380 women</td>
<td>NA</td>
<td>NA</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Checkmark indicates a significant inverse relationship between hip/thigh circumference and a health outcome after statistical control for waist circumference or BMI. In other words, for a given waist circumference or BMI, an increasing hip or thigh circumference is associated with decreased risk of a given health outcome. NA, not assessed. X, no association.
In 1997, Seidell and colleagues first noted that for a given level of obesity, diabetics have smaller hip circumferences compared to non-diabetics, irrespective of their age and lifestyle factors (76). A more recent study investigated the prospective risk of coronary heart disease and premature mortality according to thigh circumference during 10-12 years of follow-up of approximately 2800 men and women in Denmark (74). The authors found that for a given level of BMI and waist circumference, those individuals who had a thigh circumference above approximately 60 cm were at lowest risk of developing heart disease or dying prematurely. These findings are in complete agreement with a prior study of over 1400 Swedish women followed over 24 years, which documented that given similar BMI or waist circumference levels, the women in the highest quartile of hip circumference consistently had lowest risk of diabetes, myocardial infarction, cardiovascular disease, and early mortality in comparison to those with the smallest hip circumference (77). In all, it appears that for a given level of obesity, a large waist circumference is associated with increased health risk, while a large thigh or hip circumference is protective against it.

2.4.2.0.0 Evidence from Body Composition Studies

2.4.2.1.0 Abdominal Subcutaneous and Visceral Adipose Tissue

The association between abdominal obesity and health risk may be explained by the accumulation of fat in two distinct adipose tissue (AT) depots within the abdomen, namely, visceral AT (VAT) and abdominal subcutaneous AT (SAT) (Figure 2). Only with the advent of imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) has the quantification of these depots become possible. In the mid 1980’s, Sparrow et al. were the first to note associations between VAT as assessed by CT and health risk (82). Since then, while a number of studies report that abdominal SAT accumulation is associated with
Figure 2. Distinguishing between abdominal SAT and VAT
Table 4. Positive association between VAT and health risk (after control for abdominal SAT)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample</th>
<th>Dyslipidemia</th>
<th>Glucose/Insulin dynamics</th>
<th>Inflammation</th>
<th>Hypertension</th>
<th>CVD</th>
<th>T2D</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pouliot (1992)</td>
<td>82 men</td>
<td>✓</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Lemieux (1996)</td>
<td>30 women</td>
<td>NA</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Banerji (1997)</td>
<td>32 men, 20 women</td>
<td>NA</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Fujimoto (1999)</td>
<td>175 men</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Boyko (2000)</td>
<td>253 men, 228 women</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>✓</td>
<td>NA</td>
</tr>
<tr>
<td>Brochu (2000)</td>
<td>44 women</td>
<td>NA</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Forouhi (2001)</td>
<td>56 men, 57 women</td>
<td>NA</td>
<td>NA</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Rendell (2001)</td>
<td>55 women</td>
<td>✓</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Janssen (2002)</td>
<td>38 women</td>
<td>✓</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ross (2002)</td>
<td>89 men</td>
<td>NA</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Nieves (2003)</td>
<td>75 men, 121 women</td>
<td>✓</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hayashi (2004)</td>
<td>150 men, 150 women</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Kuk (2006)</td>
<td>291 men</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>✓</td>
</tr>
</tbody>
</table>

Checkmark indicates a significant positive relationship between VAT and a health outcome after statistical control for subcutaneous abdominal AT. In other words, for a given amount of subcutaneous abdominal AT, increasing VAT is associated with increased risk of a given health outcome. NA, not assessed. X, no association.
cardiometabolic risk (83-85), the preponderance of literature suggests that the amount of VAT is the best correlate of dyslipidemia, glucose and insulin dynamics (including insulin resistance), systemic inflammation, incidence of hypertension, cardiovascular disease, type-2 diabetes, and mortality, independent of abdominal SAT (Table 4) – even though it explains only 30% of the variation in health risk (86). This evidence, along with the distinct anatomical location of VAT, in particular its drainage by the portal vein, as well as the relatively unique lipolytic/endocrine activity of visceral adipocytes (discussed below), has led to the notion that VAT accumulation may explain the link between abdominal obesity and health risk (87-90). However, as direct evidence of a causal relationship between VAT accumulation and cardiometabolic risk is scarce, many have questioned the proposed pathogenic role of VAT (91-95).

Although attempts to define a critical cut-point of VAT accumulation beyond which health risk is elevated (akin to those established for BMI and waist circumference) have been reported, to date, no age and sex specific VAT threshold values have been firmly established. In an early attempt to delineate such a VAT cut-point in young Caucasian adults, Despres and Lamarche (96) found that a cross sectional area of VAT at the L4-L5 intervertebral space beyond 100 cm² was associated with deteriorations in metabolic profile and glucose tolerance.

2.4.2.2.0 Lower Body Subcutaneous Adipose Tissue

While the detrimental effect of a large waist circumference is thought to be primarily explained by excess accumulation of VAT, the health protective effect of larger buttocks, hips or thighs has largely been attributed to greater SAT deposition in the lower body. Indeed, as shown in Table 5, numerous body composition studies have shown that for a given amount of abdominal fat (abdominal SAT and VAT), those with greater amounts of lower body or gluteofemoral fat, specifically SAT, are at reduced risk of dyslipidemia, glucose intolerance,
Table 5. Inverse association between lower-body adipose tissue and health risk (after control for total or abdominal adipose tissue)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample</th>
<th>Dyslipidemia</th>
<th>Glucose/Insulin Dynamics</th>
<th>Vascular disease</th>
<th>Inflammation</th>
<th>Blood Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terry et al. 1991</td>
<td>133 men, 130 women</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Williams et al. 1997</td>
<td>224 women</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>X</td>
</tr>
<tr>
<td>Tatsukawa et al. 2000</td>
<td>50 men, 50 women</td>
<td>✓</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Van Pelt et al. 2002</td>
<td>166 women</td>
<td>✓</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Tanko et al. 2003</td>
<td>1356 women</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>NA</td>
<td>X</td>
</tr>
<tr>
<td>Snijder et al. 2004</td>
<td>275 men, 281 women</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Snijder et al. 2004</td>
<td>244 men, 240 women</td>
<td>NA</td>
<td>NA</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ferreira et al. 2004</td>
<td>161 men, 175 women</td>
<td>NA</td>
<td>NA</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Snijder et al. 2005</td>
<td>1019 men, 1087 women</td>
<td>✓</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Vega et al. 2006</td>
<td>873 men, 1061 women</td>
<td>✓</td>
<td>✓</td>
<td>NA</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Rocha et al. 2008</td>
<td>140 women</td>
<td>✓</td>
<td>✓</td>
<td>NA</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Yim et al. 2008</td>
<td>104 men, 215 women</td>
<td>✓</td>
<td>✓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Checkmark indicates a significant inverse relationship between lower-body AT and a health outcome after statistical control for total or abdominal AT. In other words, for a given amount of total or abdominal AT, an increasing lower-body AT is associated with decreased risk of a given health outcome. NA, not assessed. X, no association.
insulin resistance, type-2 diabetes, and arterial stiffness (17-23, 73, 97-101). For example, in a sample of 224 women, Williams et al (23) were the first to show that for a given amount of abdominal SAT and VAT, greater total leg AT (as measured by DEXA) was associated with lower levels of total and LDL-cholesterol and triglycerides. However, as eloquently reported by Goodpaster et al. (102), fat in the lower body can be stored in at least two distinct locations. Approximately 90% of the AT in the lower body is stored in the large SAT depot, whereas the remaining ~10% is stored in AT located beneath a layer of muscle fascia and interspersed between the muscle bellies, in what is often termed intermuscular, interstitial or subfascial AT (Figure 3). Since most early studies in this area utilized DEXA to quantify lower body adiposity, these two AT depots were often not distinguished (17, 18, 20, 21, 97). A subsequent study on over 2100 participants utilized a CT scan of the thigh to delineate these two depots, and found that greater thigh SAT (but not intermuscular AT) was associated with more favorable glucose and lipid levels independent of abdominal SAT and VAT (19). These findings have since been confirmed by at least 2 studies (73, 98). Further, it has been shown that in contrast to the health protective effect of greater lower body SAT, excess lower body intermuscular AT appears to be associated with a deteriorated metabolic profile (98). However, there is an important caveat to the observation of lower body SAT being metabolically protective: lower body SAT is only inversely associated with metabolic parameters after statistical control for total or abdominal adiposity. That is, simple correlation analyses reveal that, just like abdominal SAT and VAT, lower body SAT is positively associated with metabolic risk (19, 21, 23, 83).

Additionally, it must be noted that hip or thigh circumference is also a strong correlate of muscle mass in the lower body, particularly among males (19, 103). Although some have suggested that the health protective effect of a larger hip or thigh circumference may also be explained by greater muscle mass in the lower body (in addition to greater SAT mass), evidence
Figure 3. Distinction between skeletal muscle, subcutaneous and intermuscular adipose tissue in the thigh
supporting this contention is inconsistent (17-19, 99). For example, Snijder et al have
documented in at least 2 separate studies that while lower body AT or SAT is independently
associated with better insulin sensitivity, glucose tolerance, and fasting glucose, lower body lean
or muscle mass shows no independent association with these outcomes (18, 19). Indeed, in a
2006 review of the literature, it was concluded that the association of a large hip circumference
(for a given waist circumference) with lower disease risk “can mostly be explained by larger
peripheral fat mass” (57).

More recently we have also shown that for a given waist circumference, a greater hip or
thigh circumference may not only represent more SAT in the lower body but also more SAT and
thus less VAT in the abdominal region (103). This latter finding is in agreement with the theory
that lower body SAT, and potentially SAT throughout the body, protects against metabolic
complications by acting as a buffer or as a metabolic sink for excess lipid, thereby reducing the
spillover and deposition of lipid in tissues ectopic to SAT, such as VAT, liver, and muscle (15, 16).
Indeed, it has previously been shown that individuals with lower body fat deposition have lower
levels of circulating plasma free fatty acids (FFA) in contrast to individuals with abdominal fat
deposition (104). Thus, it is plausible that the beneficial effect of large stores of lower body SAT
may be partly mediated by less fat storage in the metabolically dangerous VAT as well as other
ectopic fat depots (103). Support for this notion arises upon examination of body composition
among Japanese sumo wrestlers (105) and in patients with multiple symmetric lipomatosis
(106). Both of these cases are characterized by massively expanded SAT depots throughout the
body, but very limited VAT accumulation, concurrent with healthy metabolic profiles.
2.4.3.0.0 How is Abdominal Obesity Different from Gynoid Obesity?

Regional fat accumulation can occur via adipocyte hypertrophy, hyperplasia or both. Abdominal obesity has been characterized primarily by fewer, but hypertrophied adipocytes, meanwhile lower-body obesity is exemplified by numerous and small subcutaneous adipocytes (59, 60, 107-109). This distinction is not trivial given it has been previously postulated that obesity developed through enlargement of existing adipocytes (hypertrophic obesity) is more likely to result in obesity-related complications as compared to obesity developed through the increase in adipocyte number (hyperplastic obesity) (110). Indeed, as initially described some 40 years ago (111), and subsequently corroborated by others (59, 60, 107, 112-114), the metabolic disturbance of excess adiposity is closely tied to the degree of subcutaneous adipocyte hypertrophy. For example, subcutaneous adipocyte size, particularly in the abdominal region has been shown to independently predict severity of insulin resistance (113, 115) and the incidence of type-2 diabetes (113, 116). Additionally, the reduction of SAT adipocyte size either via weight loss treatment or via pharmacological treatment with thiazolidinediones (PPAR-γ agonists) is associated with an improvement in insulin sensitivity and fasting lipid levels (114, 117).

It is suggested that adipocyte hypertrophy may be an indicator of exhaustion of the ability of SAT to buffer excess energy due to impaired adipogenesis (118) and/or adipocyte insulin resistance (119) during energy surplus. Indeed, the absolute number of new adipocytes generated per year has been shown to be 70% lower in individuals with hypertrophic versus hyperplastic obesity (120). Additionally, hypertrophied SAT adipocytes have been reported to correlate with excess deposition of fat into tissues external to SAT such as VAT (108, 114) and the liver (114), but a limited accumulation of lower body SAT. In other words, gynoid obesity may simply represent a phenotype of obesity that can more easily accommodate excess energy,
that is, via hypercellular expansion of the adipose organ, without deleterious consequences (Figure 4). Furthermore, evidence also suggests that adipocytes from the lower body may be functionally different from those in the abdominal region.

2.4.4.0.0 Are Abdominal Adipocytes Different from Lower Body Adipocytes?

2.4.4.1.0 Lipolysis

One of the main functions of adipose tissues throughout the body is the short and long-term storage of excess energy in the form of triglyceride, which can subsequently be liberated from adipocytes in the form of free fatty acids to be used as fuel by other tissues during periods of fasting, starvation and exercise. Interestingly, the rate at which adipocytes may take up or release free-fatty acids (FFA) into the circulation, and thus contribute to the systemic level of plasma FFA, is location dependent.

For example, abdominal SAT is the major determinant of systemic FFA levels, with lipolysis from this depot contributing approximately 70% of all FFA in the circulation (121). Simply put, abdominal subcutaneous adipocytes are much more lipolytically active than are adipocytes in the lower body. In comparison to gluteal subcutaneous adipocytes, abdominal subcutaneous adipocytes have a 4- to 5-fold greater increase in the rate of lipolysis in response to a similar catecholamine stimulation (122), a difference at least partly mediated by the significantly higher (approximately 2-fold) expression of β-adrenergic receptors among abdominal subcutaneous adipocytes (123). Further, the rate of action of hormone-sensitive lipase, the key lipolytic enzyme, appears to be much higher (87%) in abdominal versus gluteal subcutaneous adipocytes (124). Lastly, it has also been observed that gluteal versus abdominal subcutaneous adipocytes are significantly more sensitive to the anti-lipolytic effects of insulin (125), one of the main inhibitory controllers of fat mobilization from adipocytes.
Abdominal obesity is characterized primarily by hypertrophy of adipocytes, meanwhile gynoid obesity is exemplified by adipocyte hyperplasia. Hypertrophied SAT adipocytes lead to FFA spillover into more deleterious depots (VAT, muscle, liver, pancreas, heart), lipotoxicity at these tissues, and consequent metabolic dysregulation.
Although the contribution of VAT lipolysis to systemic FFA levels is quite minimal (~5%) (121), visceral adipocytes may be the most lipolytically active in the body. Indeed, visceral adipocytes show significantly elevated rates of lipolysis upon catecholamine stimulation in comparison to abdominal subcutaneous adipocytes (126), a difference likely mediated by the greater number of β-adrenergic receptors on visceral adipocytes (127). Visceral adipocytes are also less sensitive to the anti-lipolytic effect of insulin (128) as compared to abdominal subcutaneous adipocytes.

2.4.4.2.0 Lipogenesis

The removal of FFA from triglyceride-rich chylomicrons in the circulation and uptake by the adipocyte depends on the expression and activity of lipoprotein lipase, the key regulator of lipogenesis. While most studies document greater lipoprotein lipase activity in gluteal versus abdominal subcutaneous adipocytes (129-131), others have failed to observe a difference (124, 132). The discrepant findings may be partly explained by a gender difference in the region specific expression and activity of lipoprotein lipase, that is, greater activity in the gluteal versus abdominal adipocytes of females but the reverse in the males (132), an effect which may be mediated by the testosterone suppression of gluteal lipoprotein lipase activity in men (133). Finally, in concert with the highest lipolytic rates of all adipocytes, it is apparent that visceral adipocytes exhibit significantly lower expression of lipoprotein lipase in contrast to abdominal subcutaneous adipocytes (134).

2.4.4.3.0 Endocrine Function

In addition to being a passive reservoir of energy, it has become recognized that AT plays an important endocrine role releasing a number of adipose-derived cytokines, or adipokines, which have various downstream metabolic effects. While it is well established that
VAT adipocytes release a greater proportion of pro-inflammatory and pro-thrombotic adipokines, but lesser quantities of insulin-sensitizing adipokines as compared to SAT adipocytes (135, 136), differences in adipokine expression or secretion between lower body versus abdominal subcutaneous adipocytes have yet to be elucidated (137). However, it has been noted that the secretion of adipokines varies directly with the size of the adipocyte, with dominance of proinflammatory adipokines being secreted by hypertrophied adipocytes (138). Thus, it is plausible that the hyperplastic expansion of AT common to gynoid obesity would be mirrored by a more benign or even health-protective adipokine milieu.

In all, it would appear that abdominal subcutaneous and visceral adipocytes contribute to elevated plasma FFA via enhanced lipolysis and blunted lipogenesis. The elevated levels of FFA in the systemic circulation may lead to lipotoxicity in the liver, skeletal muscle, and pancreas and resultant hepatic and peripheral insulin resistance, altered hepatic metabolism, decreased insulin secretion and finally, overt metabolic disturbance (118, 119, 139). Conversely, gluteal adipocytes are more likely to maintain normal plasma FFA levels via enhanced lipogenesis and limited lipolysis, thereby limiting lipotoxicity in various tissues and maintain a normal metabolic profile (Figure 5). Additionally, although less clear, abdominal adipocytes, particularly those in VAT, appear more likely than gluteal adipocytes to contribute to elevated levels of pro-inflammatory and pro-thrombotic adipokines, but reduced levels of protective adipokines.
Figure 5. Functional differences between adipocytes from different regions of the body

Lower body SAT adipocytes

Abdominal SAT adipocytes

VAT adipocytes

Lipolytic Activity

<

Lipogenic Activity

>

Secretion of Prothrombotic/Proinflammatory adipokines

≤

Please refer to text for details.
2.4.5.0.0 Implications of Fat Loss from Different Regions of the Body

2.4.5.1.0 Regional Fat Loss/Redistribution in Disease States

2.4.5.1.1 Cushing’s Syndrome

The loss of lower body SAT in response to chronic glucocorticoid excess, in patients with Cushing’s syndrome is often associated with a redistribution of fat into the abdominal region, and ectopic fat storage in concert with metabolic deterioration (137). The excess cortisol in Cushing’s syndrome is thought to selectively augment lipoprotein lipase activity in abdominal AT but not in lower-body SAT (140), thereby leading to preferential abdominal fat storage, particularly in the VAT depot (141). However, how such a hormonal milieu also results in a loss of lower-body SAT is not well understood. This redistribution of fat from the lower-body to the VAT depots in patients with Cushing’s syndrome is accompanied by a deterioration in metabolic status (142).

2.4.5.1.2 Lipodystrophy

Lipodystrophy encompasses a heterogeneous group of disorders associated with generalized (whole body) or partial (depot specific) lack of AT, which can be inherited (genetic-origin) or acquired (through immunological, inflammatory, or drug-induced means) (143). Not only are patients with lipodystrophy at increased cardiometabolic risk, but the severity of metabolic complications observed is closely related to the extent of their fat loss (144).

For example, approximately 40-50% of HIV patients receiving highly active antiretroviral therapy (HAART) including protease inhibitors develop lipodystrophy (145, 146). This most common form of lipodystrophy is characterized by the gradual loss of SAT from the face, limbs, and the gluteal region (145, 146). The reduction in subcutaneous fat mass occurs via an
unfavorable apoptosis of existing adipocytes (147) combined with a reduced adipocyte
differentiation capacity (148). Concurrent with the gradual loss of SAT, these patients commonly
exhibit a compensatory hypertrophy of VAT (149, 150), as well as ectopic fat deposition in the
liver (151) and muscle (152). Not surprisingly, the large majority of HIV patients with
lipodystrophy exhibit dyslipidemia (146), impaired glucose tolerance (153), hypertension, insulin
resistance and endothelial dysfunction (145). The metabolic complications observed among
lipodystrophic HIV-patients have been shown to be related to increased VAT (150), reduced
lower-body SAT (154) or both (155).

In contrast, acquired partial lipodystrophy (Barraquer-Simons syndrome) is
characterized by gradual AT loss which progresses inferiorly from the face towards the
abdomen, but a spares lower-body SAT (156). In fact, in this form of lipodystrophy, SAT
accumulation in the lower body is often excessive, leading to an exaggerated gynoid fat
distribution (143). Interestingly, possibly due to the presence of functional SAT in the lower
body, plasma FFA levels in these patients are normal and are readily suppressed by insulin (157),
and VAT amount is normal or reduced (156). Accordingly, cardiometabolic disorders are quite
uncommon among these patients in comparison to other types of lipodystrophy (156).

2.4.5.2.0 Regional Fat Loss in Response to Exercise- and/or Diet-Induced Weight Loss

In accordance with the cross-sectional evidence showing a positive association between
abdominal fat amount and metabolic risk, the reduction of abdominal fat, particularly VAT, in
response to diet and or exercise intervention is associated with improvements in various
metabolic markers (13, 14, 158-163). For example, in a study of 40 obese premenopausal
women who underwent dietary weight loss, the decrease in VAT volume was a stronger
correlate of the improvement in plasma glucose and lipid levels compared to the changes in
body weight, BMI, total AT and abdominal SAT (13). In another study examining the effects of
diet or exercise weight loss in a sample of 52 obese men, the degree of VAT reduction was
positively correlated ($r = 0.49$, $P < 0.01$) with improvements in insulin sensitivity, as assessed via
the hyperinsulinemic-euglycemic clamp procedure (14). Similarly, in a weight loss study of 32
obese men and women, Goodpaster et al. (158) found that of all the regional AT depots
quantified, reduction in VAT was the only significant predictor of improvement in insulin
sensitivity. Finally, an independent relationship between VAT reduction and improvement in
insulin sensitivity has also been documented in type-2 diabetics (161, 162).

Although greater relative lower-body SAT deposition may be health protective, the
extrapolation of this cross-sectional finding to suggest that selective loss of lower-body SAT
during weight-reduction may lead to deterioration in cardiometabolic profile counters current
knowledge. In contrast to the subcutaneous adipocyte apoptosis observed in lipodystrophy
(described above), the loss of lower-body SAT among healthy individuals in response to a
negative energy balance (as induced by diet or exercise) is a physiologically distinct
phenomenon, as it occurs via a reduction in adipocyte size, meanwhile the number of
adipocytes remains unchanged (164). In comparison to their larger counterparts, small SAT
adipocytes are more insulin sensitive (165), secrete less prothrombotic cytokines (166), and are
associated with less fat spillover into ectopic tissues (114). While loss of functional adipocytes in
lipodystrophy is metabolically deleterious, the shrinking of adipocytes during negative energy
balance is generally metabolically beneficial (114). In other words, the reduction of lower-body
SAT through diet or exercise in healthy subjects should result in metabolic improvement much
akin to that observed in response to reduction of abdominal AT.
Nevertheless, the only study to date to test this hypothesis reports that independent of alterations in trunk fat, reductions in leg fat are associated with increased metabolic risk after 14 weeks of weight loss (99). Unfortunately, in that study: 1) trunk fat was represented by the combination of abdominal and gluteal AT – distinct AT depots which may carry opposite effects on cardiometabolic risk (as described above); 2) dual-energy x-ray absorptiometry (DEXA) measurement of leg fat did not distinguish between SAT and inter-muscular AT depots, which have independent and possibly opposing effects on cardiometabolic risk (described above); and 3) the analyses did not specifically adjust for changes in VAT and abdominal SAT; and 4) the sample consisted of both pre- and post-menopausal women. Therefore, the limitations inherent to this earlier study confound interpretation and preclude definitive conclusions. Indeed, it remains to be properly studied whether loss of lower-body SAT during exercise or diet induced weight-reduction leads to deterioration in metabolic profile.

2.5.0.0 Breast Size: a Novel Marker of Obesity Heterogeneity in Women

As described above, it is fairly well established that the health risk associated with a given BMI category or a given degree of adiposity is largely dependent on the regional distribution of body fat as measured by anthropometric markers such as waist circumference and hip circumference, or body composition markers such as abdominal VAT and lower body SAT. However, as eloquently reported by Vega and colleagues (86), the various combinations of these anthropometric or body composition markers at best explain only 38% of the variance in any metabolic outcome. Thus, there remains room for elucidation of novel anthropometric or body composition measures that can help explain metabolic risk beyond that achieved by currently established markers.
In a recent study, Ray and colleagues (24) suggested that a new anthropometric marker of health risk among women be considered: breast size. In the prospective epidemiological study, over 92,000 women from the Nurses Health Study II were followed for 10 years. During the follow-up period, 1,844 of the women were diagnosed with type-2 diabetes. The investigators reported that bra cup size at age 20 was a significant predictor of future risk of type-2 diabetes, with women with the largest cup (≥ D cup) size having almost a 5-fold risk of diabetes in contrast to women with the smallest cup size (≤ A cup). Although this association was weakened after further control for BMI, waist circumference, diet, physical activity and other confounders in the analyses, women with a D cup or greater were still at 58% increased risk of developing type-2 diabetes in contrast to women with an A cup or smaller during the 10 years of follow up. Thus, Ray and colleagues (24) concluded that “there may be additional benefit to include breast size in the assessment of risk factors for type-2 diabetes.”

While these findings are intriguing, and the strengths of the study, including a large cohort and meticulously controlled analyses, cannot be discounted, a number of limitations inherent to this study must also be acknowledged. First, in the study of Ray et al., breast size was ascertained via self-recall of bra cup size at age 20 among women who at time of assessment were on average 38.1 years of age. Given that self-report of current anthropometric characteristics is fraught with significant error and influenced by social desirability (167), the assessment of bra cup size via self-report is a clear limitation – one which is further exacerbated by the 18.1 year recall. Additionally, it is suggested that bra cup size may be a poor estimate of breast volume given that an estimated 50% of women do not wear the correct bra cup for their breast size (168). In other words, even if the women in this study accurately recalled and truthfully reported their bra size at age 20, this measure may not have given a precise estimate of actual breast size. Finally, it must also be noted that the women in this study were
predominantly lean or slightly overweight, with average BMIs of 22.4, 24.4, 25.8, and 27.0 kg/m² for women with a ≤ A cup, B cup, C cup, and ≥ D cup, respectively. Indeed, when the authors examined the ability of bra cup size to predict diabetes risk within different strata of BMI, the cut off BMI for the heaviest strata was greater than or equal to a BMI of 23.1 kg/m² – well within the normal BMI category. Additionally, another study investigating the association between breast cup size and breast cancer risk in the same cohort of women, reported a positive relationship between the two variables only in the women with a BMI less than 25 kg/m² (169). Thus, it remains largely unknown what predictive value breast size may provide in determining health risk among women with a BMI greater than 30 kg/m². In light of these limitations, in an adjoining commentary, Sorisky (25) cautioned that it is “too early to say whether breast size will be a meaningful indicator to identify women at increased risk for type-2 diabetes.”

2.5.1.0.0 Breast Size and Metabolic Risk: Potential Mechanisms

It is currently unknown how a large breast size, independent of overall adiposity, predisposes women to metabolic risk. Although it has been postulated that the primary mediating mechanism between breast size and diabetes risk might be early menarche and associated metabolic disturbance, different lines of evidence suggest that large breasts may also act as a surrogate for a deleterious body fat distribution.

2.5.1.1.0 Precocious Puberty and Early Insulin Resistance

It has been shown that bra cup size among adult women may be an indicator of precocious, or early onset, puberty (24). In addition to the well established relationship between high adiposity and early pubertal development (170), childhood-onset hyperinsulinemia and insulin resistance also often precede precocious puberty among female adolescents (171). Additionally, girls who experience precocious puberty carry their deleterious metabolic profiles,
including insulin resistance and elevated blood pressure, throughout adolescence and into early adulthood (172). Most recently, it has been revealed that early age at menarche is associated with an increased risk of type-2 diabetes (173), as well as cardiovascular disease and related mortality in adulthood (174), independent of total adiposity. Thus, it is plausible that for a given level of obesity, women with larger breasts may have had an earlier onset of puberty, high adiposity in childhood, as well as an early onset of metabolic abnormalities – all of which may predispose to development of type-2 diabetes and cardiovascular disease.

2.5.1.2.0 Disordered Fat Storage

2.5.1.2.1 Insights from Women with HIV-Associated Lipodystrophy

As described above, the most common form of lipodystrophy occurs in HIV patients receiving highly active antiretroviral therapy (HAART) which includes protease inhibitors. This type of lipodystrophy is characterized by the gradual loss of peripheral SAT (via apoptosis of existing adipocytes (147) and a reduced adipocyte differentiation (148)), but a compensatory increase in VAT and intermuscular AT (145, 146, 149, 150, 175). Paradoxically, in addition to the peripheral loss of SAT and the augmented ectopic fat storage, HIV positive women who undergo HAART also often exhibit a significant enlargement in breast size (176-179). For example, in the study by Dong et al. (177), of the 21 HIV-positive women on HAART who expressed concern regarding changes in body habitus, 15 noticed a significant increase in breast size. Upon clinical examination of these women, the authors reported that the “peripheral fat wasting is associated with massive accumulation of fat over the abdomen as well as the breasts” (177). Although some have postulated on potential mechanisms (180), it remains largely unknown how HAART among HIV positive women can cause a simultaneous loss of peripheral SAT in concert with excess accumulation of AT in the breasts.
2.5.1.2.2 Insights from Adipose Tissue Liposuction

Additional evidence of a connection between limited peripheral SAT, augmented ectopic fat deposition and increased breast size comes from follow-up studies investigating long-term sequelae of cosmetic liposuction of SAT. In recent years liposuction has become the most popular aesthetic surgical procedure in the United States (181). Due to the improvement in liposuction techniques, it is now possible to remove more than 10 l of AT, through a process called mega-liposculpture (182). Interestingly, it has been reported that approximately 30-50% of women undergoing liposuction of SAT from the hips, thighs, or abdomen present with a paradoxical enlargement of breast size of at least one cup (183-185) as well as an increase in VAT accumulation post-surgery (186). For example, in a retrospective analysis of women who had undergone SAT liposuction at a single center, 25 of the 73 women assessed (34%) experienced an increase in breast size (185). Two factors which are correlated with a greater likelihood of breast enlargement post liposuction include the removal of a large quantity of AT and liposuction from the gluteofemoral and abdominal region (184, 185). These findings support the notion of a liposuction-induced compensatory recovery of body fat that occurs through enlargement of non-excised AT mass, rather than regrowth of excised AT – a finding that has been consistent across numerous animal studies (187).

Overall, it appears that breast size and storage of fat in ectopic depots such as VAT often increase in concert as a potential compensatory mechanism when peripheral SAT stores become limited (either via surgical removal or through apoptosis). Thus, a large breast size in otherwise healthy women may be an indicator of dysfunctional or limited peripheral SAT, and by extension, the excess storage of energy in ectopic and metabolically deleterious depots –
themselves, strong predictors of metabolic dysregulation. However, this hypothesis remains to be tested.

2.5.2.0.0 Methods for Quantifying Breast Size

Since breast size is not a commonly measured outcome, in contrast to the study of breast tissue quality, there currently exists no gold standard method of breast size quantification. Indeed, in many of the studies discussed above (24, 184, 185), breast size was most frequently assessed via a simple self-report of bra cup size. However, if actual breast tissue volume is the outcome of interest, bra cup size may be too unreliable of a measure given that an estimated 50% of women do not wear the correct bra cup for their breast size (168). In one of the earliest studies to directly quantify breast volume, Campagne and colleagues (188) used fast setting plaster bandages applied directly to the chest of participants to produce a plaster cast which could subsequently be used to determine the volume of each breast. More recent attempts at quantifying breast volume have utilized radiographic imaging techniques, primarily MRI (189-191). Hussain and colleagues (189) have developed an MRI protocol which includes the placement of the breasts into separate coils with the subject lying prone inside the MRI system. In contrast to scanning the entire breast with contiguous images (no spaces between images), the authors found that 3, 5, and 10 sequential but spaced axial images of the breasts were associated with a 10%, 5%, and 3% error in breast volume estimation. This study also noted the importance of menstrual cycle stage on the estimate of breast volume, as it was shown to vary by 13.6%, peaking pre-menses and achieving the lowest volume during ovulation. Nevertheless, there currently exist no studies investigating the relationship between directly quantified breast tissue volume and metabolic risk.
2.6.0.0.0 Metabolically-Healthy Versus Metabolically Abnormal Obesity

In contrast to the application of anthropometric and body composition measures to delineate the high-risk from the low-risk obese individual (discussed above), a different approach for distinguishing high versus low risk forms of obesity involves the direct assessment of metabolic status. Credit for the first observation of the heterogeneity in health profile among obese individuals is often given to Vague (9), the originator of the android versus the gynoid obesity phenotypes (discussed above). In 1965, in an investigation of factory workers, Albrink and Meigs similarly noted that “many obese men had normal triglycerides” (192). In 1973, after reviewing available evidence on the relationship between obesity and heart attacks, Keyes (36) suggested that while “gross” obesity was obviously associated with health risk, the evidence base for an association between moderate levels of excess weight and heart attacks was rather tenuous. In 1980, Andres (193) drew similar conclusions regarding the impact of obesity on mortality risk, concluding that “major population studies of obesity and mortality fail to show that overall obesity leads to greater risk.” In response to the accumulating evidence, Sims (36) included the designation of “healthy obese” subtype in his classification of obesity in 1982, thereby first identifying a unique subset of obese individuals that appear to be at least partially protected from the development of the cardiometabolic disturbances generally attributed to obesity (Figure 6). Indeed, numerous studies have shown that despite an obese state, many individuals have high insulin sensitivity as well as normal glucose tolerance, blood lipid levels, and blood pressure (32, 33) and a favourable cytokine profile (194). According to prospective evidence, these individuals, described commonly as metabolically healthy obese (MHO), appear to be at no greater risk for developing type-2 diabetes or cardiovascular disease than are normal weight subjects (30, 195). Specifically, in a 13 year follow up of 1824 men, St-Pierre and colleagues (195) concluded that in contrast to healthy normal weight men, obese men had an
Figure 6. Metabolically healthy vs. metabolically abnormal obesity

**Metabolically Abnormal Obese**
- ~70% of obese individuals

Features:
- High BMI
- High Fat Mass
- Low Insulin Sensitivity
- High Triglycerides
- Low HDL-cholesterol

Potential Mechanisms:
- High fat storage in VAT, liver, muscle
- Late-onset obesity (adulthood)
- Physically inactive
- Caucasian bias

**Metabolically Healthy Obese**
- ~30% of obese individuals

Features:
- High BMI
- High Fat Mass
- High Insulin Sensitivity
- Low Triglycerides
- High HDL-cholesterol

Potential Mechanisms:
- Low fat storage in VAT, liver, muscle
- Early-onset obesity (childhood)
- Physically active
- Black bias

Adapted from Karelis et al. 2004
elevated risk of ischemic heart disease only when their obesity was accompanied by the presence of elevated cardiometabolic risk factors.

Recent estimates suggest that anywhere between 6-38.4 % of obese individuals can be classified as MHO (29-31, 196, 197), that is, void of the common metabolic complications of obesity. The large variation in estimations of MHO prevalence may be explained by the inconsistent criteria used to define or categorize MHO versus metabolically abnormal obese (MAO). Despite the knowledge of the MHO phenotype for close to 30 years, there currently exist no established criteria for the definition of MHO individuals (35). Some have used arbitrary cut-points of insulin sensitivity (31, 33, 198), cardiometabolic risk factor clustering (30, 31), or the complete absence of any metabolic aberration (29, 197) to delineate MHO from MAO individuals. For example, Kuk and Ardern (197) recently reported that the prevalence of MHO individuals in a large representative sample of the US can vary by 6-fold, depending on the definition used. One of the most common definitions of MHO is the presence of obesity without a clustering of cardiometabolic risk factors (i.e. ≤ 2 risk factors, or absence of metabolic syndrome as per established criteria). According to this definition, it is estimated that approximately 1 in 3 obese individuals are MHO (30, 31, 197).

2.6.1.0.0 What Differentiates Metabolically-Healthy from Metabolically-Abnormal Obese Individuals?

2.6.1.1.0 Body Composition

As a plausible mechanism for the normal cardiometabolic profile, it has been reported that for the same BMI, MHO subjects tend to have a lower waist circumference (31), and specifically less VAT accumulation (33, 199) in contrast to MAO individuals, therefore closely resembling the metabolically-benign gynoid obesity phenotype (9) (described above). Indeed,
one study showed that despite similar levels of total body fat, MHO women had 49% less VAT than MAO women (33). Nevertheless, it should be noted that in this study the level of VAT even among the MHO individuals was still considerably high (141 ± 51 cm^2), that is, above the cut-point previously suggested for defining an elevated VAT level (200). Additionally, the finding of low VAT among MHO individuals could not be corroborated by a significantly larger follow-up study (198). In contrast, this latter study reported significantly less fat storage in ectopic depots such as liver and muscle in MHO versus MAO men and women (198). However, although these findings suggest a clear parallel between the MHO phenotype with that of gynoid obesity, or hyperplastic obesity (as described above) there currently exists no evidence for greater fat storage in the subcutaneous AT among MHO, particularly in the lower body (33, 198). Overall, while the evidence remains scarce, it appears that for a given level of adiposity, MHO individuals may be less prone to fat storage in the metabolically dangerous and ectopic depots such as VAT, liver and muscle.

2.6.1.2.0 Age of Obesity Onset

Other defining characteristics of the MHO phenotype, in contrast to obese individuals with cardiometabolic risk, include an earlier onset of obesity as well as greater physical activity levels. For example, Brochu et al (33) noted that a large proportion of MHO women (48%) reported an early onset of obesity (between 13-19 yr of age). In this regard, it is interesting to note that childhood-onset obesity is marked by a combination of adipocyte hyperplasia and hypertrophy, while adult-onset obesity is accomplished primarily via adipocyte hypertrophy (201). Indeed, some have suggested that the capacity to grow new adipocytes via differentiation of pre-adipocytes decreases progressively with age (202). This again suggests a parallel between
the MHO phenotype and that of gynoid obesity, which is thought to occur predominantly via the metabolically safer hyperplasia of adipocytes.

### 2.6.1.3.0 Others

While the study of Brochu et al (33) found no difference in resting metabolic rate or physical activity levels between MHO and MAO women, others reported a significant association between self-report levels of leisure-time physical activity and the MHO phenotype (31). Conversely, a difference in cardiorespiratory fitness between MHO and MAO subjects has not been observed (198). Lastly, the only study to consider the influence of race on the likelihood of being MHO versus MAO, suggests that obese blacks are 18% more likely to be MHO in contrast to white obese individuals (31).

### 2.6.2.0.0 Should Metabolically-Healthy Obese Individuals Lose Weight?

Given that MHO individuals appear to be largely void of the health risks commonly attributed to individuals of excess weight, it has often been questioned whether obese individuals who are otherwise healthy should be counseled to lose weight (8, 37). For example, Sims (36), the originator of the MHO concept, previously suggested that attempts to reduce the weight of MHO individuals via diet and/or exercise intervention may be counterproductive or even deleterious, stating that MHO individuals “should not be urged to achieve a normal weight.” He went on to argue that the poor record of weight loss and weight loss maintenance among obese individuals may be driven by the strong tendency of MHO individuals to regain lost weight. McGlaughlin et al (203) suggested that since not all obese individuals are at similar risk for developing diabetes and cardiovascular disease, intensive weight-loss interventions should only be provided to the insulin-resistant, or metabolically abnormal, subset of the obese population. These authors further extend their argument by stating that only metabolically
abnormal obese individuals can benefit from weight loss (203). Most recently, in describing a novel clinical staging system for obesity, Sharma and Kushner argued that weight maintenance, rather than weight loss, should be the clinical strategy of choice when dealing with obese individuals who are otherwise healthy (37).

Although scarce, existing evidence appears to support the general notion that weight-loss among MHO individuals may in fact be needless or even counterproductive. For example, in one small study, 24 women were divided into insulin sensitive or insulin resistant groups (that did not differ on other metabolic outcomes) and underwent 4 months of weight loss treatment via caloric restriction combined with Sibutramine administration (204). While the insulin resistant obese women improved their insulin sensitivity, no change was observed among the insulin sensitive obese women. These early results are consistent with more recent findings of Shin et al (199), who specifically investigated the effect of 3 months caloric restriction (300kcal/day) on MHO versus MAO women, and reported no significant improvement in any of the cardiometabolic outcomes measured among the MHO women. Most recently, Karelis et al. (38) studied the effect of a 6 month diet intervention (500-800 kcal/day) in postmenopausal MHO versus MAO women (highest vs. lower quartile of insulin sensitivity). While the MAO women demonstrated a 26.1% improvement in insulin sensitivity, in response to the same intervention and similar magnitude of weight loss, the MHO women had a surprising 13 % deterioration in insulin sensitivity (38). However, the authors of this study acknowledge that the attenuation in insulin sensitivity in response to a reduction in weight, and more specifically fat mass, is counter-intuitive and lacks a plausible physiological mechanism (38).

The notion that weight reduction among a third of obese patients may be unnecessary calls to question the uniform treatments currently recommended for all obese patients, which
suggest that all individuals exceeding a BMI of 30 kg/m² should lose 5-10% of body weight (Figure 7) (34, 35). Given the potential implications for clinical practice and public health, further inquiry in this area is clearly warranted. Specifically, it remains unclear whether exercise- and diet-induced weight reduction among MHO men and women improves or deteriorates cardiometabolic status.

2.6.3.0.0 Are Metabolically-Healthy Obese Individuals Really Healthy?

While MHO individuals may be at lower relative risk of metabolic disturbance and morbidity in comparison to MAO individuals, emerging evidence suggests their health may not be optimal. In fact, a number of studies now suggest that the health status of MHO individuals lies somewhere between that of healthy lean individuals and MAO individuals. These recent findings question the proposed recommendation that MHO individuals should not lose weight via lifestyle intervention.

For example, it has been reported that despite their normal metabolic profiles MHO individuals may show signs of subclinical vascular disease (205). Specifically, in comparison to lean and healthy women matched on age and metabolic status, MHO women were shown to have a significantly greater intima media thickness of the common carotid artery as well as a poorer endothelial function (reduced flow-mediated dilatation). Further, although Stefan et al. (198) did not find a significant difference in carotid artery intima media thickness, the MHO individuals in this study were at greater risk of excess fat deposition in the liver as compared to their lean and healthy counterparts, despite identical metabolic profiles.

More recently, two separate studies have cast uncertainty on the prior observation that MHO individuals are at no greater prospective health risk than healthy lean individuals (197, 206). Using approximately 9 years of follow-up data on over 6000 men and women, Kuk and
Figure 7. Simplified obesity treatment algorithm

Adapted from National Institutes of Health, National Heart, Lung, and Blood Institutes (1998)
Ardern (197) reported that MHO and MAO individuals were at similarly elevated risk of mortality in comparison to metabolically healthy and lean individuals. These findings were then corroborated by Arnlov et al who studied the risk of mortality and cardiovascular events over 30 years of follow-up in over 1700 Swedish men (206). Overall, this emerging evidence base suggests that while many obese individuals may be metabolically healthy, and thus at less risk than their MAO counterparts, they may still be at greater risk of disease and mortality in comparison to those who are metabolically healthy as well as lean. Such notions suggest that, in contrast to prior assertions, MHO individuals should be counseled to reduce body weight to further improve their health status.

2.7.0.0.0 Summary

The prevalence of obesity is increasing globally, with fewer and fewer regions of the world remaining unaffected by the problem. As obesity is known to substantially increase the risk of various diseases and premature mortality, our collectively expanding waistlines present a major global public health problem. An ongoing challenge in the field is that of proper clinical classification of health risk among obese individuals, specifically, the identification and treatment prioritization of obese individuals at highest risk of developing disease and early mortality. Indeed, as discussed in detail above, it is apparent that the mere presence of excess body fat does not always indicate an elevated disease risk; nor does its absence guarantee a lack thereof.

However, a complete understanding of the inherent heterogeneity among obese individuals has not been achieved as a number of important questions remain unanswered. Is the loss of body fat from the metabolically-healthy lower body associated with an exacerbation in metabolic health? Independent of other measures, do large breasts define obese women
who may be at higher metabolic risk due to excess fat storage in metabolically dangerous depots? Does weight loss among otherwise healthy obese individuals result in no appreciable benefit or even a worsening in metabolic status? The following three studies will examine these pertinent questions. The findings from these studies may have important implications for the clinical classification of health risk among obese individuals, and may improve our understanding of the effect of regional fat loss on health risk as well as the effect of weight loss interventions among different subtypes of obesity.
Chapter 3: Manuscript 1

Is the reduction of lower body subcutaneous adipose tissue associated with elevations in risk factors for diabetes and cardiovascular disease?

This manuscript has been published in the journal *Diabetologia*. The co-authors of this manuscript are Jennifer L. Kuk and Robert Ross. This work was supported in part by the Canadian Institutes of Health Research doctoral award to Peter M. Janiszewski and the Canadian Institutes of Health Research to Robert Ross (MT13448).
ABSTRACT

Background: Since the accumulation of lower-body subcutaneous adipose tissue (SAT) is associated with decreased cardiometabolic risk, we evaluated whether reductions in lower-body SAT independent of changes in VAT and abdominal SAT are associated with elevations in diabetes and cardiovascular disease risk factors.

Methods: Overweight and obese men (n = 58) and premenopausal women (n = 49) with elevated cardiometabolic risk underwent 3 months of diet and/or exercise induced weight-loss treatment; regional body composition assessment by magnetic resonance imaging (MRI); and cardiometabolic risk assessment including an OGTT.

Results: After control for potential confounders, reductions in VAT, abdominal and lower-body SAT were all associated with improvements in selective cardiometabolic risk factors, including fasting glucose levels, lipid status and OGTT glucose and insulin. Independent of changes in the other AT depots, reductions in VAT and abdominal SAT, but not lower-body SAT, remained associated with improvement in fasting glucose levels, glucose tolerance and lipid status.

Conclusion: Among overweight and obese adults with increased cardiometabolic risk, the selective reduction of lower-body SAT is not associated with elevations in risk factors for diabetes and cardiovascular disease. Thus, the reduction of excess AT conveys health benefit regardless of origin.
INTRODUCTION

Abdominal adiposity is a strong predictor of type-2 diabetes and cardiovascular disease risk independent of total obesity (65, 72). Specifically, VAT has emerged as a powerful predictor of type-2 diabetes (10), cardiovascular disease (11), and mortality (12) independent of other fat depots. In accordance with the cross-sectional evidence, the reduction of abdominal obesity and specifically VAT through diet and/or exercise is associated with substantial improvements in obesity-related cardiometabolic risk factors (13, 14).

Conversely, lower-body SAT deposition may actually be protective against cardiometabolic risk (15, 16). To that effect, several studies report that after control for abdominal AT and/or VAT, greater levels of lower-body SAT are associated with reduced risk of glucose intolerance, insulin resistance, dyslipidemia, and arterial stiffness (17-23). However, extrapolation of this notion to suggest that, controlled for changes in abdominal adiposity, reductions in lower-body SAT during weight-reduction may lead to deterioration in cardiometabolic profile, and thus increase the risk of type-2 diabetes and cardiovascular disease, counters current knowledge. Indeed, during negative energy balance, SAT throughout the body decreases in mass due to a reduction in adipocyte size (164). This reduction in adipocytes size may lead to improved insulin sensitivity (165), reduced secretion of prothrombotic cytokines (166), reduced plasma triglyceride levels, as well as decreased ectopic fat storage in the liver and VAT (114). These observations suggest that, rather than being detrimental, the reduction of lower-body SAT stores should actually improve cardiometabolic profile.

Nevertheless, Okura et al. report that independent of alterations in trunk fat, reductions in leg fat are associated with increased cardiometabolic risk after 14 weeks of weight loss (99). Unfortunately, in that study: 1) trunk fat was represented by the combination of abdominal and
gluteal AT – distinct AT depots which may carry opposite effects on cardiometabolic risk (102); 2) dual-energy x-ray absorptiometry (DEXA) measurement of leg fat did not distinguish between SAT and inter-muscular AT depots, which have independent and possibly opposing effects on cardiometabolic risk (102); 3) the analyses did not specifically adjust for changes in VAT and abdominal SAT; and 4) the sample consisted of both pre- and post-menopausal women. Therefore, the limitations inherent to this earlier study confound interpretation and preclude definitive conclusions.

In response, we investigated the independent effects of reductions in VAT, and abdominal SAT and lower-body SAT on changes in risk factors for type-2 diabetes and cardiovascular disease in response to diet and/or exercise interventions in a sample of overweight or obese men and premenopausal women with elevated cardiometabolic risk.
METHODS

Subjects

Subjects for this study included 58 Caucasian men and 49 Caucasian premenopausal women without overt disease who were recruited from the general public and participated in previously published exercise and/or diet-induced weight loss studies at Queen’s University (Kingston, Canada) (14, 207-209). Methodological details of these individual studies are previously reported (14, 207-209). Briefly, a total of 33 men and 38 women were recruited to participate in a study on the effects of diet and resistance and aerobic exercise on body composition and metabolic status (207, 208). In this study, within each gender, subjects were randomized to a diet-only, diet plus resistance exercise, or a diet plus aerobic exercise group (see “Interventions” below for further assignment information). Additionally, a total of 52 men and 54 women participated in 2 other studies (1 in each gender) comparing the effect of diet and aerobic exercise-induced weight loss on regional body composition and metabolic status (14, 209). In these studies, within each gender, subjects were randomized to a control, diet-induced weight loss, exercise-induced weight loss, or an exercise without weight loss group (see “Interventions” below for further assignment information). The inclusion criteria for these studies were: a body mass index (BMI) > 27 kg/m$^2$ and a waist circumference > 102 cm or a waist:hip circumference ratio (WHR) > 0.95 in the men, and (BMI) > 27 kg/m$^2$ and a waist circumference > 88 cm or a waist:hip circumference ratio (WHR) > 0.85 in the women. Additionally, all subjects were weight stable (± 2 kg) for at least 6 months prior to study, were nonsmokers who consumed an average of fewer than two alcoholic beverages per day, had a sedentary lifestyle and were not taking medications known to influence the cardiometabolic risk factors measured. All participants gave informed consent for their respective studies before
participation in accordance with the ethical guidelines set by Queen’s University (See Appendix 1 and 2 for more details).

Subjects for the current analysis included 58 men and 49 women from these studies who were overweight or obese (body mass index [BMI] ≥ 27 kg/m²) and were at elevated cardiometabolic risk at baseline. Elevated cardiometabolic risk was defined by the presence of one or more of the following: impaired fasting glucose, impaired glucose tolerance, high or borderline high triglycerides, very high, high or borderline high low-density lipoprotein cholesterol (LDL-C), low high-density lipoprotein cholesterol (HDL-C), and high or borderline high total cholesterol, as defined according to current clinical guidelines (210, 211).

Interventions

Thirty one of the 58 men were randomly assigned to a diet-only (DO; n = 10), diet plus resistance exercise (DR; n = 10), or diet plus aerobic exercise (DA; n = 11) program designed to induce a daily 1000-1250 kcal energy deficit for 16 weeks, described in detail elsewhere (208). The remaining 27 men were randomly assigned to a diet (n= 13) or exercise (n = 14) program designed to induce a daily 700 kcal energy deficit for 12 weeks (14).

Twenty three of the 49 women were randomly assigned to a DO (n = 8), DR (n = 8), and DA (n = 7) program aimed at inducing a daily 1000-1200 kcal energy deficit for 16 weeks (207). The remaining 26 women were randomly assigned to a diet (n = 13) or exercise (n = 13) program aimed at inducing a 500 kcal/day energy deficit for 14 weeks (209).

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 using a wall-mounted stadiometer. Waist circumference
was taken at the level of the last rib to the nearest 0.1 cm after a normal expiration. (Please refer to Appendix 5 for further details)

Maximal Oxygen Consumption

Maximal oxygen consumption (VO$_2$max) was determined by using a graded treadmill test and standard open-circuit spirometry techniques (Sensor-Medics, Yorba Linda, California). (Please refer to Appendix 4 for further details).

Measurement of total and regional fat and skeletal muscle by magnetic resonance imaging

Whole-body (41 to 47 equidistant images) magnetic resonance imaging (MRI) data was obtained with a General Electric, 1.5 Tesla magnet using a procedure described in detail elsewhere (208). The MRI data was analyzed with a specially designed image analysis software (Tomovision Inc, Montreal, Canada) using an established protocol (208, 212).

Total AT and skeletal muscle (SM) volume were determined using all 41 to 47 images. The image at 5 cm below L4-L5 was used to divide the upper and lower body. VAT and abdominal SAT were calculated using the five images extending from 5 cm below to 15 cm above L4-L5. AT and SM volumes (L) were converted to mass units (kg) using assumed constant densities (0.92 kg/L and 1.04 kg/L, respectively) (213). (Please refer to Appendix 3 for further details)

Metabolic risk factors

Blood samples used to determine fasting lipid and lipoprotein values were obtained in the morning after a 12- to 14-hour overnight fast. Serum total cholesterol and triglyceride levels were determined using standard techniques. High-density lipoprotein cholesterol was assayed after isoelectric-polyanionic precipitation. The LDL cholesterol was subsequently determined using the Friedewald equation:
LDL cholesterol = cholesterol – [HDL cholesterol + (0.46 x triglycerides)].

Before and approximately 6 days after treatment, a 2 hour, 75g OGTT was administered the morning after an overnight fast. Blood samples were collected from the antecubital vein at 0, 60, and 120 minutes. Glucose was measured using an automated glucose analyzer (YSI), and insulin was measured by a radioimmunoassay kit (Intermedico, Toronto, Canada). The glucose and insulin area under the curve (AUC) was determined by using a trapezoid model (214).

(Please refer to Appendix 6 for further details on the OGTT).

Statistical analyses

Baseline differences in anthropometric, MRI, and cardiometabolic risk factors between males and females were assessed using independent samples Student’s t-test. Changes in variables in response to treatment (collapsed across treatment type) were assessed using paired t-tests. Linear regression analyses were performed to determine the univariate and multivariate relationships between changes in regional fat depots (abdominal SAT, VAT, and lower-body SAT) and cardiometabolic risk factors with control for confounders: gender, age, type of weight-loss treatment, and change in VO₂max. To create the most parsimonious model, non-significant confounder terms were removed from the model. All non-normally distributed variables were normalized prior to analysis using transformations. All statistical procedures were performed using SPSS 15.0 software.
RESULTS

Details of exercise and diet adherence are described in detail elsewhere (14, 207-209). Briefly, the subject attendance of the exercise programs ranged between 92-98 % in the men and 92-96% in the women. Among the men, the calculated average diet-induced energy deficits among the different study groups ranged from 662 - 1147 kcal/day. Among the women, the calculated average diet-induced energy deficits among the different study groups ranged from 400 - 1299 kcal/day.

The pre- and post-treatment values for the anthropometric, body composition, cardiometabolic risk factors, and VO$_2$max in men and women are presented in Table 1. At baseline, the men were older and had higher values for body weight, waist circumference, total and lower-body skeletal muscle, VAT, and VO$_2$max, but lower values for total and lower-body total AT, total, lower-body and abdominal SAT (P < 0.05). The values for all cardiometabolic risk factors (p > 0.05) with the exception of lower HDL-cholesterol in the men (P < 0.05) were not different between genders. The prevalence of the various cardiometabolic risk factors in the men and women at baseline is presented in Table 2. As shown in Figure 1, while a significant positive association was present between abdominal and lower-body SAT in both men and women (P < 0.05), neither of these depots were significantly associated with VAT (P > 0.05).

Both the men and women had significantly reduced weight, body mass index, waist circumference, whole body, abdominal and lower-body total and subcutaneous AT, VAT and total skeletal muscle mass, but increased VO$_2$max post-treatment (P < 0.05). While the men also had less lower-body skeletal muscle mass post-treatment (P < 0.05), the women showed no change from baseline (P > 0.05). With the exception of HDL-cholesterol (P > 0.05 in both genders), significant improvements in all cardiometabolic risk factors were observed post-
treatment in both genders, including reduction in OGTT glucose and insulin, fasting plasma glucose and insulin, triglycerides, and total and LDL-cholesterol (P < 0.05).

As shown in Table 3, after control for gender and age, of the three depots (VAT, abdominal and lower-body SAT), only VAT was a significant positive correlate of plasma insulin, TG, OGTT glucose (P < 0.05) and plasma glucose levels (P = 0.06), but a negative correlate of HDL-cholesterol level (P < 0.05).

Independent of the other AT depots, VAT was a significant positive correlate of plasma glucose and insulin, OGTT glucose and insulin (P < 0.05) and TG (P = 0.06). In the same models, abdominal SAT was a significant negative correlate of HDL-cholesterol (p < 0.05). Lastly, independent of the abdominal AT depots, greater lower-body SAT was correlate of lower TG but higher HDL-cholesterol levels (P < 0.05).

As shown in Table 4, after control for confounders, reductions in VAT were associated with reduction in fasting plasma glucose, and the OGTT glucose and insulin (P < 0.05). Also, reductions in abdominal SAT were associated with reductions in total and LDL-cholesterol, and OGTT insulin (P < 0.05). Lastly, reductions in lower-body SAT were associated with reductions in OGTT insulin (P < 0.05).

After further control for changes in abdominal and lower-body SAT, reductions in VAT remained significantly associated with reductions in fasting plasma glucose and OGTT glucose (P < 0.05). Moreover, reductions in abdominal SAT remained independently associated with reductions in total and LDL-cholesterol (P < 0.05). In the same models, reductions in lower-body SAT were not associated with cardiometabolic risk factor changes (P > 0.05).
Table 1: Selected Anthropometric, Body Composition, and Metabolic Risk Factors at Baseline and Post-Treatment

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 58)</th>
<th>Women (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>Anthropometric</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (y)</strong></td>
<td>43.9 ± 9.1</td>
<td>39.9 ± 6.8 b</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>101.3 ± 12.0</td>
<td>91.3 ± 11.1 a</td>
</tr>
<tr>
<td><strong>Body mass index (kg/m²)</strong></td>
<td>32.1 ± 3.0</td>
<td>28.9 ± 2.7 a</td>
</tr>
<tr>
<td><strong>Waist circumference (cm)</strong></td>
<td>108.8 ± 7.9</td>
<td>99.5 ± 6.7 a</td>
</tr>
<tr>
<td><strong>Magnetic resonance imaging</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total AT (kg)</strong></td>
<td>35.4 ± 7.9</td>
<td>27.3 ± 7.1 a</td>
</tr>
<tr>
<td><strong>Total SAT (kg)</strong></td>
<td>27.3 ± 7.1</td>
<td>21.4 ± 6.0 a</td>
</tr>
<tr>
<td><strong>Total skeletal muscle (kg)</strong></td>
<td>33.4 ± 4.1</td>
<td>31.9 ± 4.2 a</td>
</tr>
<tr>
<td><strong>Abdominal AT (kg)</strong></td>
<td>9.1 ± 2.1</td>
<td>6.5 ± 1.8 a</td>
</tr>
<tr>
<td><strong>Abdominal SAT (kg)</strong></td>
<td>3.5 ± 1.3</td>
<td>2.4 ± 1.0 a</td>
</tr>
<tr>
<td><strong>Abdominal SAT (kg)</strong></td>
<td>5.2 ± 1.7</td>
<td>4.0 ± 1.4 a</td>
</tr>
<tr>
<td><strong>Lower body AT (kg)</strong></td>
<td>14.7 ± 3.7</td>
<td>11.7 ± 3.3 a</td>
</tr>
<tr>
<td><strong>Lower body SAT (kg)</strong></td>
<td>12.6 ± 3.6</td>
<td>10.0 ± 3.0 a</td>
</tr>
<tr>
<td><strong>Lower body skeletal muscle (kg)</strong></td>
<td>18.7 ± 2.3</td>
<td>17.7 ± 2.6 a</td>
</tr>
<tr>
<td><strong>Metabolic risk factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>OGTT glucose AUC (mmol/L · 2 h)</strong></td>
<td>30.5 ± 9.8</td>
<td>27.2 ± 8.2 a</td>
</tr>
<tr>
<td><strong>OGTT insulin AUC (pmol/L · 2 h)</strong></td>
<td>360.9 ± 241.7</td>
<td>219.3 ± 136.0 a</td>
</tr>
<tr>
<td><strong>Fasting glucose level (mmol/L)</strong></td>
<td>5.6 ± 0.7</td>
<td>5.1 ± 0.8 a</td>
</tr>
<tr>
<td><strong>Fasting plasma insulin level (pmol/L)</strong></td>
<td>95.7 ± 70.4</td>
<td>70.6 ± 50.0 a</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/L)</strong></td>
<td>2.3 ± 1.3</td>
<td>1.6 ± 0.7 a</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/L)</strong></td>
<td>5.0 ± 1.0</td>
<td>4.6 ± 0.8 a</td>
</tr>
<tr>
<td><strong>HDL-cholesterol (mmol/L)</strong></td>
<td>0.9 ± 0.3</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td><strong>LDL-cholesterol (mmol/L)</strong></td>
<td>3.4 ± 0.7</td>
<td>3.1 ± 0.7 a</td>
</tr>
<tr>
<td><strong>VO₂max (ml/kg/min)</strong></td>
<td>34.3 ± 7.0</td>
<td>40.2 ± 7.4 a</td>
</tr>
</tbody>
</table>

Data presented as the group means ± SD.  a Significantly different from baseline (P < 0.05).  b Significantly different from men (p < 0.05).
**Table 2: Prevalence of Metabolic Risk Factors in the Study Sample**

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Men (n= 58)</th>
<th>Women (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Overweight</td>
<td>14 (24)</td>
<td>12 (25)</td>
</tr>
<tr>
<td>Obese</td>
<td>44 (76)</td>
<td>37 (76)</td>
</tr>
<tr>
<td>Abdominally obese</td>
<td>47 (81)</td>
<td>43 (88)</td>
</tr>
<tr>
<td>Impaired fasting glucose</td>
<td>25 (43)</td>
<td>18 (37)</td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>10 (17)</td>
<td>8 (16)</td>
</tr>
<tr>
<td>High/borderline high TG</td>
<td>39 (67)</td>
<td>23 (47)</td>
</tr>
<tr>
<td>Very high/high/borderline high LDL-cholesterol</td>
<td>30 (52)</td>
<td>23 (47)</td>
</tr>
<tr>
<td>Low HDL-cholesterol</td>
<td>28 (48)</td>
<td>16 (33)</td>
</tr>
<tr>
<td>High/borderline high total cholesterol</td>
<td>25 (43)</td>
<td>31 (63)</td>
</tr>
<tr>
<td>Overweight/Obese + 1 risk factor</td>
<td>9 (16)</td>
<td>15 (31)</td>
</tr>
<tr>
<td>Overweight/Obese + 2 risk factors</td>
<td>16 (28)</td>
<td>11 (22)</td>
</tr>
<tr>
<td>Overweight/Obese + 3 or more risk factors</td>
<td>33 (57)</td>
<td>23 (47)</td>
</tr>
</tbody>
</table>

Overweight (BMI ≥ 25kg/m²); obese (BMI ≥ 30kg/m²); abdominally obese (waist circumference > 102 and 88 cm in men and women, respectively); metabolic risk factors defined as per American Diabetes Association and National Cholesterol Education Program guidelines (210, 211).
Table 3: Standardized β-coefficients for associations between baseline regional AT deposition and metabolic risk factors

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>Insulin</th>
<th>TG</th>
<th>Cholesterol</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>OGTT Glucose</th>
<th>OGTT Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAT</td>
<td>0.19</td>
<td>0.25</td>
<td>0.28</td>
<td>-0.07</td>
<td>0.00</td>
<td>-0.24</td>
<td>0.39</td>
<td>0.19</td>
</tr>
<tr>
<td>Abdominal SAT</td>
<td>0.06</td>
<td>0.14</td>
<td>0.03</td>
<td>0.09</td>
<td>-0.01</td>
<td>-0.05</td>
<td>0.11</td>
<td>0.13</td>
</tr>
<tr>
<td>Lower body SAT</td>
<td>0.03</td>
<td>0.07</td>
<td>-0.16</td>
<td>0.07</td>
<td>-0.03</td>
<td>0.14</td>
<td>-0.01</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAT</td>
<td>0.20</td>
<td>0.26</td>
<td>0.20</td>
<td>-0.08</td>
<td>-0.01</td>
<td>-0.17</td>
<td>0.28</td>
<td>0.21</td>
</tr>
<tr>
<td>Abdominal SAT</td>
<td>0.04</td>
<td>0.12</td>
<td>0.28</td>
<td>0.12</td>
<td>0.03</td>
<td>-0.30</td>
<td>0.17</td>
<td>0.09</td>
</tr>
<tr>
<td>Lower body SAT</td>
<td>0.04</td>
<td>0.04</td>
<td>-0.33</td>
<td>-0.04</td>
<td>-0.05</td>
<td>0.34</td>
<td>-0.08</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*Model 1*, control for age and gender; *Model 2*, model 1 plus control for other regional AT depots. ^a^ p ≤ 0.05,  ^b^ p = 0.06.
Table 4: Standardized β-coefficients for associations between changes in regional fat deposition and changes in metabolic risk factors

<table>
<thead>
<tr>
<th></th>
<th>Glucose Δ</th>
<th>Insulin Δ</th>
<th>TG Δ</th>
<th>Cholesterol Δ</th>
<th>LDL-C Δ</th>
<th>HDL-C Δ</th>
<th>OGTT Glucose Δ</th>
<th>OGTT Insulin Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAT Δ</td>
<td>0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12</td>
<td>0.09</td>
<td>-0.14</td>
<td>-0.10</td>
<td>-0.09</td>
<td>0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Abdominal SAT Δ</td>
<td>-0.04</td>
<td>0.04</td>
<td>-0.01</td>
<td>0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.11</td>
<td>0.14</td>
<td>0.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lower body SAT Δ</td>
<td>-0.01</td>
<td>-0.01</td>
<td>-0.06</td>
<td>0.11</td>
<td>0.11</td>
<td>-0.04</td>
<td>0.14</td>
<td>0.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

|               |           |           |        |               |         |         |                |                |
| **Model 2**   |           |           |        |               |         |         |                |                |
| VAT Δ         | 0.22<sup>a</sup> | -0.13    | 0.10   | -0.17         | -0.16   | -0.07   | 0.19<sup>a</sup> | 0.11           |
| Abdominal SAT Δ | -0.08     | 0.07     | 0.01   | 0.23<sup>a</sup> | 0.24<sup>a</sup> | -0.11 | 0.07 | 0.19           |
| Lower body SAT Δ | -0.01     | 0.02     | -0.07  | 0.03          | 0.03    | 0.05    | 0.07           | 0.19           |

*Model 1, control for age, gender, treatment group, and change in VO<sub>2max</sub>; Model 2, model 1 plus control for changes in other regional AT depots. <sup>a</sup> p ≤ 0.05.
Figure 1: Associations between lower-body SAT, abdominal SAT, and VAT in men and women
DISCUSSION

The primary finding of this study is that the reduction of lower-body SAT consequent to weight-loss was not associated with deterioration or an attenuated improvement in risk factors for type-2 diabetes and cardiovascular disease independent of changes in VAT and abdominal SAT. However, independent of changes in lower-body SAT, reductions in VAT and abdominal SAT were associated with improvements in cardiometabolic risk.

A number of cross-sectional studies have previously shown that after control for abdominal AT and/or VAT, greater levels of lower-body AT, specifically SAT, are associated with reduced risk of glucose intolerance, insulin resistance, dyslipidemia, and arterial stiffness (17-23). While we could not corroborate this finding for glucose tolerance, the results of the current report demonstrate that independent of VAT and abdominal SAT, greater lower-body SAT is associated with lower TG and higher HDL-cholesterol levels. It has been reported that the lower-body (gynoid) obesity phenotype is associated with a hyperplasic expansion of the adipose organ, exemplified by numerous small adipocytes – unlike the few, hypertrophied adipocytes of the abdominal obesity phenotype (59, 60). Enlarged adipocytes that are filled to capacity may predispose to elevations of plasma FFA levels (215), possibly through poor buffering of FFA flux in the postprandial state (15), leading to spillover of lipid into the liver, muscle and VAT (114), and consequent insulin resistance (113, 115). Additionally, lower-body SAT adipocytes are known to have relatively high rates of lipoprotein lipase activity (enhanced lipogenesis) but diminished rates of lipolysis (129). Thus, it is suggested that through the buffering of circulating lipid during periods of energy surplus, the small and sensitive subcutaneous adipocytes of the lower body could decrease cardiometabolic risk by limiting excess lipid deposition within tissues ectopic to lower-body SAT, all of which are associated with increased health risk (16). Indeed,
clinical examples of subcutaneous adipocyte hypercellularity (i.e. multiple symmetric lipomatosis) are associated with minimal lipid accumulation in the liver, muscle and VAT, and a normal cardiometabolic profile despite a frank obese state (106), while SAT deficiency (i.e. lipodystrophy) is associated with fat storage in the liver and muscle and an insulin resistant state (216). Additionally, implantation of SAT into lipodystrophic rodents has been shown to reverse ectopic fat deposition and associated cardiometabolic risk (217).

While limited relative lower-body SAT deposition may represent an inadequate buffering capacity of excess calories predisposing to cardiometabolic dysregulation, our findings suggest that this cross-sectional observation should not be extrapolated to indicate that the reduction of this depot through weight-loss is associated with deterioration in cardiometabolic profile. Indeed, during times of negative energy balance, AT decreases in mass due to a reduction in adipocyte size, while the number of adipocytes is unchanged (164). In comparison to their larger counterparts, small adipocytes are more insulin sensitive (165), secrete less prothrombotic but more antithrombotic cytokines (166), and are associated with reduced ectopic fat storage (114), while the reduction in size of subcutaneous adipocytes is associated with decreased plasma triglyceride levels and VAT storage (114). Thus, counter to being detrimental, the reduction of lower-body SAT stores may actually improve the function of this tissue, thereby improving the cardiometabolic profile. Indeed, our results revealed that reductions in lower-body SAT are associated with improvement in OGTT insulin independent of age and gender but not changes in other fat depots.

Nonetheless, our findings contrast with those of Okura et al. who documented that independent of alterations in trunk fat, reductions in leg fat as measured by DEXA are correlated with a worsening of LDL-cholesterol and plasma glucose levels after 14 weeks of diet and
exercise in a mixed sample of pre- and post-menopausal women (99). A number of key differences between these two studies could explain the discrepant results. First, in the continuous analysis by Okura et al., trunk fat was represented by the combination of abdominal and gluteal AT – distinct AT depots which carry opposite effects on cardiometabolic risk (20). Secondly, we distinguished between the different AT depots within the lower body, namely SAT and inter-muscular AT, which have independent and possibly opposing effects on cardiometabolic risk (102). Thirdly, unlike the analyses performed in the current study, the analyses performed by Okura et al. did not adjust for changes in VAT or abdominal SAT. Lastly, our study included only pre-menopausal women, which eliminates the established confounding effect of menstrual status on regional body composition as well as cardiometabolic risk (218).

Consistent with prior reports (83, 84), we found that both VAT and abdominal SAT are independent correlates of cardiometabolic risk. This finding was most consistent for VAT, which was independently associated with five of eight cardiometabolic risk factors assessed in this study (fasting glucose and insulin, TG, and OGTT glucose and insulin). Additionally, our results indicate that reductions in VAT and abdominal SAT, controlled for changes in lower-body SAT, are independently associated with improvements in glucose tolerance and dyslipidemia, respectively. These results agree with previous studies (13, 14), and highlight the importance of reducing abdominal obesity in the treatment of obesity-related cardiometabolic risk.

Our findings are derived from a relatively homogeneous sample of sedentary, obese, middle aged Caucasian men and women from middle- to upper-class. This may limit the generalizability of the results of our study, but should not affect the internal validity. Additionally, it is now recognized that adipose tissue acts as an endocrine organ secreting various cytokines which have numerous effects on cardiometabolic status (219). As emerging
research has noted that the secretion of specific adipokines varies according to location of AT (220) as well as the size of individual adipocytes (138), it is plausible that some of our findings may be explained by changes in depot specific secretion of these bioactive molecules. These notions require further study.

In summary, our findings confirm that abdominal AT deposition, specifically excess VAT, is associated with increased cardiometabolic risk, while lower-body SAT deposition may be cardiometabolically protective. However, consequent to weight-loss, not only is the selective reduction of lower-body SAT not associated with a deterioration in cardiometabolic profile, it may be beneficial in a manner similar to reductions in VAT and abdominal SAT. Thus, among overweight and obese men and women the reduction of excess AT is likely to convey health benefit regardless of origin.
Breast Volume is an Independent Predictor of Visceral and Ectopic Fat in Premenopausal Women

This manuscript has been published in the journal *Obesity*. The co-authors of this manuscript are Travis J. Saunders and Robert Ross. This work was supported in part by the Canadian Institutes of Health Research doctoral award to Peter M. Janiszewski and the Canadian Institutes of Health Research to Robert Ross (MT13448).
ABSTRACT

Background: It is suggested that a large breast size among women may predict type-2 diabetes risk independent of body mass index (BMI) and waist circumference. The purpose of this study was to determine the independent associations between breast volume with cardiometabolic risk factors and regional fat distribution.

Methods: Ninety-two overweight or obese premenopausal women (age = 39.9 ± 6.8 years) underwent full-body magnetic resonance imaging (MRI) for the assessment of breast volume, VAT, abdominal SAT and lower-body SAT, and intermuscular AT (IMAT), a 2-hour oral glucose tolerance test (OGTT) and fasting phlebotomy for assessment of triglyceride, total, HDL-, and LDL-cholesterol levels.

Results: Breast volume was not associated with any of the cardiometabolic risk factors assessed (P > 0.05). However, VAT was consistently associated with a number of cardiometabolic risk factors (OGTT glucose, OGTT insulin, and triglyceride levels) after control for age, BMI, waist circumference, breast volume and the other AT depots. In univariate models, breast volume was positively associated with VAT, IMAT, and abdominal SAT and lower-body SAT (P < 0.05). After control for age, BMI and waist circumference level, breast volume remained positively associated with VAT and IMAT (P < 0.05), such that women with the highest breast volume had approximately 1.1 and 1.3 kg more VAT and IMAT, respectively, but no more abdominal SAT or lower-body SAT, by comparison to women with the smallest breast volume.

Conclusion: The previously documented association between breast size and type-2 diabetes risk may be in part explained by excess VAT and/or IMAT deposition.
INTRODUCTION

Body mass index (BMI) and waist circumference are established independent anthropometric predictors of type-2 diabetes risk in men and women (66). A recent report suggests that a large breast size among young women may predict the risk of developing type-2 diabetes in middle-age independent of BMI and waist circumference (24). However, it remains unknown whether directly assessed breast size is associated with established cardiometabolic antecedents for type-2 diabetes among women independent of more common measures of anthropometry and body composition. These issues must be addressed before clinical assessment of breast size for disease risk screening in women is advocated (25).

With respect to plausible mechanisms, limited evidence suggests that a large breast size may predict cardiometabolic risk independent of BMI and waist circumference by acting as a surrogate for excess ectopic fat deposition. For example, approximately 40-50% of women undergoing liposuction of subcutaneous adipose tissue (SAT) from the hips, thighs, or abdomen present with a paradoxical enlargement of breast size of at least one cup (183, 184) as well as a relative increase in VAT accumulation post-surgery (186). Additionally, highly active antiretroviral therapy (HAART) among HIV-positive women is associated with a peripheral loss of functional SAT but a compensatory increase in VAT and intermuscular AT (IMAT) (149, 150, 175), in association with a significant enlargement in breast size (176). Thus, it is plausible that large breasts may indicate a phenotype characterized by the augmented deposition of fat in ectopic depots, such as VAT and IMAT – each of which is independently associated with diabetes and cardiovascular disease risk factors (98, 221).

Therefore, the purpose of the current study in a sample of overweight or obese premenopausal women was twofold: 1) to determine the independent associations between
breast volume as measured directly by magnetic resonance imaging (MRI) with cardiometabolic risk factors, and 2) to explore the associations between breast tissue volume, VAT, IMAT, and abdominal and lower body SAT.
METHODS:

Subjects

Subjects included 92 premenopausal Caucasian women without overt cardiovascular disease and type-2 diabetes who were recruited from the general public and participated in previous studies at Queen’s University (Kingston, Canada) (207, 209). The inclusion criteria for these studies were: a BMI > 27 kg/m$^2$ and a waist circumference > 88 cm or a waist:hip circumference ratio (WHR) > 0.85. Additionally, all women were weight stable (± 2 kg) for at least 6 months prior to study, were nonsmokers, consumed an average of fewer than two alcoholic beverages per day, had a sedentary lifestyle and were not taking medications known to influence the cardiometabolic risk factors measured. All participants gave informed consent for their respective studies before participation in accordance with the ethical guidelines set by Queen’s University.

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 using a wall-mounted stadiometer. Waist circumference was taken at the level of the last rib to the nearest 0.1 cm after a normal expiration.

Measurement of total and regional fat, skeletal muscle, and breast volume by magnetic resonance imaging

Whole-body (41 to 47 equidistant images) magnetic resonance imaging (MRI) data was obtained with a General Electric, 1.5 Tesla magnet with participants lying prone with arms outstretched above their head using a procedure described in detail elsewhere (208). The MRI data was analyzed with a specially designed image analysis software (Tomovision Inc, Montreal,
Canada) using an established protocol (208, 212).

Total AT, IMAT (AT intertwined between bundles of skeletal muscle fibers) and skeletal muscle (SM) volume were determined using all 41 to 47 images. The image at 5 cm below L4-L5 was used to divide the upper and lower body. VAT and abdominal SAT were calculated using the five images extending from 5 cm below to 15 cm above L4-L5. Lower-body SAT was measured starting at 5 cm below L4-L5 and moving inferiorly. AT and SM volumes (L) were converted to mass units (kg) using assumed constant densities (0.92 kg/L and 1.04 kg/L, respectively) (213).

Breast tissue volume measures using imaging techniques such as MRI are typically performed with the breasts of the participant suspended in a receiver coil (189). As the current study uses previously acquired MRI data which was not originally obtained for the purpose of specifically quantifying breast tissue volume, no receiver coil was applied. Instead, breast tissue was analyzed as the combined cross-sectional area of SAT, mammary ducts, and connective tissue anterior to the pectoral musculature and/or the sternum medially, and the latissimus dorsi musculature laterally (Figure 1 A and B). Total breast tissue volume (L) was determined by applying the truncated cone model for tissue volume estimation on 3-5 images of the chest beginning at the right humeral head moving inferiorly until no breast tissue was visible (total distance ranging from 9.8 to 20.9 cm). It has previously been shown that the use of 3-5 MR images to estimate breast volume is associated with a 3-5 % error compared to methods using continuous images of the entire breast (189). Due to the fact that breast tissue is composed of both SAT as well as mammary ducts and connective tissue, the calculated volume was not converted into a mass unit. Since the volume of breast tissue was assessed from MRI scans of participants lying prone on the MRI table, potential exists for total breast tissue volume to be underestimated due to tissue compression. If indeed present, such a serial underestimation of
Figure 1: Assessment of breast tissue volume using magnetic resonance imaging

A) Raw MRI scan of the chest

B) Analyzed MRI scan for assessment of breast tissue
breast tissue volume is unlikely to influence the associations reported between breast volume, cardiometabolic risk factors, and regional AT deposition.

**Metabolic risk factors**

Blood samples used to determine fasting lipid and lipoprotein values were obtained in the morning after a 12–14 h overnight fast. Total cholesterol, HDL-cholesterol and triglycerides were measured using enzymatic methods on the Roche Modular analytical system (Roche Diagnostics, Indianapolis, IN, USA). The LDL-cholesterol concentration was subsequently determined using the Friedewald equation (222).

A 2-hour 75 gram oral glucose tolerance test (OGTT) was administered the morning after an overnight fast. Blood samples were collected from the antecubital vein at 0, 60 and 120 min. Glucose was measured using an automated glucose analyser (YSI, Yellow Springs, OH, USA), and insulin was measured by a radioimmunoassay kit (Intermedico, Toronto, ON, Canada). The glucose and insulin area under the curves (AUCs) were determined using a trapezoid model (214).

**Statistical analyses**

Initially, 3 separate linear regression models were used to determine the relationships between breast volume, regional and ectopic fat depots (abdominal SAT, lower body SAT, VAT, and IMAT) and cardiometabolic risk factors; Model 1, controlled for age only; Model 2, controlled for age, BMI and waist circumference; and Model 3, controlled for age and other fat depots. Finally, linear regression analyses were performed to determine the relationships between breast volume and the regional and ectopic fat depots before and after control for BMI and waist circumference. All non-normally distributed variables were normalized prior to
analysis using transformations. All statistical procedures were performed using SPSS 16.0 software.

RESULTS:

The anthropometric, MRI, and cardiometabolic risk factor data are presented in Table 1. Breast volume was positively correlated with all anthropometric measures including, body weight \((r = 0.54, p < 0.05)\), BMI \((r = 0.58, p < 0.05)\), waist circumference \((r = 0.55, p < 0.05)\), and HC \((r = 0.54, p < 0.05\); data not shown), as well as all measures of regional AT deposition, including VAT \((r = 0.42, p < 0.05)\), IMAT \((r = 0.44, p < 0.05)\), abdominal \((r = 0.57, p < 0.05)\) and lower body SAT \((r = 0.47, p < 0.05)\).

As shown in Table 2, breast volume was not significantly associated with any of the cardiometabolic risk factors assessed \((p > 0.05)\) in any of the three statistical models employed. The same was true of IMAT, which was not a significant predictor of cardiometabolic risk in any of the statistical models \((p > 0.05)\).

After control for age, VAT was a significant positive predictor of triglycerides, total cholesterol, OGTT glucose and insulin AUC, but a negative predictor of HDL-cholesterol \((p < 0.05)\). Further, VAT remained a significant predictor of triglycerides, total cholesterol and OGTT glucose and insulin AUC after additional control for BMI and waist circumference \((p < 0.05)\). Finally, after control for age and the other depots measured (including breast volume), VAT remained a significant predictor of triglycerides as well as OGTT glucose and insulin AUC \((p < 0.05)\). Thus, for a given BMI and waist circumference or amount of breast volume, abdominal and lower body SAT, increasing VAT was associated with elevated cardiometabolic risk.
Although abdominal SAT was not significantly associated with any cardiometabolic risk factor after controlling for age (p > 0.05), after further control for BMI and waist circumference, abdominal SAT became a negative predictor of both OGTT glucose and insulin AUC (p < 0.05). Similarly, lower body SAT was a significant negative predictor of triglycerides, and OGTT glucose and insulin independent of age, BMI, and waist circumference (p < 0.05) despite no associations with any cardiometabolic risk factor in the initial statistical model controlling for age only (p > 0.05). In other words, for a given BMI or waist circumference, those women with greater abdominal or lower body SAT had lower levels of cardiometabolic risk factors. Finally, lower body SAT remained a significant negative predictor of OGTT glucose AUC independent of age, breast volume and the other AT depots (p < 0.05).

As illustrated in Figure 2, breast volume was a significant predictor of VAT, IMAT, and abdominal and lower body SAT in univariate analyses (p < 0.05). Breast volume remained a significant positive predictor of VAT and IMAT after control for BMI and waist circumference in the multivariate model (Figure 2 A and B, p < 0.05). On the other hand, once BMI and waist circumference were controlled for, breast volume showed no association with abdominal and lower body SAT (Figure 2 C and D, p > 0.05). Thus, for a given BMI and waist circumference level, women with larger breasts had a greater amount of AT storage in the visceral and intermuscular depots.
Table 1: Selected Anthropometric, Body Composition, and Metabolic Risk Factors in Study Sample of Women

<table>
<thead>
<tr>
<th></th>
<th>n = 92</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>39.9 ± 6.8</td>
</tr>
<tr>
<td>Anthropometric</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>89.0 ± 13.1</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>32.9 ± 4.5</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>109.1 ± 12.2</td>
</tr>
<tr>
<td>Magnetic resonance imaging</td>
<td></td>
</tr>
<tr>
<td>Breast volume (l)</td>
<td>2.7 ± 0.9</td>
</tr>
<tr>
<td>Abdominal AT (kg)</td>
<td>8.0 ± 2.6</td>
</tr>
<tr>
<td>VAT (kg)</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>Abdominal SAT (kg)</td>
<td>6.4 ± 2.1</td>
</tr>
<tr>
<td>Lower body SAT (kg)</td>
<td>19.4 ± 5.1</td>
</tr>
<tr>
<td>IMAT (kg)</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>Metabolic risk factors</td>
<td></td>
</tr>
<tr>
<td>OGGT glucose AUC (mmol/L ∙ 2 h)</td>
<td>27.1 ± 6.4</td>
</tr>
<tr>
<td>OGGT insulin AUC (pmol/L ∙ 2 h)</td>
<td>274.1 ± 174.1</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.6 ± 1.0</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.0 ± 0.8</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>3.1 ± 0.7</td>
</tr>
</tbody>
</table>

Data presented as the group means ± SD.
Table 2: Standardized β-coefficients for associations between regional AT deposition and metabolic risk factors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TG</th>
<th>Cholesterol</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>OGTT Glucose</th>
<th>OGTT Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast volume</td>
<td>0.12</td>
<td>0.04</td>
<td>0.09</td>
<td>-0.10</td>
<td>0.10</td>
<td>0.09</td>
</tr>
<tr>
<td>VAT</td>
<td>0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15</td>
<td>-0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IMAT</td>
<td>0.07</td>
<td>0.06</td>
<td>0.08</td>
<td>-0.10</td>
<td>0.11</td>
<td>0.02</td>
</tr>
<tr>
<td>Abdominal SAT</td>
<td>0.11</td>
<td>0.03</td>
<td>-0.01</td>
<td>-0.03</td>
<td>-0.02</td>
<td>0.13</td>
</tr>
<tr>
<td>Lower body SAT</td>
<td>-0.03</td>
<td>-0.07</td>
<td>-0.06</td>
<td>0.06</td>
<td>-0.16</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast volume</td>
<td>0.02</td>
<td>0.04</td>
<td>0.17</td>
<td>-0.07</td>
<td>0.08</td>
<td>-0.11</td>
</tr>
<tr>
<td>VAT</td>
<td>0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.20</td>
<td>-0.23</td>
<td>0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IMAT</td>
<td>0.00</td>
<td>0.06</td>
<td>0.11</td>
<td>-0.08</td>
<td>0.09</td>
<td>-0.10</td>
</tr>
<tr>
<td>Abdominal SAT</td>
<td>-0.26</td>
<td>-0.02</td>
<td>0.02</td>
<td>0.20</td>
<td>-0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lower body SAT</td>
<td>-0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.12</td>
<td>-0.03</td>
<td>0.25</td>
<td>-0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Model 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast volume</td>
<td>0.04</td>
<td>0.00</td>
<td>0.12</td>
<td>-0.06</td>
<td>0.09</td>
<td>-0.02</td>
</tr>
<tr>
<td>VAT</td>
<td>0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.22</td>
<td>0.13</td>
<td>-0.23</td>
<td>0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IMAT</td>
<td>-0.10</td>
<td>-0.02</td>
<td>0.02</td>
<td>-0.01</td>
<td>-0.07</td>
<td>-0.13</td>
</tr>
<tr>
<td>Abdominal SAT</td>
<td>0.11</td>
<td>0.05</td>
<td>-0.05</td>
<td>-0.02</td>
<td>0.02</td>
<td>0.15</td>
</tr>
<tr>
<td>Lower body SAT</td>
<td>-0.20</td>
<td>-0.17</td>
<td>-0.13</td>
<td>0.14</td>
<td>-0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.15</td>
</tr>
</tbody>
</table>

Model 1, control for age

Model 2, control for age, waist circumference and BMI

Model 3, control for age and other AT depots

<sup>a</sup> p ≤ 0.05,  <sup>b</sup> p = 0.06

HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; TG, triglycerides
**Figure 2:** Associations between breast volume and adipose tissue distribution

Associations determined using simple and multiple linear regressions in 92 women before (solid lines) and after (dotted lines) control for BMI and waist circumference.

\[ \beta, \text{standardized beta coefficients} \]
DISCUSSION

The results of the current study suggest that breast volume, as assessed directly by MRI, is not significantly associated with cardiometabolic risk among overweight and obese premenopausal women. However, for a given BMI and waist circumference level, a large breast volume appears to indicate a greater propensity for fat storage in the visceral and intermuscular, but not subcutaneous depots. Given that VAT and IMAT are independently associated with health risk, excess VAT and/or IMAT may in part explain the previously documented association between breast size and type-2 diabetes (24).

In a recent epidemiological study including over 92,000 women from the Nurses Health Study II, Ray and colleagues reported that bra cup size at age 20 was a significant predictor of future risk of type-2 diabetes independent of BMI, waist circumference, diet, physical activity and other confounders (24). Specifically, it was reported that after adjustment for various confounders (including BMI and waist circumference) women with a D cup or greater were at 58% increased risk of developing type-2 diabetes in contrast to women with an A cup or smaller during the 10 years of follow up. However, our results suggest that MRI-measured breast volume is not associated with any of the cardiometabolic risk factors assessed, themselves antecedents for type-2 diabetes, including insulin and glucose response during an OGTT as well as fasting levels of triglycerides, total, HDL- and LDL- cholesterol. While it is possible that the narrow BMI range of the current sample may have limited our ability to demonstrate an association between breast volume and cardiometabolic risk factors, the readily documented association between VAT and numerous cardiometabolic risk factors in the same sample argues against such a possibility.
Interestingly, as illustrated in Figure 2, given the same age, BMI, and waist circumference, women with the highest breast volume had approximately 1.1 and 1.3 kg more VAT and IMAT, respectively, but no more abdominal or lower-body SAT, by comparison to women with the smallest breast volume. The ability of breast volume to predict fat storage in the visceral depot independently of BMI and waist circumference may be relevant given the previously reported independent associations between VAT and health risk. Indeed, we have previously shown that VAT is associated with cardiometabolic risk (221) and mortality (12) independent of other AT depots. More importantly, VAT has also been shown to predict prospective risk of developing type-2-diabetes independent of other AT depots, family history of diabetes, fasting glucose and insulin, and glucose tolerance at baseline (10). In other words, while VAT may be associated with traditional cardiometabolic risk factors (as shown here), it also appears to predispose to type-2 diabetes risk via mechanisms which are independent of these markers. Additionally, while we could not corroborate the findings in the current study, some (98) but not all (207) previous studies have also shown excess IMAT to be an independent predictor of cardiometabolic risk. Thus, given these observations, it remains plausible that the findings of Ray et al. (24) of increased type-2 diabetes risk associated with larger breast size may have been mediated, at least in part, by a greater relative fat storage in VAT and IMAT, themselves independent risk factors for disease.

Although there is a clear parallel between our findings of a larger breast volume being associated with increased VAT and IMAT despite a similar BMI and waist circumference, and the body composition changes observed among HIV-positive women undergoing HAART (149, 150, 175, 176), as well as women undergoing subcutaneous liposuction (183, 184, 186), it is unclear if and how these observations are related. In the case of HIV-positive women on HAART, it has been reported that the disease itself and more prominently the associated therapy leads to
subcutaneous adipocyte dysfunction, characterized by impaired differentiation and adipogenesis, particularly in the peripheral depots (145). According to the metabolic sink hypothesis, the inability of peripheral SAT stores to accommodate energy during times of excess is thought to result in a compensatory spillover of lipid into ectopic depots such as VAT, IMAT, and liver, accompanied by an insulin resistant, prothrombotic and proinflammatory state (16, 114, 223). The observation that breasts, which are predominantly composed of SAT, increase in unison with VAT when other SAT stores become limited (either via surgical removal or through dysfunction), is paradoxical and seems to run counter to the above hypothesis. These issues clearly warrant further elucidation.

Given that waist circumference measurement cannot distinguish between the relative contribution of VAT and abdominal SAT to total abdominal adiposity, the clinical assessment of breast size may be of potential use therein. Indeed, we previously reported that other simple anthropometric measures, such as hip circumference, may also be used to provide information above and beyond waist circumference regarding the relative contribution of VAT to abdominal adiposity, albeit in the opposite direction from that observed with breast volume (103). Specifically, among both men and women, a larger hip circumference for a given waist circumference is associated with less visceral or a more subcutaneous abdominal adiposity.

Limitations of the current study warrant mention. The study sample consisted of a relatively homogeneous group of sedentary, obese, premenopausal women from the middle-to-upper socioeconomic class. This characteristic may limit the generalizability of the results of our study but should not affect the internal validity. Indeed, the homogeneity of our study group on demographics, socioeconomic factors, and lifestyle characteristics is a benefit because it reduces the likelihood of confounding by these factors. Additionally, given that the current analysis used
previously acquired MRI data, we were unable to measure breast tissue volume by the use of a receiver coil, as is preferable (189). Nevertheless, while not ideal, our direct assessment of breast volume using 3-5 contiguous MRI scans, is a considerable improvement in methodology over the reliance on self-recall of bra size performed in the only other study, to our knowledge, which has investigated similar issues (24). Lastly, the current study was unable to examine the association between breast tissue composition (fat versus gland versus connective tissue) and cardiometabolic risk; a potential area of inquiry for future studies.

In conclusion, the results of the current study in a sample of overweight/obese premenopausal women suggest that breast volume, as assessed directly by MRI, is not associated with cardiometabolic risk factors. However, a large breast volume appears to indicate a greater propensity for fat storage in the visceral and intermuscular, but not subcutaneous depots, independent of established anthropometric measurements (BMI and waist circumference). Thus, it remains possible that the previously documented association between breast size and type-2 diabetes risk may be partly explained by a deleterious body fat distribution.
Chapter 5: Manuscript 3

Effects of weight loss among metabolically healthy obese men and women

The co-author of this manuscript is Robert Ross. This work was supported in part by the Canadian Institutes of Health Research doctoral award to Peter M. Janiszewski and the Canadian Institutes of Health Research to Robert Ross (MT13448).
ABSTRACT

Background: An estimated 30% of obese individuals are metabolically-healthy obese (MHO); exhibiting high insulin sensitivity, normal plasma lipid levels, and low risk of diabetes and cardiovascular disease despite their excess adiposity. In contrast to the established cardiometabolic benefits of modest weight loss among metabolically-abnormal obese (MAO) individuals, it has been suggested that weight loss among MHO individuals may be unnecessary or even result in elevated cardiometabolic risk. We studied the effects of exercise- and diet-induced weight loss on cardiometabolic risk among MHO and MAO men and women.

Methods: Sixty three MHO men and women (≤ 2 metabolic syndrome risk factors as per NCEP ATPIII) and 43 MAO men and women participated in 3-6 months of exercise or diet weight-loss intervention. Changes in anthropometry, body composition by magnetic resonance imaging, and cardiometabolic risk, including insulin sensitivity by hyperinsulinemic-euglycemic clamp, were assessed.

Results: Post intervention, body weight, waist circumference, and total and abdominal adipose tissue were significantly reduced in all subjects (P < 0.05). Improvements in insulin sensitivity were also observed in MHO and MAO men and women (P < 0.05). Among MHO subjects, a reduction in fasting insulin was the only other cardiometabolic improvement in response to intervention, meanwhile the improvements among MAO subjects were more numerous (triglycerides, fasting glucose and insulin, HDL-cholesterol, and total cholesterol; P < 0.05 for all).

Conclusion: Weight loss among MHO subjects is not associated with an increase in cardiometabolic risk, but may lead to modest improvement in certain risk factors.
INTRODUCTION

While obesity is an established risk factor for type-2 diabetes, cardiovascular disease, and mortality (5, 7, 55), the presence of excess body fat does not always indicate an immediate disease risk; nor does its absence guarantee a lack thereof (8). For instance, it is estimated that one in three obese individuals has no known history of disease and presents with a normal cardiometabolic profile (29-31). Termed metabolically healthy obese (MHO), such individuals are reported to exhibit high insulin sensitivity, normal blood pressure, healthy blood lipid levels (32, 33), a favorable cytokine profile (194) and experience a low prospective risk of type-2 diabetes and cardiovascular disease (30) despite their excess adiposity. However, more recent evidence suggests that despite being metabolically healthy, MHO individuals may still experience an elevated risk of mortality (197, 206), as well as early signs of vascular disease (205). Indeed, in a study of over 6000 men and women followed for approximately 9 years, Kuk and Ardern (197) reported that MHO individuals had more than two-fold the risk of premature mortality in comparison to metabolically healthy and lean individuals.

Although leading health authorities currently recommend weight reduction as the primary treatment strategy for all obese patients, regardless of cardiometabolic status (26-28), it has been argued that weight reduction among obese patients who are otherwise healthy may not only be unnecessary (36, 199, 204), but may also potentially increase health risk (38). For example, Sims (36) previously suggested that MHO individuals “should not be urged to achieve a normal weight.” More recently, Karelis et al. demonstrated that a modest weight reduction (5.0-6.5 kg or 6-7 %) achieved via caloric restriction resulted in a 13% deterioration in insulin sensitivity among a group of postmenopausal MHO women, in contrast to the 26% improvement in insulin sensitivity among metabolically abnormal obese (MAO) women undergoing the same intervention. The notion that weight reduction among a third of obese
patients may be needless or counterproductive is at odds with the uniform 5-10% weight loss treatment currently recommended for all obese patients (34, 35). Given the potential implications for clinical practice and public health, further inquiry in this area is clearly warranted.

The objective of the present study was to investigate the effects of exercise- and diet-induced weight reduction on cardiometabolic risk factors among MHO as well as MAO men and women.
METHODS:

Subjects

Subjects for this study included Caucasian men (n = 46, age = 57.8 ± 13.7 years) and premenopausal (n = 29, age = 42.6 ± 5.7 years) and post-menopausal women (n = 34, age = 66.9 ± 5.6 years) without overt disease who were recruited from the general public and participated in previously published exercise and/or diet-induced weight loss studies at Queen’s University (Kingston, Canada) (14, 209, 224). Methodological details of these individual studies are previously reported (14, 209, 224). Briefly, a total of 52 men and 54 premenopausal women participated in 2 separate studies (1 in each gender) comparing the effect of diet and aerobic exercise-induced weight loss on regional body composition and metabolic status (ClinicalTrials.gov registration numbers: NCT00664547 and NCT00664495) (14, 209). In these studies, within each gender, subjects were randomized to a control, diet-induced weight loss, exercise-induced weight loss, or an exercise without weight loss group. For the current investigation, data from those men (n = 20) and women (n = 29) who were randomized into either the diet-induced or the exercise-induced weight loss were used (see “Interventions” below for details). Additionally, a total of 136 abdominally obese men and women, 60 to 80 years of age, were recruited to participate in a study on the effects of resistance and/or aerobic exercise on risk factors for disease and disability (ClinicalTrials.gov registration number: NCT00520858)(224). In this study, within each gender, subjects were randomized to 1 of 4 groups for approximately 6 months: resistance exercise, aerobic exercise, resistance and aerobic exercise combined, or non-exercise control group (see “Interventions” below). For the current investigation, data from those men (n = 26) and women (n = 34) randomized to either the aerobic exercise or the resistance and aerobic exercise combined were included.
The inclusion criteria for these studies were: a BMI > 27 kg/m$^2$ and a waist circumference > 102 cm in the men, and BMI > 27 kg/m$^2$ and a waist circumference > 88 cm in the women. All subjects were weight stable (± 2 kg) for at least 6 months prior to study, were nonsmokers who consumed an average of fewer than two alcoholic beverages per day, had a sedentary lifestyle and were not taking medications known to influence the cardiometabolic risk factors measured. All participants gave informed consent for their respective studies before participation in accordance with the ethical guidelines set by Queen’s University.

**Definition of MHO versus MAO**

There exist no established criteria for the definition of MHO individuals (35). Some have used arbitrary cut-points of insulin sensitivity (31, 33, 198), or cardiometabolic risk factor clustering (30, 31) to delineate MHO from MAO subjects. Initially, we delineated our sample into MHO and MAO based on levels of insulin sensitivity at baseline. However, separating the sample based on this one outcome alone resulted in two groups which differed in insulin sensitivity, but were quite similar on other cardiometabolic outcomes of interest. Accordingly, we chose to define our MHO and MAO groups using a more comprehensive clustering of cardiometabolic risk factors. Specifically, we defined as MHO men and women who were abdominally obese (waist circumference > 88 cm in women and 102 cm in men) but had one or less of the following cardiometabolic risk factors: fasting plasma glucose ≥ 5.6 mmol/L, triglycerides ≥ 1.7 mmol/L, HDL-cholesterol < 1.0 mmol/L in men and < 1.3 mmol/L in women, and blood pressure ≥ 130/85 mmHg. In contrast, all abdominally obese men and women meeting two or more of the above cardiometabolic risk factor criteria were classified as MAO. Importantly, our primary observations regarding the effect of weight-loss on cardiometabolic risk remained the same regardless of the definition used to delineate the MHO and MAO groups.
Interventions

Of the 46 men in the current study, 20 were randomly assigned to a diet \((n = 11)\) or exercise \((n = 9)\) program designed to induce a daily 700 kcal energy deficit for 12 weeks (14). The remaining men were randomized to either 6 months of aerobic exercise \((n = 13)\) performed for 30 minutes, 5 times per week or to resistance and aerobic exercise combined \((n = 13)\) performed 3 times per week (30 minutes of aerobic exercise plus ~30 minutes of resistance exercise per session), as described in detail elsewhere(224).

Of the 63 women in the current study, 29 premenopausal women were randomly assigned to a diet \((n = 13)\) or exercise \((n = 16)\) program aimed at inducing a 500 kcal/day energy deficit for 14 weeks (209). The remaining postmenopausal women were randomized to either 6 months of aerobic exercise \((n = 16)\) performed for 30 minutes, 5 times per week or to resistance and aerobic exercise combined \((n = 18)\) performed 3 times per week (30 minutes of aerobic exercise plus ~30 minutes of resistance exercise per session), as described in detail elsewhere (224).

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 using a wall-mounted stadiometer. Waist circumference was taken at the level of the last rib to the nearest 0.1 cm after a normal expiration.

Measurement of total and regional adipose tissue and skeletal muscle by magnetic resonance imaging

Whole-body (41 to 47 equidistant images) magnetic resonance imaging (MRI) data was obtained with a General Electric, 1.5 Tesla magnet using a procedure described in detail
elsewhere (208). The MRI data was analyzed with a specially designed image analysis software (Tomovision Inc, Montreal, Canada) using an established protocol (208, 212). Total adipose tissue (AT) and skeletal muscle (SM) volume were determined using all 41 to 47 images. The image at 5 cm below L4-L5 was used to divide the upper and lower body. VAT and abdominal SAT were calculated using the five images extending from 5 cm below to 15 cm above L4-L5 (kg). AT and SM volumes (L) were converted to mass units (kg) using assumed constant densities (0.92 kg/L and 1.04 kg/L, respectively).

**Metabolic risk factors**

Blood samples used to determine fasting glucose, insulin, and lipid values were obtained in the morning after a 12- to 14-hour overnight fast. Glucose was measured using an automated glucose analyzer (YSI), and insulin was measured by a radioimmunoassay kit (Intermedico, Toronto, Canada). Serum total cholesterol and triglyceride levels were determined using standard techniques. HDL-cholesterol was assayed after isoelectric-polyanionic precipitation. The LDL-cholesterol was subsequently determined using the Friedewald equation(222).

Subjects consumed a weight maintaining diet consisting of at least 200 grams of carbohydrate for a minimum of 4 days before measurements of insulin sensitivity and were asked to avoid strenuous physical activity for 3 days preceding the studies. Post-treatment data were obtained 36 to 96 hours after the last exercise session. All studies were performed at ~8 am after a 10- to 14-hour overnight fast. An antecubital vein was catheterized for infusion of insulin and 20% glucose, and a hand vein was cannulated in a retrograde fashion and placed in a heating pad for sampling of arterialized blood. Insulin was infused at a rate of 40 mU/m²/min for 3 hours. Blood glucose was measured using an automated glucose analyzer (YSI 2300 Glucose Analyzer, YSI, Yellow Springs, OH) every 5 minutes in arterialized blood. Insulin
sensitivity was calculated using the average exogenous glucose infusion rate during the final 30 minutes of euglycemia.

**Statistical analyses**

Prior to analysis, data was assessed for normality using the Shapiro-Wilk test. In the case of a non-normal distribution, data was normalized using log-transformations. Baseline differences in anthropometric, MRI, and cardiometabolic risk factors between groups were assessed using a 2-by-2 (sex-by-risk category) analysis of co-variance (ANCOVA). Due to significant difference in age between MAO and MHO subjects, age was used as a covariate in thes analyses. Where an interaction term was significant, post-hoc analysis was performed using independent-samples t-tests with a Bonferroni correction for multiple comparisons. Changes in variables (pre-post) in response to intervention were assessed using a repeated-measures analysis of co-variance (ANCOVA) with gender and metabolic stratification as the between-subjects factors, and inclusion of significant age and treatment modality terms as covariates. Where significant interactions were present, subsequent comparisons were performed with a Bonferroni correction for multiple comparisons. Secondary analyses were performed to examine the separate effects of the different weight-loss modalities utilized on changes in insulin sensitivity among MHO and MAO subjects using repeated-measures ANOVA (collapsed across gender). Finally, men and women were also separated into sex-specific tertiles of baseline insulin sensitivity, and the effect of weight loss intervention on changes in insulin sensitivity was examined by repeated-measures ANOVA (collapsed across modality). All statistical procedures were performed using SPSS 17.0 software (Chicago, IL).

**RESULTS**
The baseline values for anthropometrics, body composition measures, and cardiometabolic outcomes in MHO and MAO men and women are presented in Table 1. The prevalence of various cardiometabolic risk factors in the MHO and MAO individuals is listed in Table 2.

At baseline, women had a lower body weight and waist circumference, less skeletal muscle mass and VAT, but greater total, lower body and abdominal SAT compared to men (p < 0.05). Further, the women had greater levels of total, HDL-, and LDL-cholesterol, as well as greater insulin sensitivity (p < 0.05).

While MHO individuals were older than their MAO counterparts (p < 0.05), no differences existed in anthropometric or body composition measures between the two groups (p > 0.05). Despite the similar obesity phenotype, MHO men and women had lower levels of fasting glucose, insulin (women only), triglycerides, but greater insulin sensitivity and HDL-cholesterol levels in comparison to their MAO counterparts (p < 0.05, Table 1). Accordingly, as shown in Table 2, clinically elevated levels of cardiometabolic risk factors were uncommon among MHO men and women.

The effect of exercise or diet intervention among MAO and MHO men and women is presented in Table 3. With the exception of skeletal muscle, all anthropometric and body composition measures changed significantly in response to intervention among MAO and MHO men and women (p < 0.05). For example, weight, waist circumference, total AT, and VAT were significantly reduced in all groups (p < 0.05). However, with the exception of abdominal SAT, the reductions in anthropometric and body composition measures were greater in the men versus the women (P < 0.05 for an interaction). Improvements in selected cardiometabolic risk factors also occurred in both MAO and MHO men and women in response to intervention, but were
more common in the MAO groups. For example, as illustrated in Figure 1, insulin sensitivity improved significantly in all groups, although this change was greater in MAO versus MHO individuals (P < 0.05 for an interaction). Among MAO subjects, additional improvements occurred in triglycerides, fasting glucose (men only), HDL-cholesterol (men only), fasting insulin (women only) and total cholesterol (women only; p < 0.05). Among MHO subjects, a reduction in fasting insulin (men only) was the only other cardiometabolic improvement in response to exercise or diet induced weight loss in addition to the enhanced insulin sensitivity.

We performed an additional analysis to illustrate the separate effects of the different weight-loss interventions on changes in insulin sensitivity. As illustrated in Figure 2, insulin sensitivity improved significantly in both MHO and MAO groups in response to the 3-4 month diet intervention (P < 0.05). Similarly, insulin sensitivity improved significantly in response to the exercise interventions in both the MHO and MAO groups (P < 0.05), with the exception of the 3 month aerobic exercise intervention in MHO and the 6 month resistance plus aerobic exercise in MAO (P > 0.05, for both).

We also examined the influence of diet or exercise weight loss intervention on insulin sensitivity in men and women who were divided into tertiles of insulin sensitivity at baseline. As illustrated in Figure 3, improvements in insulin sensitivity were observed in both men and women in response to weight loss regardless of baseline insulin sensitivity level (P < 0.05 for all except P = 0.06 for highest tertile of insulin sensitivity in men). For example, in response to weight-loss intervention, insulin sensitivity improved by 13.8% (P = 0.06) among the men in the highest tertile of insulin sensitivity at baseline and by 9.8 % (P < 0.05) among the women in the highest tertile of insulin sensitivity at baseline.
Table 1: Selected Anthropometric, Body Composition, and Metabolic Outcomes at Baseline in MAO and MHO men and women

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAO (n = 20)</td>
<td>MHO (n = 26)</td>
</tr>
<tr>
<td><strong>Age (yr)</strong></td>
<td>53.1 ± 14.8</td>
<td>61.4 ± 11.8 b</td>
</tr>
<tr>
<td><strong>Anthropometric</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>98.1 ± 9.4</td>
<td>95.6 ± 12.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.4 ± 2.7</td>
<td>31.0 ± 3.1 b</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>109.2 ± 6.0</td>
<td>111.7 ± 8.3</td>
</tr>
<tr>
<td><strong>MRI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total AT (kg)</td>
<td>32.6 ± 6.0</td>
<td>33.6 ± 8.2</td>
</tr>
<tr>
<td>Total SM (kg)</td>
<td>33.5 ± 4.5</td>
<td>30.9 ± 3.7</td>
</tr>
<tr>
<td>Visceral AT (kg)</td>
<td>4.0 ± 1.2</td>
<td>4.4 ± 1.2 a</td>
</tr>
<tr>
<td>Visceral AT (cm³)</td>
<td>208.7 ± 64.8</td>
<td>222.8 ± 63.7</td>
</tr>
<tr>
<td>Abdominal SAT (kg)</td>
<td>4.6 ± 1.5</td>
<td>4.5 ± 1.9</td>
</tr>
<tr>
<td>Abdominal SAT (cm³)</td>
<td>301.7 ± 103.1</td>
<td>294.6 ± 130.2</td>
</tr>
<tr>
<td>Lower body SAT (kg)</td>
<td>11.9 ± 3.3</td>
<td>12.5 ± 3.5</td>
</tr>
<tr>
<td><strong>Metabolic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting Glucose (mmol/L)</td>
<td>5.3 ± 0.5</td>
<td>4.9 ± 0.5 b</td>
</tr>
<tr>
<td>Fasting Insulin (UI)</td>
<td>9.8 ± 5.2</td>
<td>9.8 ± 5.2</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.3 ± 1.0</td>
<td>4.3 ± 0.8</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>0.8 ± 0.3</td>
<td>1.1 ± 0.2 b</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>2.5 ± 0.8</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>2.3 ± 0.9</td>
<td>1.4 ± 0.5 b</td>
</tr>
<tr>
<td>Insulin Sensitivity</td>
<td>(mg/kg·SM/min)</td>
<td>10.8 ± 3.4</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>128.4 ± 16.0</td>
<td>122.0 ± 17.7</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>82.2 ± 10.8</td>
<td>76.2 ± 9.3</td>
</tr>
</tbody>
</table>

Data presented as the group means ± SD.

Age included as a covariate in analyses.

*Significantly different from men (P < 0.05).

bSignificantly different from at-risk group of same gender (P < 0.05).

MAO, metabolically abnormal obese; MHO, metabolically healthy obese; BP, blood pressure
Table 2: Prevalence of metabolic risk factors among MAO and MHO men and women.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAO (n = 20)</td>
<td>MHO (n = 26)</td>
</tr>
<tr>
<td>Abdominal obesity</td>
<td>20 (100.0)</td>
<td>26 (100.0)</td>
</tr>
<tr>
<td>Impaired fasting glucose</td>
<td>7 (35.0)</td>
<td>1 (3.8)</td>
</tr>
<tr>
<td>Elevated triglycerides</td>
<td>16 (80.0)</td>
<td>6 (23.1)</td>
</tr>
<tr>
<td>Elevated LDL-cholesterol</td>
<td>4 (20.0)</td>
<td>4 (15.3)</td>
</tr>
<tr>
<td>Low HDL-cholesterol</td>
<td>18 (90.0)</td>
<td>8 (30.1)</td>
</tr>
<tr>
<td>Elevated total cholesterol</td>
<td>5 (25.0)</td>
<td>4 (15.4)</td>
</tr>
<tr>
<td>Elevated blood pressure</td>
<td>6 (30.0)</td>
<td>1 (3.8)</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>20 (100.0)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

Abdominal obesity (waist circumference > 88 cm in women and 102 cm in men), impaired fasting glucose (fasting plasma glucose ≥ 5.6 mmol/L), elevated triglycerides (triglycerides ≥ 1.7 mmol/L), low HDL-cholesterol (HDL-cholesterol < 1.0 mmol/L in men and < 1.3 mmol/L in women), elevated LDL-cholesterol (>2.6 mmol/L), elevated total cholesterol (>5.2 mmol/L), elevated blood pressure (Systolic/diastolic blood pressure ≥ 130/85 mmHg), and metabolic syndrome (3 or more of abdominal obesity, impaired fasting glucose, elevated triglycerides, low HDL-cholesterol, and elevated blood pressure).
# Table 3: Effect of exercise or diet intervention among MAO and MHO men and women.

<table>
<thead>
<tr>
<th></th>
<th>Men MAO (n = 20)</th>
<th>Men MHO (n = 26)</th>
<th>Women MAO (n = 23)</th>
<th>Women MHO (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>-6.0 ± 2.8 a</td>
<td>-4.0 ± 2.8 a</td>
<td>-4.9 ± 2.4 a, c</td>
<td>-3.0 ± 2.3 a, c</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-1.9 ± 0.9 a</td>
<td>-1.3 ± 1.0 a</td>
<td>-1.8 ± 1.0 a, c</td>
<td>-1.1 ± 0.8 a, c</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>-6.2 ± 2.8 a</td>
<td>-5.6 ± 3.2 a</td>
<td>-4.6 ± 3.2 a, c</td>
<td>-4.1 ± 3.3 a, c</td>
</tr>
<tr>
<td><strong>MRI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total AT (kg)</td>
<td>-5.1 ± 2.2 a</td>
<td>-4.1 ± 2.0 a</td>
<td>-4.7 ± 2.9 a, c</td>
<td>-3.2 ± 2.3 a, c</td>
</tr>
<tr>
<td>Total SM (kg)</td>
<td>-0.8 ± 1.3</td>
<td>-0.2 ± 1.3</td>
<td>-1.0 ± 1.2</td>
<td>0.1 ± 1.0</td>
</tr>
<tr>
<td>Visceral AT (kg)</td>
<td>-0.8 ± 0.4 a</td>
<td>-0.6 ± 0.4 a</td>
<td>-0.4 ± 0.4 a, c</td>
<td>-0.3 ± 0.3 a, c</td>
</tr>
<tr>
<td>Visceral AT (cm³)</td>
<td>-40.1 ± 26.3 a</td>
<td>-40.5 ± 34.4 a</td>
<td>-18.2 ± 24.6 a, c</td>
<td>-19.0 ± 21.7 a, c</td>
</tr>
<tr>
<td>Abdominal SAT (kg)</td>
<td>-0.7 ± 0.5 a</td>
<td>-0.5 ± 0.4 a</td>
<td>-0.7 ± 0.6 a</td>
<td>-0.4 ± 0.4 a</td>
</tr>
<tr>
<td>Abdominal SAT (cm³)</td>
<td>-40.5 ± 35.4 a</td>
<td>-28.8 ± 33.8 a</td>
<td>-39.5 ± 49.0 a, c</td>
<td>-21.3 ± 40.1 a, c</td>
</tr>
<tr>
<td><strong>Metabolic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting Glucose (mmol/L)</td>
<td>-0.6 ± 0.7 a</td>
<td>-0.1 ± 0.4</td>
<td>-0.3 ± 0.8</td>
<td>0.0 ± 0.4</td>
</tr>
<tr>
<td>Fasting Insulin (UI)</td>
<td>-1.9 ± 4.2</td>
<td>-2.0 ± 3.2 a</td>
<td>-3.8 ± 4.0 a</td>
<td>-0.6 ± 3.9</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>-0.0 ± 0.6</td>
<td>-0.2 ± 0.4</td>
<td>-0.4 ± 0.5 a</td>
<td>-0.1 ± 0.5</td>
</tr>
</tbody>
</table>
Data presented as the group means of change scores (post-pre) ± SD

Analyses controlled for age and treatment modality (diet vs. exercise)

a Significant change in variable from pre- to post-intervention

b Change in variable different in MHO versus MAO

c Change in variable different in men versus women

For further details of the repeated measures ANCOVA analyses, including p values for main effects and interactions, and the significance of covariates, please refer to Appendix 8.
Figure 1. Individual and group changes in insulin sensitivity among MAO and MHO men and women in response to diet or exercise induced weight reduction.

Effect of diet- or exercise induced weight loss on insulin sensitivity in MAO (Panel A) and MHO (Panel B) men and MAO (Panel C) and MHO women (Panel D). Each line represents the change in insulin sensitivity of a single participant from pre- to post-intervention. Black dashed line within each panel represents the mean change in that group. Changes in insulin sensitivity were significant for all groups, but significantly greater in the MAO versus MHO subjects (refer to table 3; P < 0.05).
Figure 2: Effect of different weight loss interventions on insulin sensitivity in MAO and MHO subjects

Each line represents the mean response in insulin sensitivity for that treatment modality (refer to inset legend). Changes in insulin sensitivity from pre-post intervention were significant for all treatment modalities in the MAO and MHO groups ($P < 0.05$), except the 3 month aerobic exercise intervention in MHO and the 6 month resistance plus aerobic exercise in MAO ($P > 0.05$, for both).
**Figure 3:** Change in insulin sensitivity in response to diet or exercise induced weight loss by tertiles of baseline insulin sensitivity in men and women

*Change in insulin sensitivity from pre-post intervention (P < 0.05)*

*Change in insulin sensitivity from pre-post intervention (P = 0.06)*
DISCUSSION

The primary finding of this investigation is that weight loss among MHO subjects is not associated with an increase in cardiometabolic risk. Accordingly, this study does not support the contention that weight reduction among otherwise healthy obese individuals is counterproductive. Nevertheless, the cardiometabolic benefit of weight loss among MHO individuals may be limited to an improvement in insulin sensitivity. Thus, while cardiometabolic benefit may be modest among the subgroup of MHO individuals, a 5-10% weight loss should still be considered an appropriate clinical treatment option for all obese men and women, regardless of initial cardiometabolic status.

McLaughlin and colleagues (204) have previously shown that while a combination of caloric restriction along with sibutramine administration results in significant weight-loss among MHO women, the treatment had no impact on insulin sensitivity. More recently, Karelis et al. demonstrated that a modest weight reduction achieved via caloric restriction resulted in a 13% deterioration in insulin sensitivity among a group of postmenopausal MHO women (38). However, as acknowledged by the authors, the observation that insulin sensitivity may be attenuated in response to a reduction in weight, and more specifically fat mass, is counter-intuitive and lacks a plausible physiological mechanism (38). Our results counter these prior observations. In fact, although the effect was more modest than among MAO individuals, insulin sensitivity improved in MHO men and women by 22 and 18.5%, respectively, regardless of weight-loss modality (diet or exercise). This observation remained true when the sample was categorized according to sex-specific tertiles of insulin sensitivity at baseline, rather than cardiometabolic risk factor clustering (Figure 3).
Conversely, we report that improvements in more established cardiometabolic risk factors (e.g. triglycerides, HDL-cholesterol, fasting glucose, etc.) are much less common among MHO than among MAO individuals. These results are in general agreement with prior studies (199, 204) which also failed to show any significant cardiometabolic benefit among MHO individuals undergoing weight loss treatment. The lack of cardiometabolic improvement in response to weight loss is not surprising when considered in light of the normal baseline levels of most cardiometabolic risk factors among MHO subjects. Alternatively, we found no evidence of deterioration in any of the cardiometabolic risk factors assessed among MHO subjects in response to weight loss. Thus, while weight reduction among MHO individuals may not result in appreciable improvement in cardiometabolic status, deterioration of cardiometabolic profile is highly unlikely.

Although leading health authorities recommend weight reduction as the primary treatment strategy for all obese patients, regardless of cardiometabolic status (26-28), it has been suggested by various authors that weight reduction among MHO individuals may be unnecessary given their rather normal metabolic profile (34-36). Indeed, it has recently been recommended that weight maintenance should be the clinical strategy of choice among MHO individuals (37). While limited health care resources dictate the need to prioritize high-risk obese individuals for aggressive treatment, to suggest that obese individuals who are metabolically healthy should not lose weight may not be the most appropriate public health message. Such a public health message may be particularly misguided at a time when the prevalence of obesity and its attendant diseases continues to increase (225, 226), despite a greater public awareness of the benefits of weight loss (227). In fact, the results of the current
study suggest that while cardiometabolic benefit may indeed be modest, a 5-10% weight loss among MHO individuals should still be considered an appropriate clinical treatment option.

Further opposition to the notion that weight loss among MHO individuals may be unnecessary comes from recent studies which document that despite being *metabolically healthy*, MHO individuals are still at greater health risk in comparison to metabolically healthy but lean counterparts (197, 205, 206). For example, Kuk and Ardern (197) report that MHO and MAO individuals are at similarly elevated risk of all-cause mortality in comparison to metabolically healthy and lean individuals – findings which were recently corroborated in a large Swedish sample (206). Additionally, in comparison to those who are metabolically healthy as well as lean, MHO subjects have been reported to show signs of subclinical vascular disease, marked by a significantly greater intima media thickness of the common carotid artery and endothelial dysfunction (205). Thus, it is important to recognize the known benefits of weight loss among obese individuals on mortality (228, 229), vascular health (230) as well as numerous other non-metabolic outcomes including knee osteoarthritis (231), fertility (232), quality of life (233), and sexual function (234).

Although the MHO phenotype has been recognized since the 1980’s (193), there exist no established criteria for the definition of MHO individuals (35). Prior investigations have used arbitrary cut-points (e.g. upper quartile of sample) of insulin sensitivity (31, 33, 198), or the absence of cardiometabolic risk factor clustering (30, 31) to delineate MHO from MAO subjects. Regardless of the definition used, as previously reviewed (8), the common cardiometabolic features of the MHO phenotype include high insulin sensitivity, high levels of HDL-cholesterol, but low levels of triglycerides. As a plausible mechanism for the normal cardiometabolic profile,
it has been reported that for the same BMI, MHO subjects tend to have less fat storage in ectopic depots such as VAT, liver and muscle in contrast to their MAO counterparts (33, 198), thereby being protected from the deleterious metabolic consequences of excess energy storage in these depots (235). However, despite markedly different cardiometabolic profiles, our MHO and MAO groups did not differ in VAT. Alternatively, it has also been suggested that individuals who preferentially store excess energy in the subcutaneous depot of the lower body may be protected against cardiometabolic risk (221). However, in agreement with one prior study (33), we found that the quantity of lower body subcutaneous AT did not differ between MHO and MAO subjects (Table 1).

Given that we used opportunistic data from previously conducted intervention studies, our analysis included the use of multiple modalities of weight loss. Although statistical control for treatment modality in our primary analyses as well as secondary analyses illustrating similar effects of different treatment modalities on insulin sensitivity suggest that weight loss modality did not influence our primary observation, it remains possible that we were underpowered to observe such differences. The definition of MHO and the method of distinguishing MHO from MAO subjects remains a contentious issue. Although we chose to define our MHO and MAO groups according to a clustering of cardiometabolic risk factors, our primary observations regarding improvement in insulin sensitivity remained even when our subjects were categorized solely by tertiles of insulin sensitivity at baseline (Figure 3). Finally, whether weight reduction among MHO individuals has a positive impact on important outcomes not assessed in the current study (i.e. systemic inflammation, levels of adipose derived cytokines, osteoporosis, vascular health, sexual function, etc.) remains unknown.
Our findings do not support the contention that weight reduction among otherwise healthy obese individuals is needless or counterproductive. While cardiometabolic benefit may be modest among the subgroup of MHO individuals, weight loss should still be considered an appropriate clinical treatment option for all obese men and women.
Chapter 6: General Discussion

6.1.0 Implications of Research

6.1.1 Assessment of Obesity Related Health Risk

While it is established that obesity substantially increases the risk of disease (5, 6) and mortality (7), as illustrated in each of the 3 studies conducted, there is considerable heterogeneity in the health risk associated with a given level of obesity. Indeed, for a given BMI level, two individuals could have markedly different health risk, depending on their relative amount of lower body AT, their breast size, or their metabolic profile. While these studies add to the body of literature which suggests that the presence of excess body fat does not always indicate an elevated disease risk, the reverse scenario is also true; the absence of excess weight does not always guarantee a healthy metabolic profile (8). Indeed, significant proportion of normal weight individuals can present with a deteriorated metabolic profile commonly thought to be exclusive to obese individuals. Despite BMIs in the normal range, these individuals, commonly termed metabolically-obese normal weight (MONW), are insulin resistant, dyslipidemic, hypertensive (236, 237) and display an adverse cytokine profile(238, 239). The MONW phenotype, in comparison to healthy normal-weight individuals, is characterized by excess VAT accumulation, fatty liver, and low physical activity levels (8, 236). Thus, it is abundantly clear that clinical assessment of health risk using only BMI criteria is exceedingly myopic.

Of the clinical tools available to aid in the identification of the high-risk obese patient, the measurement of waist circumference along with BMI is one that has received the most
support. Indeed, over a decade ago, the National Institutes of Health had published sex specific waist circumference cut-points to help characterize health risk associated with a given BMI level (Table 2) (27). Despite this positive step forward, a number of issues continue to limit the widespread clinical adoption of waist circumference assessment (240). First, there exists no accepted measurement landmark for measuring waist circumference (241). Additionally, it is suggested that not all individuals with an elevated waist circumference are at increased health risk (242, 243). This latter issue may be partly driven by the inability of waist circumference measurement to distinguish between the two AT depots within the abdomen, VAT and abdominal SAT. In this regard, the findings from study #2 suggest the possible use of breast size as a means to qualify a waist circumference measurement; for a given waist circumference measurement, those women with larger breasts tend to have greater contribution of VAT to the total adiposity in the abdomen. Of course, given the preliminary nature and the inherent limitations of this work (i.e. small sample size, Caucasian subjects, MRI quantification of breast size, etc.) the adoption of breast size measurement in clinical obesity screening is unlikely to occur any time soon. Further, in coining the phenotype of the hypertriglyceridemic waist, others have suggested the combined use of waist circumference measurement along with a fasting triglyceride measure to discriminate between healthy and unhealthy abdominally obese individuals (244). Indeed, it was subsequently reported that for the same waist circumference level, the presence of elevated triglycerides discriminates the high-VAT from the high-SAT abdominally obese phenotypes (245).

Finally, as reported in numerous studies (17-23, 73, 97-101), and corroborated by results in study #1, greater amounts of lower body SAT (for a given amount of total or abdominal obesity) are associated with a healthier metabolic profile. While radiographic
measurement of lower body SAT in clinical practice is unfeasible, hip or thigh circumference measurement can be used as an inexpensive and easy surrogate (103). In fact, as we reported in a prior study, not only does a large hip or thigh circumference (for a given waist circumference or BMI) indicate greater amounts of lower body SAT, these measures also predict less VAT storage (103). Thus, it is possible that hip or thigh circumference could be used to distinguish the high-VAT from the high-SAT abdominally obese patient, much akin to the use of fasting triglycerides or breast size discussed above. Future studies using large representative samples should investigate the utility of combining a measurement of BMI, waist circumference, and hip or thigh circumference in the assessment of obesity-related health risk.

6.1.2 Effects of Weight Loss Intervention

As illustrated in Figure 7 (Chapter 2), current obesity treatment guidelines suggest that all individuals exceeding a BMI of 30 kg/m$^2$ should be counseled to lose 5-10% of their baseline body weight in order to improve current health and reduce future risk of overt disease (34, 35). However, some prior studies have suggested that intentional fat loss among obese individuals may not always be an appropriate clinical option. For example, Okura et al previously documented that in contrast to fat loss from other body regions, fat loss from the lower body may actually result in an exacerbation in metabolic status (99). Given that short of regional liposuction, it is impossible to lose fat from one region of the body (e.g. abdominal) without losing fat from another (lower body), the observations of Okura et al can be simplified to suggest that losing fat per se may be detrimental to metabolic health. Additionally, a number of authors have cautioned against the necessity of weight loss treatment among MHO individuals, as weight loss in these patients may be unnecessary (36, 37, 203) if not outright harmful (38).
In contrast to these prior arguments, the results of studies #1 and #3 fully support current obesity treatment guidelines which recommend modest weight loss for all obese individuals. Indeed, in contrast to the original findings of Okura et al. (99) and the effect of disease driven fat loss (140, 143), we found that the loss of lower body SAT in men and women undergoing exercise and/or diet weight loss intervention is independently associated with improvements in metabolic profile. These findings support the contention that regardless of location, fat loss is likely to benefit metabolic health. Additionally, the primary finding of study #3 of an improved insulin sensitivity among MHO individuals reinforces the benefits of modest weight reduction among all obese individuals, even those who may be classified as metabolically healthy. While our findings of no significant improvement in other metabolic risk factors (i.e. lipids, blood pressure, etc.) were in general agreement with prior weight loss studies among MHO individuals (199, 204), we found no support for the contention that weight loss among MHO subjects may be harmful. The notion that even MHO can benefit from modest weight loss is further supported by recent studies which indicate that despite being metabolically healthy, obese individuals can still be at greater risk of premature mortality and vascular disease (197, 205, 206).

6.2.0 Limitations of Current Research

Given that all three studies in this thesis were conducted using opportunistic data that has been previously collected, the study designs and/or the methodology used within these new studies was limited by the design of the original studies. For example, in the studies looking at the influence of weight loss intervention (studies #1 and #3), we pooled subject data from a number of different weight loss interventions which varied in their mode (diet, aerobic exercise,
resistance/aerobic exercise) as well as in duration. Although the mode of intervention was not a primary focus of these studies, and despite our best efforts to account for these factors in the analyses, it remains possible that some of our findings may be partially confounded by the different interventions. Nevertheless, it is important to note that the original studies from which data was pooled have generally shown similar subject responses regardless of intervention type (14, 209). Additionally, the assessment of breast volume in study #2 was limited by the MRI image acquisition protocol of previous studies which required all participants to lie prone with arms stretched overhead. However, during a time when success rates for acquisition of external research funding are at record lows, and funding for novel research is scarce, the use of data previously collected to answer novel scientific questions certainly has its value, particularly in terms of maximizing scientific output for a given quantity of financial investment.

Finally, as our results are based on studies conducted almost exclusively in abdominally obese and sedentary Caucasian subjects, predominantly from middle-to high socioeconomic class, who all live in the general vicinity of Kingston, Ontario, it is not possible to generalize these findings to other populations. While this is a key limitation given the known influence of ethnicity and socioeconomic status on obesity risk (246), body composition (247) and susceptibility to disease risk (248), it is one that is inherent to research conducted in a university setting in a city with a fairly homogenous population.

6.3.0 Consideration of the Public Health Message

One area which is infrequently considered by researchers is the public health message derived from, or the media’s interpretation of the results of their work. In the areas of research investigated in this thesis, there exists much work with conclusions that cannot be easily
translated into concise health messages, often leading to misinterpretation of findings, and public confusion.

For example, much research which suggests that big hips or thighs or excess lower body adiposity is *health protective* has been often misrepresented in the media and thus misunderstood by the public. Indeed, in response to a recent review on the topic of lower body adiposity (137), a number of news outlets ran stories with captivating and yet misleading headlines such as, “Fat Butts May Be Healthy” or “Fat thighs may benefit health, say researchers.” Unfortunately, as discussed in Chapter 2, lower body SAT is only inversely associated with metabolic parameters after statistical control for total or abdominal adiposity. That is, simple correlation analyses reveal that, just like abdominal SAT and VAT, lower body SAT is positively associated with metabolic risk (19, 21, 23, 83). Unfortunately, this very important caveat is rarely considered. If indeed lower body fat is healthy, as simply suggested by the headlines, such messages may be misconstrued by the public to erroneously indicate that more must be better (presumed benefit of fat gain) while less must be deleterious (presumed danger of fat loss).

The same issues arose when Ray and colleagues originally published their findings indicating that women with large breasts, independent of their BMI or waist circumference level, tend to have greater risk of developing diabetes (24). While the headlines were equally misleading (e.g. “Larger breast size may raise risk of diabetes”), the lack of context in the reports on the study resulted in many large-breasted women responding to the story with suggestions that they will undergo breast reduction to reduce their risk of developing type-2 diabetes (249). Of course, as was originally cautioned by the authors of that study (24), and as the results of
study #2 suggest, breast size may simply be a proxy for other more important causative factors in disease development, such as early onset insulin resistance or increased ectopic fat storage. That is, all current evidence suggests that large breasts are not innately pathological in themselves, and thus their reduction via surgery is unlikely to result in any health benefit.

Finally, the concept of the MHO phenotype and the suggested implications for treatment also pose difficulties in terms of a public health message. While it is clear that there is marked heterogeneity in terms of metabolic health and prospective disease risk among obese individuals, recent studies suggest that despite a healthy metabolic profile, obesity per se can still confer health risk (197, 205, 206). In other words, while some obese individuals can be healthier than others due to differences in genetics, body composition, physical activity levels, age, ethnicity, and others – the same individuals are also generally at higher risk of disease and premature mortality in comparison to equally healthy but lean individuals. Additionally, the results of study #3 suggest that in opposition to previous warnings (36, 38, 204), diet or exercise induced weight loss among MHO individuals is not harmful and may in fact result in further health improvement.

The above scenarios illustrate the need for greater clarity in published research, with more emphasis on the important caveats to the findings, as well as the contextualization of new findings in terms of what is already known. Additionally, it is equally important for researchers to continue their work beyond publishing in peer-review publications by engaging the media and public to help ensure the resulting public message is consistent with the findings of their work.
6.4.0 Directions for Future Research: Connecting the Dots Versus Splitting Hairs

While showcasing the significant heterogeneity of health risk among obese individuals, the obesity literature has become heavily saturated with countless dichotomous categorization systems to delineate the high-risk from the low-risk obese individual; abdominally obese versus gynoid obese, hypertrophic obese versus hyperplastic obese, MHO versus MAO, hypertriglyceridemic versus normotriglyceridemic waist, fit obese versus unfit obese, insulin resistant versus insulin sensitive obese, visceral obese versus subcutaneous obese, and many others. Unfortunately, even though there is considerable overlap between the factors that define each phenotype, these various categorization systems are rarely considered in tandem. For example, high VAT storage is a key defining trait of abdominal obesity, hypertrophic obesity, unfit obesity, and MAO. The same can be argued for hypertrophied SAT adipocytes. Conversely, high levels of physical activity are common to MHO, fit obese, insulin sensitive obese, and subcutaneous obese phenotypes. Rather than developing even more isolated schemes to categorize obese individuals into arbitrary groups, it will be important for future research to bring together the most common and critical features of these overlapping systems. Only when these notions are untangled, can we hope for a significantly improved capacity of primary health care to accurately identify and prioritize for treatment obese individuals at the highest health risk.

6.5.0 Unraveling Conflicting Research Findings

All three of the studies in this thesis were conducted as a response to prior preliminary research findings. In all three cases, our findings were at varying degrees of odds with those of
the original studies. As is often suggested, differences in methodology between our work and that of prior studies may explain the conflicting findings.

In study #1 our assessment of lower body SAT loss independently of changes in abdominal SAT and VAT, was a significant improvement on the methodology previously used by Okura and colleagues (99), the limitations of which were discussed elsewhere. Additionally, given the known effects of aging and menopause among women on loss of peripheral adiposity, we only utilized data on premenopausal women. Thus, our study population was different from that of Okura et al, who combined data from pre and post-menopausal women.

In study #2, while our methodology was not ideal, it was an advancement on the retrospective self-recall of bra cup size used by Ray and colleagues to assess breast size in their study (24). Additionally, it must be noted that the primary outcomes in these two studies (prospective risk of diabetes versus levels of metabolic risk factors or body composition) are sufficiently different from one another as to not permit direct comparisons between the studies. That is, despite the fact we found no significant association between breast volume and metabolic risk factors, our findings do not preclude the possibility that breast volume may independently predict the prospective risk of diabetes. Additionally, differences in the characteristics of subjects used in these two studies further confound direct comparisons. Indeed, in the study of Ray et al the sample of women was predominantly lean, with very few women in the overweight or obese categories. In contrast, our sample consisted exclusively of abdominally obese women. To that effect, another study in the same cohort of women studied by Ray et al found a significant association between breast cup size and breast cancer that was only present in women with a BMI less than 25kg/m² (169).
In study #3, our findings of an improvement in insulin sensitivity in response to weight loss among MHO individuals is in direct contrast to the decrease in insulin sensitivity observed by Karelis and colleagues (38). As a potential reason for the discrepant findings, the means of defining MHO versus MAO differed between the current study and that of Karelis et al. In our study we performed 2 separate analyses with MHO subjects defined by either no clustering of metabolic risk factors, or by insulin sensitivity in the upper tertile within each gender. Karelis et al defined their MHO subjects as those in the upper quartile of insulin sensitivity at baseline. Thus, it remains possible that the MHO group in the prior study was closer to a theoretical ceiling for insulin sensitivity than were the MHO subjects in our study. However, examination of individual subject responses in our study (Study #3, Figure) suggests that even the most insulin sensitive of the MHO subjects showed the same general trend for an increase in insulin sensitivity in response to weight loss.

Additionally, although conflicting results are often chalked up to methodological differences between studies, it must be underscored that such differences, especially when sample sizes are limited, may also be driven by normal physiological variability among human subjects. Indeed, in a series of studies, Bouchard and colleagues have eloquently illustrated that physiological response to a intervention differs significantly between individuals, and is largely determined by genetic predisposition (250, 251). For example, in response to an given exercise intervention, the improvement in cardiorespiratory fitness among sedentary adults can range from absolutely no change to 100% improvement (251). Additionally, in response to the same energy restriction, reductions in VAT and weight can differ by 5-fold between individuals (250).
Chapter 7: Summary and Conclusions

In contrast to healthy and lean individuals, obese individuals are at greater risk of disease and premature mortality. However, a burgeoning literature base, along with the studies presented within this thesis, highlight the considerable heterogeneity in the health risk associated with a given level of obesity. Thus, the continuing challenge in the obesity field will be one of establishing proper clinical classification of health risk, and identifying and prioritizing for treatment those individuals at highest risk of developing disease and early mortality. Despite the heterogeneity in health risk between obese individuals, the findings of the present research fail to support previous contentions that weight loss treatment in certain situations or in certain populations may be unnecessary or harmful. Indeed, the present research suggests that fat loss from all regions of the body is associated with metabolic benefit, and that weight-loss induced metabolic improvements are observed even among otherwise healthy obese individuals. These observations fully support current obesity treatment guidelines which dictate that all obese individuals should be counseled to lose 5-10% of body weight. While the benefits of lifestyle-based weight-loss are clear, motivating the general public to adopt a healthy lifestyle remains a challenge.
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Appendix 1: Example of Consent Form for Participation in Ross Lab Research Study
CONSENT TO VOLUNTEER FOR PARTICIPATION IN A STUDY

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

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

CO-INVESTIGATORS:

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

Robert Hudson, M.D., Ph.D., FRCPC
Kingston General Hospital
Medicine, Division of Endocrinology and Metabolism
Kingston, Ontario, K7L 3N6
533-2973
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 2½-hour appointment for the oral glucose tolerance test (Hotel Dieu Hospital); one 5-hour appointment for the insulin sensitivity and blood lipid/cholesterol tests (Hotel Dieu Hospital); one 2-hour appointment to complete the MRI, MRS, (Kingston General Hospital) and CT tests (Hotel Dieu Hospital); and one 30-minute appointment for the DEXA test. All of these appointments will be scheduled at a time that is convenient for you. Further, each of these tests will be performed twice, once at the beginning and once at the
end of the treatment period.

Risks and Benefits

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

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

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

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

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

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

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

6) The exercise test may cause muscle soreness or fatigue. In any individual there is a minute risk of a heart attack or death from the exercise test. A trained paramedic or medical
doctor will be present. If you develop chest pain the test will be stopped immediately.

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

CONFIDENTIALITY

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

In the event you 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 2: Example of Medical Questionnaire
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 physician 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

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Has your doctor ever said you have heart trouble?</td>
<td>___</td>
</tr>
<tr>
<td>B. Do you get pains, pressure or tightness in your chest?</td>
<td>___</td>
</tr>
<tr>
<td>C. Do you often feel faint or experience dizziness?</td>
<td>___</td>
</tr>
<tr>
<td>D. Has you doctor ever told you that you have high blood pressure?</td>
<td>___</td>
</tr>
<tr>
<td>E. Is there a good reason, not mentioned above, why you should avoid exercise?</td>
<td>___</td>
</tr>
</tbody>
</table>
F. Do you have, or have you ever had, problems with any of the following?

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. Heart or blood vessels</td>
<td></td>
</tr>
<tr>
<td>ii. Nerves or brain</td>
<td></td>
</tr>
<tr>
<td>iii. Breathing or lungs</td>
<td></td>
</tr>
<tr>
<td>iv. Hormones, thyroid, or diabetes</td>
<td></td>
</tr>
<tr>
<td>v. Muscles, joints, or bones</td>
<td></td>
</tr>
<tr>
<td>vi. Other (please list)</td>
<td></td>
</tr>
</tbody>
</table>

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 on erectile function, body composition, metabolic and psychological health. 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, erectile function 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 Kinesiology and Health Studies, Queen’s University (613 533-6583).

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\textsubscript{2}max test)

Cardiovascular fitness is assessed using a maximal oxygen uptake (VO\textsubscript{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
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
Appendix 3: MRI Data Acquisition and Analysis
**Magnetic Resonance Imaging**

**Acquisition of Data**

Whole body MRI is considered by many to be a gold standard technique for determining whole body muscle and total and regional fat deposition. The MR images are obtained within the Department of Radiology at Kingston General Hospital. Whole-body MRI was performed on each participant at study start and end.

**List of Measured Variables**

- Total adipose tissue
- Total subcutaneous adipose tissue
- Visceral adipose tissue
- Abdominal subcutaneous adipose tissue
- Skeletal muscle

**Measurement Device**

General Electric 1.5 Tesla magnet (Milwaukee, WI)

**Measurement Procedures**

The Project Manager will schedule appointments for whole body testing with the MRI technicians at KGH. These appointments are made outside of the normal hours of medical use on the magnet (Saturday mornings).

*Preparing the participant*

A graduate student meets the participant at the main entrance of KGH and escort them to the imaging department. The participant changes into hospital gowns to eliminate clothing-related artifact in the images.
Image Acquisition:

The data acquisition process takes 30-40 minutes. During this time, the technician performs at least 11 scans (including scouts) to acquire cross-sectional images of each participant (see next page for detailed image acquisition protocol).
Figure: An example of the revised Ross protocol that landmarks at the femoral and humeral head.

**Protocol (Abdomen)**

- T1-weighted, spin-echo pulse sequence
- Each image = 10 mm thickness, 40 mm spaces
- TR = 210 ms; TE = 17 ms; 1/2 NEX
- FOV = 48 cm x 36 cm (Rectangular)
- Matrix = 256 x 256
- Each acquisition = 7 images
- Time = 26 seconds (breath hold)

**Sequence of Series to Acquire Images**

1. Sagittal scout to locate L4-L5 & Right Femoral Head
2. L4-L5 down (abdomen protocol)
3. Femoral Head down (appendicular protocol, rectangular FOV)
4. 35 cm below Femoral Head down (appendicular protocol, 1/2 FOV)
5. 70 cm below Femoral Head down (appendicular protocol, 1/2 FOV)
6. Sagittal scout to locate L4-L5
7. Coronal scout to locate Right Humeral Head
8. L4-L5 up (abdomen protocol)
9. 35 cm above L4-L5 up (appendicular protocol, rectangular FOV)
10. Humeral head up (appendicular protocol, rectangular FOV)
11. 35 cm above humeral head up (appendicular protocol, 1/2 FOV)

*For each series of images data is acquired over 310 mm (155 cm above isocentre of magnet, 155 cm below isocentre)*
MRI Data Analysis

The acquired images are burned to compact disk and taken back to an image analysis computer at Queen’s University. For each set of scans, the graduate student will examine the images, identify the images to be used for analysis, arrange them sequentially from inferior (foot images) to superior (finger images), and adapt a program (script file) for the computer to retrieve the images in sequence for tissue analysis.

Determination of tissue area (muscle, fat, organ, etc.) on each image was accomplished using specially-designed image analysis software called Slice-O-Matic, a product of Tomovision (Montreal, Canada). A technician viewed the cross-sectional image and identified muscle, fat, and other lean tissues shown in the image, tracing the gray-level image and tagging the visible tissue area with specific colors (see below).

Figure: An example of a raw and analyzed MR image of the thigh
A completely analyzed mid-thigh image, for example, would have tag colors identifying lower extremity bone, muscle, lean tissue, intramuscular fat, and subcutaneous AT, and the voxel area of the tag labeling each tissue could be quantified, indicating the volume of tissue found in that cross-section of the body that was 1 cm thick.

Since the images are not contiguous, but rather placed at 5 cm centers, the 4 cm of body tissue between images is interpolated mathematically using data from adjacent images. Using voxel summary data from all the images on each whole body scan, Excel spreadsheets are used to run interpolation formulas and calculate whole body tissue volumes (see following page for details). Because each tissue varies in density, we employ tissue-specific conversions to express volumes as masses (in kilograms). For example, skeletal muscle and adipose tissue volumes were converted to masses by multiplying them by 1.04 and 0.92, respectively.
MRI Tissue Area and Whole Body Volume Calculation

1. Area (cm²)
   
   A. Grey Level Histogram
   
   - Grey Level Image
   
   B. Edge Detection

   Voxel Size = \( \frac{FOV}{Matrix} \times \text{Image Thickness} \)

   - FOV = 480 x 480 mm
   - Matrix = 256 x 256
   - Image Thickness = 1 cm
   
   Voxel size = 1.88 mm³

   - Skeletal Muscle (115 cm²)
   - Subcutaneous AT (117 cm²)
   - Interstitial AT (11 cm²)
   - Bone (6 cm²)

2. Volume (cm³, liters) and Mass (kg)

   **Equation 1**
   
   \[
   \text{Volume} = \sum_{i=1}^{N} A_i + \frac{h}{3} \sum_{i=2}^{N} (A_{i-1} + A_i + \sqrt{(A_{i-1} - A_i)})
   \]

   **Equation 2**
   
   \[
   \text{Volume} = \sum_{i=1}^{N} \left( \frac{A_i + A_{i+1}}{2} \right) \times h
   \]

   **Equation 3**
   
   \[
   \text{Mass (kg)} = \text{Volume} \times \text{Tissue Density}
   \]

   - Muscle = 1.04 kg/L
   - Adipose Tissue = 0.92 kg/L
Appendix 4: Measurement of Maximal Oxygen Consumption During Graded Exercise Test
List of Measured Variables

- Maximal oxygen consumption (L/min)
- Maximal oxygen consumption per kg body weight (L/kg/min)

Measurement Devices

- Sensor Medics Vmax29 Metabolic Cart
- Laptop computer
- Treadmill
- Calibration gas tanks (with sufficient gas)
- Polar HR monitor
- USB stick

Measurement Procedures

Cardiorespiratory fitness tests were performed at the Ross Laboratory in the Physical Education Center at the beginning, midway, and end of the study for each participant.

The VMax system (used to measure breath-by-breath gases) was turned on 30 minutes in advance of attempting system calibration. Flow-volume and gas calibration took 30 minutes on average. Each test took approximately 30 to 45 minutes per participant. The participants changed into an athletic shirt and shorts, and wore a pair of comfortable shoes suitable for brisk walking or jogging (they were reminded to bring all items on the day of the test). Subjects wear a Polar heart rate monitor to record heart rates every 20 seconds throughout the test. The test usually lasted 12-15 minutes, beginning with a relatively brisk pace at level grade, increasing grade to 5% at the 3rd minute, and then further increasing the grade by 2% every 2 minutes thereafter. If after 2 minutes at the maximal incline of 15% the subject had not reached exhaustion, the speed was increased (generally by 0.2 mph). Heart rates were observed and recorded on the VO₂ Data Collection Sheet by a Research Assistant, who held a receiver watch.
while standing close to the participant. Breath-by-breath analysis of respiratory gases was recorded throughout the test.

Criteria for a successful VO\textsubscript{2}max test:

There are a number of popular criteria in the literature which are to be used to assess whether the participant being tested has actually achieved VO\textsubscript{2}max.

- Plateau in VO\textsubscript{2} (oxygen uptake) with increasing work rate (increasing treadmill incline, speed or both)
  - Note: Approximately 50% of individuals undergoing VO\textsubscript{2}max testing never reach a true plateau.
- RQ > 1.10: This suggests non-metabolic production of CO\textsubscript{2} and reliance on anaerobic metabolism.
- Heart rate (beats per minute or bpm) exceeding age predicted max HR (220-age) minus 12bpm. For example, for a 20 year old, the HR to be exceeded = 188 bpm (220-20 -12)
- Borg scale=10. This gives the perception of effort by the participant during the test.

*A successful test should meet at least 3 of the above criteria.*

Familiarization

Measuring changes in aerobic capacity requires a comparison of maximal performance on a graded exercise test. For people who have never been on a treadmill and are unaccustomed to pushing themselves physically, obtaining meaningful results can prove difficult. During the run-in period, each participant will be familiarized with the treadmill while their ability and balance are assessed while they demonstrate a few minutes of walking on the treadmill. For some, this required more than one session of practice. The more they comprehend the test and what is required of them, the better the participants respond when encouraged to exercise “to fatigue” during the test.
Appendix 5: Anthropometric Measurement Protocol
Measurement Devices
- Anthropometric tape (Gullick II) - contains a tension indicator device
- Harpendon Calipers
- Detecto Weight Scale
- Stadiometer

Measurement Procedures

WEIGHT AND HEIGHT

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

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

SKINFOLDS (mm)

General Procedure:
1. Do one complete round of all skinfold measurements before repeating the procedure to obtain another measure. Two measures of each site will be obtained (or three if necessary).
2. All measurements are taken on the right side of the body.
3. Ensure participant is relaxed when taking measurements (for each skinfold, position participants as seen in pictures).
4. A fold of skin plus the underlying fat is grasped between your thumb and index finger with the back of your hand facing you. The fingers are placed on the skin
~ 8cm apart and then drawn together to form a fold (see picture below). This fold is pinched 1 cm above the mark you made to locate the spot for measurement.

5. Keep the jaws of the calipers at right angles (perpendicular) to the body surface (the skinfold).

6. Jaws of the caliper are placed 1 cm below the point where the skinfold is raised (directly on the landmark).

7. Maintain pressure of the fingers on the skinfold, fully release caliper trigger, and take measurement within 4 seconds of pinch (longer results in fluid displacement in tissue).

8. Record value to the nearest 0.2mm.

9. The accuracy of skinfold measurement is highly dependent upon the measurer’s ability to rigorously follow the procedure above.

10. Calculate the mean of 2 values for each measure unless the difference between the first and second measure is greater than the intra-measure error listed above. If so, take a third measure of that site and take a mean of the two values that are closest in value. If the three values are equidistant, take a mean of all three.
a) **SUBSCAPULAR**—Locate the inferior angle of the scapula. You may ask the participant to hold their right hand behind their back momentarily to make the scapula protrude, making it easier to landmark. The pinch is taken on a diagonal fold (~45 degree angle), 3cm below the inferior angle of the scapula.
b) **TRICEP** – With the subject standing, right arm bent at a 90-degree angle, palm facing up, use the anthropometric tape to mark the midpoint between the acromion process and olecranon process of the elbow on the lateral side of the right arm. Use the tape to then transfer that mark to the posterior aspect of the arm (midline of back of arm – tricep). The skinfold is taking at a vertical angle with the subject standing relaxed with arm at side.
c) **ILIAC CREST** – Landmark the Iliac crest and make a pen mark 3 cm superior at the mid-axillary line. Get the subject to stand relaxed looking straight ahead with right arm held up on shoulder. Take the skinfold on a 45-degree angle running forward and slightly downward.

![Image showing the Iliac Crest landmark](image)

---

d) **THIGH** – Measure with the anthropometric tape and landmark at the midpoint between the inguinal crease and proximal patella. To obtain this landmark, have participant stand with most of their weight on the left leg. Next, instruct the participant to bring their right leg forward, touch their toe to the floor, and bend the leg. After obtaining the landmark have the subject stand with feet shoulder width apart, keeping slightly more weight on their left leg (this makes it easier to get a skinfold). Take a vertical fold right on the landmark.

![Image showing thigh measurement](image)
CIRCUMFERENCE MEASUREMENTS

For all circumferences, position the tape directly around the body part so that the inferior edge of the tape is at the level of the landmarked point. Ensure there is no clothing under tape (except in Hip Measurement).

a) HIP – should be called “buttocks” measure – at the level of farthest posterior protrusion of the buttocks. Be sure tape does not sag. Ensure the participant is standing with their feet together.

b) GENERAL WAIST PROCEDURE

1. Clear the client’s abdomen of all clothing and accessories. If you find resistance to the suggestion to fully remove shirt, roll up the shirt to allow free access to measurement sites and hold in place with a clip (i.e. hair clip).
2. Position the client with feet shoulder width apart and arms crossed over the chest in a relaxed manner.

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

4. Position the tape directly around the abdomen so that the inferior edge of the tape is at the level of the landmarked point. Use a cross-handed technique to bring the zero line of the tape in line with the measuring aspect of the tape. Ensure that the measuring tape is positioned in a horizontal plane around the abdomen. Apply tension to the tape to ensure it is snug, without causing indentation to the skin. Walk around the participant to ensure the tape is straight all around the abdomen. Alternatively, if a mirror is available – use this to ensure proper tape alignment.
5. At the end of a normal expiration, take the measurement.

**WAIST – ILIAC CREST** - top of the iliac crest. To find this landmark, palpate the upper right hipbone and draw a line where you locate the uppermost lateral border of the iliac crest.

**WAIST – LAST RIB** – bottom of rib cage on right side. To find this landmark, palpate the lower right rib cage and draw a line where you locate the lowest lateral border of the ribs.

**WAIST – MIDPOINT BETWEEN ILIAC CREST AND LAST RIB** – midpoint between the bottom of the rib cage and the top of the iliac crest (use the landmarks from the previous two waist circumference measures to locate this).

c) **BICEP** - Horizontally around upper arm; Right side, use previously made skinfold mark from tricep measurement; Left side, create mark halfway between olecranon and acromion.

d) **PROXIMAL THIGH** - Horizontally around thigh, immediately distal to gluteal furrow.

e) **MID-THIGH** – Horizontally at level of previously made skinfold mark.

f) **CALF** – Horizontally at the widest part of the calf
Appendix 6: Oral Glucose Tolerance Test Protocol
**Background:** The 2-hour oral glucose tolerance test (OGTT) is obtained at our metabolic facilities in the Hotel Dieu Hospital. Briefly, participants are given a specially-prepared drink containing exactly 75g of glucose and were asked to drink it all in less than 10 minutes. When they had finished drinking, a “0-time” blood sample (5mL volume) was taken from the catheter. Samples were taken every 30 minutes thereafter until 120 minutes had passed. The two most important samples for diagnosing Type 2 diabetes are the fasting or “0-time” sample and the sample taken 2 hours after ingestion of the glucose drink. Fasting samples which were greater than 7.0 mmol/L or a 2-hour glucose level greater than 11.1 mmol/L is diagnostic of diabetes, according to the 1999 World Health Organization (WHO) diabetes criteria. Diagnosis of diabetes at baseline was considered cause for exclusion from the studies.

**List of Measured Variables**

- Fasting glucose
- Fasting insulin
- 2-hour glucose
- 2-hour insulin

**Measurement Equipment**

- 2 X 6 mL mauve top tubes: labelled -15 and 0 (mauve top for insulin)
- 2 x 3 mL grey top tubes labelled -15 and 0 – these will be sent to the lab ASAP (i.e. within 2 hours; grey top for glucose)
- 4 Mauve top 3 mL tubes labelled 30, 60, 90 and 120. These are the times you need to take the blood.
- 1 x 3 mL grey top tubes labelled 120
• 20-gauge angio
• 1 interlink cap used to put on the end of the angio
• 1 X10 mL normal saline flush
• Alcohol swab
• 4 x 4 gauze
• 2 x 10 mL syringes with blunt plastic cannula attached to ends
• 1 x 5 mL syringe with blunt plastic cannula attached to end for discarded blood
• 4 x 5mL syringes with blunt plastic cannula attached to end for drawing blood
• 1 opsite to apply over angio
• Tape
• COLD Glucodex (75 g 300 mL) with glass
• Timer
• Paper and pen to keep record of times for blood draw
• Bucket with ice to keep blood cold

**Measurement Procedures**

*Preparing the participant*

Participants are instructed to eat a normal meal the evening before, followed by a 12-hour fast prior to testing. Participants are introduced to the study nurse, who would seat them and briefly explain the test.
OGTT Procedure

Ask the participant when the last time they had something to eat or drink. Do not continue with procedure if they have not fasted for at least 12 hours. Explain to the participant the procedure for taking blood.

1. Start IV with 20 angio with Interlink (cap) in the antecubital vein.
2. Have a 10mL syringe of flush ready.
3. Take your first blood sample with a 10mL syringe, making sure you have a full 10 mL of blood. This will be your –15 time. Put 6mL of blood in the mauve 6mL tube and 3mL in the 3mL mauve top tube.
4. Inject 2.5mL of flush in the angio to keep the line patent.
5. Take your 0 time blood 15 minutes after your –15 time. Follow the above procedure in #3.
6. Give the participant Glucodex 75g 300mL in a cup. They have only 10 minutes to drink it. Sometimes you must encourage them to drink the Glucodex. They must drink ALL of it within the 10 minutes.
7. As soon as they are finished start the timer for 30 minutes. This will start the OGT. You will now take blood samples every 30-minute.
8. Send the –15 and 0 time blood to the CORE lab.
9. Keep the blood samples on ICE at all times.
10. When you have taken your last 120 sample, offer juice, crackers and cheese or peanut butter.
11. Send the 120 blood sample to the CORE lab to be tested.
12. Explain to the participant the importance of having some food after the OGT.
13 Explain hypoglycaemia and the symptoms to the participant. Explain there might be a small chance of feeling weak after the OGT.

14 Once the participant has left, the blood now needs to be tested.

15 Once tested, the needs to be spun in the centrifuge.

16 Each mauve 6 mL top tube requires two aliquots (4 in total) and the 3 mL requires one aliquot for each time taken.

17 Make SURE blood tubes are labelled with code number and times (-15 0 30 60 90 120)

18 Store blood in freezer
Appendix 7: Examples of Statistical Analyses used to Derive Results for Manuscripts 1-3
A: Example of Normalization of Data

Manuscript 1: Histogram of VAT not-normally distributed

VAT Descriptive Statistics: Excessive Skewness and Kurtosis Present

<table>
<thead>
<tr>
<th>Statistic</th>
<th>VATwt1</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2.90273</td>
<td>.120859</td>
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<tr>
<td>Median</td>
<td>2.76000</td>
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<tr>
<td>Variance</td>
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<tr>
<td>Std. Deviation</td>
<td>1.250178</td>
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<tr>
<td>Minimum</td>
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<td>Maximum</td>
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<tr>
<td>Range</td>
<td>7.351</td>
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</tr>
<tr>
<td>Interquartile Range</td>
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<tr>
<td>Skewness</td>
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<tr>
<td>Kurtosis</td>
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<td>.463</td>
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</table>
VAT Tests of Normality. Significant Shapiro-Wilk test (P < 0.001); VAT not normally distributed.

<table>
<thead>
<tr>
<th>Tests of Normality</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Kolmogorov-Smirnov</td>
<td>Shapiro-Wilk</td>
<td></td>
<td></td>
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<td>Sig.</td>
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</table>

a. Lilliefors Significance Correction

Histogram of VAT after log-transformation
Log-transformed VAT Descriptive Statistics: Normal Skewness and Kurtosis

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Statistic</th>
<th>Std. Error</th>
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<td>Variance</td>
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<td>Std. Deviation</td>
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<td>Minimum</td>
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<tr>
<td>Maximum</td>
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<td>Interquartile Range</td>
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<td>Kurtosis</td>
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</tbody>
</table>

Log-transformed VAT Tests of Normality. Non-significant Shapiro-Wilk test (P > 0.05); log-transformed VAT normally distributed.

Tests of Normality

<table>
<thead>
<tr>
<th></th>
<th>Kolmogorov-Smirnova</th>
<th>Shapiro-Wilk</th>
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<tbody>
<tr>
<td></td>
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<td>Statistic df Sig.</td>
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<tr>
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</table>

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.
B. Example of Linear Regression Analyses

**Manuscript 1, Table 3, Model 1:** Cross-sectional association between log-transformed VAT and OGTT glucose, controlling for age and gender

<table>
<thead>
<tr>
<th>Variables Entered/Removed</th>
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<tbody>
<tr>
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</table>

a. All requested variables entered.

<table>
<thead>
<tr>
<th>Model Summary</th>
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<tbody>
<tr>
<td>Model</td>
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</tr>
</tbody>
</table>

a. Predictors: (Constant), Gender, Age, LOGVat1

<table>
<thead>
<tr>
<th>Coefficients*</th>
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a. Dependent Variable: OGTgluAUC1
Manuscript 2, Table 3, Model 3: Independent associations between Abdominal SAT, Lower-body SAT, VAT, IMAT, and breast volume (independent variables) with OGTT glucose (dependent variable), after control for age.

<table>
<thead>
<tr>
<th>Variables Entered/Removed</th>
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<tbody>
<tr>
<td>Model</td>
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<table>
<thead>
<tr>
<th>Model Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
</tr>
<tr>
<td>1</td>
</tr>
</tbody>
</table>

a. Predictors: (Constant), Tot Brst Vol, AGE, LOGLSAT1, TIMAT1, LOGVAT, LOGAbSAT

<table>
<thead>
<tr>
<th>Coefficients²</th>
</tr>
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<tbody>
<tr>
<td>Model</td>
</tr>
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<tr>
<td></td>
</tr>
</tbody>
</table>

a. Dependent Variable: OGTglu1
B. Example of Analysis of Co-variance (ANCOVA)

**Manuscript 3, Table 1**: $2 \times 2$ (gender x metabolic risk category) ANCOVA for baseline differences in VAT at L4L5, co-varying for age

### Between-Subjects Factors

<table>
<thead>
<tr>
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<th>N</th>
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<tbody>
<tr>
<td>SEX</td>
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<tr>
<td>1</td>
<td>46</td>
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<td>2</td>
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### Descriptive Statistics

**Dependent Variable: VATL4L5a**

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<tr>
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### Tests of Between-Subjects Effects

**Dependent Variable:** VATL4L5a

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<th>Mean Square</th>
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</table>

*a. R Squared = .464 (Adjusted R Squared = .443)*

**Note of Interpretation:**
Significant effect of “SEX” term indicates differences in VAT at L4L5 by gender. No significant effect of “metsyn0no1yes” – the factor dividing MHO from MAO groups. Finally no significant interaction of SEX * metsyn0no1yes.
B. Example of Repeated-Measures Analysis of Co-variance (ANCOVA)

Manuscript 3:

Screenshot above illustrates the setup of SPSS to run a mixed factor repeated measures ANCOVA.

The independent variable is insulin sensitivity (MKGSM)

The within subjects dependent variable is time (Pre vs Post intervention), thus in the Within Subjects box the 2 levels (pre/post) of the log transformed insulin sensitivity variable are added (LOGMKGSM1 and LOGMKGSM2).

Given the mixed design, this analysis also includes 2 between subjects variables, SEX and metabolic risk category.

Lastly, the analysis co-varies for age and intervention type
## Output for entire sample:

### Within-Subjects Factors

Measure: MEASURE_1

<table>
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<th>IS</th>
<th>Dependent Variable</th>
<th>MEASURE_1 IS Dependent Variable</th>
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### Descriptive Statistics

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>LOGMKGSM1</td>
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Tests of Within-Subjects Contrasts

Measure: MEASURE_1

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<tr>
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<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
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</thead>
<tbody>
<tr>
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<td>.005</td>
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</tr>
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<td>Linear</td>
<td>.720</td>
<td>103</td>
<td>.007</td>
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<td></td>
</tr>
</tbody>
</table>

Note:
- Main effect of time on IS (log transformed insulin sensitivity)
- Significant time by metabolic risk interaction
- Non-significant covariates

Plot of IS change by metabolic risk category

Estimated Marginal Means of MEASURE_1

Covariates appearing in the model are evaluated at the following values: exORdiet = 1.2202, AGE = 56.61
Primary Output with non-significant co-variates removed from original model:

Tests of Within-Subjects Contrasts

<table>
<thead>
<tr>
<th>Source</th>
<th>IS</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
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</thead>
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<td>.730</td>
<td>105</td>
<td>.007</td>
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</table>

Note:
- Significant effect of time (IS)
- Significant interaction by metabolic risk, so MHO and MAO respond differently

Next step:

Split sample by gender and perform mixed design repeated measures ANCOVA, but with metabolic risk as the only between-subjects factor. This is similar to performing a pair-wise t-test for post-hoc purposes, but allows for the inclusion of covariates in the model (if significant). As per Bonferroni adjustment, significant p value is reduced to <0.025 for the multiple (2) comparisons.
Output for MHO men and women (metsyn0no1yes = 0)

metsyn0no1yes = .00

### Between-Subjects Factors

<table>
<thead>
<tr>
<th>SEX</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
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</tbody>
</table>

### Descriptive Statistics

<table>
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<tr>
<th>SEX</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>N</th>
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<tr>
<td>LOGMKGSM1</td>
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<td>1.1282</td>
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<td></td>
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<td>Total</td>
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<td>.17484</td>
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### Tests of Within-Subjects Contrasts

<table>
<thead>
<tr>
<th>Measure:MEASURE_1</th>
<th>Source</th>
<th>IS</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
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<tr>
<td></td>
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a. metsyn0no1yes = .00
Plot of insulin sensitivity change in MHO men and women, separated by sex

Estimated Marginal Means of MEASURE_1

Note:
- Main effect of time on insulin sensitivity among MHO subjects that does not vary by gender (no SEX interaction)
Output for MAO men and women (metsyn0no1yes = 1)

metsyn0no1yes = 1.00

**Between-Subjects Factors**

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<td>SEX 2</td>
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a. metsyn0no1yes = 1.00

**Descriptive Statistics**

<table>
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<th>Std. Deviation</th>
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<td>LOGMKGSM1 1</td>
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<td>LOGMKGSM1 2</td>
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<td>LOGMKGSM2 1</td>
<td>1.2036</td>
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<td>LOGMKGSM2 2</td>
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a. metsyn0no1yes = 1.00

**Tests of Within-Subjects Contrasts**

<table>
<thead>
<tr>
<th>Source</th>
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<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
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<td>IS</td>
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<td>Error(IS)</td>
<td>Linear</td>
<td>.346</td>
<td>41</td>
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</table>

a. metsyn0no1yes = 1.00
Plot of insulin sensitivity in MAO men and women, separated by sex

Estimated Marginal Means of MEASURE_1

Note: main effect of time on insulin sensitivity among MAO men and women, that did not vary by gender (no significant interaction by SEX)
Appendix 8: Detailed Table of Repeated Measures ANCOVA for Manuscript 3
Table: Effect of exercise or diet intervention among MAO and MHO men and women.

<table>
<thead>
<tr>
<th></th>
<th>Men MAO (n = 20)</th>
<th>Men MHO (n = 26)</th>
<th>Women MAO (n = 23)</th>
<th>Women MHO (n = 40)</th>
<th>Significant Covariate</th>
<th>Main Effect Significance</th>
<th>Time x Sex Significance</th>
<th>Time x Met Risk Significance</th>
<th>Time x Sex x Time Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric</strong></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>-6.0 ± 2.8</td>
<td>-4.0 ± 2.8</td>
<td>-4.9 ± 2.4</td>
<td>-3.0 ± 2.3</td>
<td>age</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.198</td>
<td>0.211</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-1.9 ± 0.9</td>
<td>-1.3 ± 1.0</td>
<td>-1.8 ± 1.0</td>
<td>-1.1 ± 0.8</td>
<td>age</td>
<td>&lt;0.001</td>
<td>0.032</td>
<td>0.175</td>
<td>0.767</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>-6.2 ± 2.8</td>
<td>-5.6 ± 3.2</td>
<td>-4.6 ± 3.2</td>
<td>-4.1 ± 3.3</td>
<td>age</td>
<td>&lt;0.001</td>
<td>0.005</td>
<td>0.738</td>
<td>0.657</td>
</tr>
<tr>
<td><strong>MRI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total AT (kg)</td>
<td>-5.1 ± 2.2</td>
<td>-4.1 ± 2.0</td>
<td>-4.7 ± 2.9</td>
<td>-3.2 ± 2.3</td>
<td>age, mod</td>
<td>&lt;0.001</td>
<td>0.015</td>
<td>0.543</td>
<td>0.831</td>
</tr>
<tr>
<td>Total SM (kg)</td>
<td>-0.8 ± 1.3</td>
<td>-0.2 ± 1.3</td>
<td>-1.0 ± 1.2</td>
<td>0.1 ± 1.0</td>
<td>age, mod</td>
<td>0.139</td>
<td>0.547</td>
<td>0.070</td>
<td>0.450</td>
</tr>
<tr>
<td>Visceral AT (kg)</td>
<td>-0.8 ± 0.4</td>
<td>-0.6 ± 0.4</td>
<td>-0.4 ± 0.4</td>
<td>-0.3 ± 0.3</td>
<td>age</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.663</td>
<td>0.469</td>
</tr>
<tr>
<td>Visceral AT (cm³)</td>
<td>-40.1 ± 26.3</td>
<td>-40.5 ± 34.4</td>
<td>-18.2 ± 24.6</td>
<td>-19.0 ± 21.7</td>
<td>age</td>
<td>&lt;0.001</td>
<td>0.008</td>
<td>0.130</td>
<td>0.277</td>
</tr>
<tr>
<td>Abdominal SAT (kg)</td>
<td>-0.7 ± 0.5</td>
<td>-0.5 ± 0.4</td>
<td>-0.7 ± 0.6</td>
<td>-0.4 ± 0.4</td>
<td>age, mod</td>
<td>&lt;0.001</td>
<td>0.099</td>
<td>0.719</td>
<td>0.998</td>
</tr>
<tr>
<td>Abdominal SAT (cm³)</td>
<td>-40.5 ± 35.4</td>
<td>-28.8 ± 33.8</td>
<td>-39.5 ± 49.0</td>
<td>-21.3 ± 40.1</td>
<td>age, mod</td>
<td>&lt;0.001</td>
<td>0.009</td>
<td>0.636</td>
<td>0.393</td>
</tr>
<tr>
<td><strong>Metabolic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting Glucose (mmol/L)</td>
<td>-0.6 ± 0.7</td>
<td>-0.1 ± 0.4</td>
<td>-0.3 ± 0.8</td>
<td>0.0 ± 0.4</td>
<td>age</td>
<td>&lt;0.001</td>
<td>0.007</td>
<td>0.036</td>
<td>0.288</td>
</tr>
<tr>
<td>Fasting Insulin (UI)</td>
<td>-1.9 ± 4.2</td>
<td>-2.0 ± 3.2</td>
<td>-3.8 ± 4.0</td>
<td>-0.6 ± 3.9</td>
<td>-</td>
<td>&lt;0.001</td>
<td>0.755</td>
<td>0.048</td>
<td>0.030</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>-0.0 ± 0.6</td>
<td>-0.2 ± 0.4</td>
<td>-0.4 ± 0.5</td>
<td>-0.1 ± 0.5</td>
<td>age</td>
<td>&lt;0.001</td>
<td>0.398</td>
<td>0.188</td>
<td>0.050</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>-0.0 ± 0.1</td>
<td>0.0 ± 0.2</td>
<td>-</td>
<td>0.111</td>
<td>0.056</td>
<td>0.076</td>
<td>0.003</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>0.1 ± 0.5</td>
<td>-0.2 ± 0.4</td>
<td>-0.2 ± 0.5</td>
<td>-0.1 ± 0.4</td>
<td>age</td>
<td>&lt;0.001</td>
<td>0.348</td>
<td>0.005</td>
<td>0.070</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>-0.5 ± 0.7</td>
<td>-0.2 ± 0.4</td>
<td>-0.3 ± 0.5</td>
<td>-0.0 ± 0.3</td>
<td>-</td>
<td>&lt;0.001</td>
<td>0.231</td>
<td>0.033</td>
<td>0.342</td>
</tr>
<tr>
<td>Insulin sensitivity (mg/kg-SM/min)</td>
<td>5.7 ± 4.0</td>
<td>3.3 ± 4.6.a,b</td>
<td>4.8 ± 4.9.a</td>
<td>4.2 ± 5.4.a,b</td>
<td>-</td>
<td>&lt;0.001</td>
<td>0.083</td>
<td>0.002</td>
<td>0.535</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>-2.1 ± 11.9</td>
<td>-3.0 ± 11.0</td>
<td>-1.9 ± 18.0</td>
<td>0.1 ± 11.3</td>
<td>mod</td>
<td>0.064</td>
<td>0.489</td>
<td>0.614</td>
<td>0.616</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>-2.9 ± 10.4</td>
<td>-2.1 ± 6.4</td>
<td>0.3 ± 9.9</td>
<td>-1.5 ± 7.1</td>
<td>age</td>
<td>0.112</td>
<td>0.169</td>
<td>0.259</td>
<td>0.341</td>
</tr>
</tbody>
</table>
Notes on Table:

\(^a\) Significant change in variable from pre- to post-intervention

\(^b\) Change in variable was different in MHO versus MAO

\(^c\) Change in variable was different in men versus women

* Variables were log-transformed

- mod, weight loss modality