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The Metabolic Impact of Resting Metabolic Rate and Body Fat Distribution of Obese Adolescents, Aged 14 to 18 Years Old

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THE METABOLIC IMPACT OF RESTING METABOLIC RATE AND BODY FAT DISTRIBUTION OF OBESE ADOLESCENTS, AGED 14 TO 18 YEARS OLD

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MSc (Human Kinetics)

Thesis submitted to the

Faculty of Graduate and Postdoctoral Studies

in partial fulfillment of the requirement for the degree of

Master's of Science in Human Kinetics

Faculty of Health Sciences, School of Human Kinetics

Your file Votre référence
ISBN: 978-0-494-79707-5
Our file Notre référence
ISBN: 978-0-494-79707-5

ACKNOWLEDGEMENTS

This master's project would not have been possible had it not been for the support, advice and encouragement from many individuals that I have had the privilege of meeting throughout the process.

Firstly, I would like to thank Dr. Glen Kenny who provided me with the opportunity to become involved in the HEARTY study. His continuous guidance and encouragement allowed for me to remain motivated and to strive for excellence in accomplishing my goals.

I am forever grateful to Angela Alberga who not only became my mentor but also a great friend. She has provided me with countless hours of guidance and support. Her kindness, openness, and intuitive suggestions have helped me to complete my thesis.

A big thanks to my knowledgeable committee members Dr. Ron Sigal and Dr. Stasia Hadjiyannakis for their time and commitment in providing me with advice to steer my project in the appropriate direction. I am grateful for your insightful suggestions and recommendations. I would also like to extend my thanks to the wonderful HEARTY team, especially Penny Philips, whose organization, patience and hard work have made my project that much easier to complete.

This experience would not have been as pleasant as it was had it not been for my lab colleagues who have provided me not only with advice and suggestions, but also great friendships. Thanks for providing me with a laugh and a listening ear during times of frustration.

Throughout the last two years, I have been lucky enough to have friends whom have been patient with my absence at times. Your endless phone calls and timely distractions have helped me to remain calm and somewhat stress free.

Finally, I would like to thank my mom, dad and my sister Lisa for their love, patience, and endless encouragement, advice and support. Thank you so much for allowing me to understand my full potential and for sharing in the frustration and pleasures that my studies have had to offer. I love you!

ABSTRACT

Background:

The prevalence of childhood obesity has increased dramatically over the past decade, highlighting the necessity to determine its effects on metabolic profile. Research has shown that abdominal obesity is strongly linked to hyperinsulinemia, dyslipidemia, insulin resistance and type II diabetes, leading to an increased risk for the development of the metabolic syndrome. Currently, little is known about the factors that may predispose individuals to the development of this syndrome. Few studies have looked at the effect of resting metabolic rate on the development of the metabolic syndrome. The effect of body fat distribution on metabolic profile is also an important consideration that warrants further investigation.

Objectives:

1) To compare the resting metabolic rate of obese adolescents, in the Healthy Eating, Aerobic and Resistance Training in Youth (HEARTY) trial, with the metabolic syndrome to those without the metabolic syndrome when matched for age, sex and BMI. A secondary objective was to compare the body fat distribution of adolescents with the metabolic syndrome to those with a healthy metabolic profile.

2) To explore the association between adipose tissue distribution (total, visceral, subcutaneous, deep subcutaneous and superficial subcutaneous adipose tissue) and metabolic risk factors (total, LDL-, and HDL- cholesterol, triglycerides, fasting insulin, fasting glucose, 2-hour glucose, HOMA-IR and blood pressure) of obese adolescents in the HEARTY study.

Methods:

1) 41 obese adolescents with the metabolic syndrome, as assessed by the International Diabetes Federation criteria, were matched for BMI, age and sex with 41 metabolically healthy obese adolescents. Resting metabolic rate was acquired using indirect calorimetry. Body composition was quantified using Magnetic Resonance Imaging (MRI).

2) 105 participants were included in the analysis. Body composition variables were determined using MRI. Blood pressure, fasting plasma glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, insulin, and 2-hour glucose were measured. Insulin resistance was assessed using the homeostasis model of assessment score (HOMA-IR).

Results:

1) Obese adolescents with the metabolic syndrome did not differ in resting metabolic rate compared to those without the syndrome. However, adolescents displaying an unhealthy metabolic profile did have greater visceral adipose tissue compared to those displaying a healthy metabolic profile.

2) Blood pressure, fasting insulin and HOMA-IR were significantly correlated with total adipose tissue, visceral adipose tissue, total subcutaneous adipose tissue and superficial subcutaneous adipose tissue. Triglyceride levels significantly correlated with total adipose tissue and visceral adipose tissue. However, on the multiple regression analysis visceral adipose tissue independently predicted total cholesterol, LDL cholesterol, 2-hour

glucose and triglyceride levels, while deep subcutaneous adipose tissue independently predicted blood pressure.

Conclusions:

1) Despite having an unhealthy metabolic profile, adolescents with the metabolic syndrome do not have an altered resting metabolic rate. However, the increased amount of visceral adipose tissue may play a part in the development of the syndrome.

2) Although all body fat depots are significantly correlated with metabolic risk factors, visceral adipose tissue remains more predictive of an adverse metabolic profile.

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1. INTRODUCTION

1.1 General Problem

Over the past 25 years, the prevalence of childhood and adolescent obesity has increased at an alarming rate. The number of overweight Canadian adolescents, aged 12 to 17 years old, has more than doubled and the number that are obese has tripled since 1978 (Shields, 2005). The World Health Organization (2009) has estimated that over 17.6 million children under the age of five are overweight worldwide. Body mass index (BMI) is used to classify youth as having a normal weight, being overweight or obese. BMI is a measure of an individual's weight in relation to his/her height and is highly correlated with body fat (Health Canada, 2003). The Centre for Disease Control and Prevention classifies a child as overweight if their BMI is greater than the 85^{th} percentile for age and sex, and those with a BMI equal to or greater than the 95^{th} percentile as obese based on BMI averages from 2004 (CDC, 2009). Using these criteria, it has been estimated that in Canada approximately 18% of children are overweight and 8% are obese (Shields, 2005).

The increasing rate of childhood obesity has become a serious health concern due to the co-morbidities associated with excess body weight. Researchers consistently find that excess adipose tissue is highly correlated with an increased risk of morbidity and mortality (Wyatt et al., 2006). Obesity in particular has been associated with cardiovascular disease (Field et al., 2001; Guh et al., 2009; Must et al., 1999), diabetes (Field et al., 2001; Guh et al., 2009; Must et al., 1999), asthma (Guh et al., 2009), hypertension (Field et al., 2001; Must et al., 1999), stroke (Field et al., 2001), gallbladder disease (Field et al., 2001; Guh et al., 2009; Must et al., 1999), osteoarthritis (Guh et al.,

2009; Must et al., 1999), sleep apnea (Young et al., 1993), metabolic syndrome (Weiss et al., 2004), and most types of cancer (Field et al., 2001; Guh et al., 2009; Lukanova et al., 2006;). Increased adiposity may also diminish quality of life, (Schwimmer et al., 2003) is associated with a decrease in self-esteem (Friedlander et al., 2003) and depression (Erickson et al., 2000).

Given the magnitude and severity of the problems associated with obesity, efforts to control weight, and promote health and wellbeing are vital. School infrastructure, curricula, and staff knowledge have been targeted to potentially influence the health of youth (Katz et al., 2009). The Government of Canada has also taken a lead role in obesity prevention by promoting healthy eating and physical activity, by implementing policies, and developing programs for obesity prevention (Public Health Agency of Canada, 2007). Obesity research has expanded to understand the predominant factors that may predispose excess adiposity in certain individuals in order to develop effective weight loss strategies. The factors suspected of contributing to obesity include genetic links, economic status, education, social factors, cultural background, and public media (Public Health Agency of Canada, 2007). These factors are all associated with one common problem: an energy imbalance.

Energy imbalances occur when there is an increase in energy intake, a decrease in energy expended or a combination of both. In the past few decades there has been an increase in the consumption of fast foods, pre-prepared meals, soft drinks and candy (Frazao, 1999; French et al., 2001). At the same time, physical activity in youth has dropped dramatically as a result of a rise in the amount of time spent watching television, playing video games and surfing the Internet (French et al., 2001; Janssen et al., 2004).

Increased caloric intake due to fast foods in combination with sedentary behaviour contributes to the upsurge in childhood obesity. Certain individual components of total daily energy expenditure might, however, play a more important role in predisposing certain individuals to excess adipose tissue. These components are resting metabolic rate (RMR), the thermic effect of food and physical activity. RMR accounts for 60-75% of total daily energy expenditure and is the largest component of energy expenditure that may be altered (Brooks et al., 2005). Therefore this component may play a significant role in the regulation of energy balance by promoting weight gain, loss or maintenance.

1.2 Specific Problem

Energy expended to maintain metabolic functions at rest account for the largest proportion of total daily energy expenditure. In fact, RMR has been identified as a potential metabolic predictor of body weight gain (Ravussin & Gautier, 1999). A general consensus exists that absolute RMR is significantly higher in obese than in non-obese subjects (Bandini et al., 1990; Kaplan et al., 1996; Molnár & Schutz, 1997; Tounian et al., 1999; Treuth et al., 1998; Verga et al., 1994). This can be attributed to the greater body size (Laessle et al., 1997) and fat-free mass of obese individuals (Buscemi et al., 2005). Fat-free mass consists of all body tissues including muscle, bone, organs and connective tissue (Brooks et al., 2005). It is well known that fat-free mass is a major determinant of RMR (Butte et al., 2007) accounting for 65-90% of its inter-individual variance (Cunningham, 1991; Elia, 1992; Garby et al., 1988; Ravussin & Bogardus, 1989). A decrease in fat-free mass may induce a significant reduction in RMR (Martin et al., 2007). With an increase in body weight, RMR per kg of fat-free mass decreases because of a disproportional increase in muscle mass (Weisier et al., 1992). When

evaluating differences in RMR between groups, fat-free mass must be taken into account to ensure differences are not solely due to larger amounts of metabolically active tissue. When fat-free mass is statistically controlled for, selected studies have found no difference between obese and non-obese subjects (Ekelund et al., 2002; Molnár & Schutz 1997; Schutz et al., 1999; Treuth et al., 1998) while others have found RMR remained significantly higher (Bandini et al., 1990; Van Mil et al., 2001) or lower (Laessle et al., 1997) in obese children and adolescents. Individuals with excess body weight, especially adiposity within the abdominal region, often display metabolic abnormalities associated with the criteria for the identification of the metabolic syndrome (International Diabetes Federation, 2007). According to the International Diabetes Federation (2007) those classified with the metabolic syndrome if they are 10-16 years old must have a waist circumference>90^{th} percentile or the adult cutoff of ≥94 cm for men and ≥80 cm for women in order to be classified as having the metabolic syndrome. If participants over the age of 16 years old had a waist circumference of ≥94cm for boys and ≥80cm for girls and also possessed two or more of the following criteria: raised triglycerides>1.7 mmol/L (>150 mg/dL), reduced HDL-cholesterol <1.03 mmol/L (<40 mg/dL), raised blood pressure with systolic blood pressure >130 mmHg and diastolic blood pressure>85mmHg, fasting plasma glucose ≥5.6 mmol/L (≥100 mg/dL), or known type 2 diabetes. The number of children exhibiting at least two of these criterions has risen in proportion with the rise in population obesity (Weiss et al., 2004). Notably, one in ten adolescents aged 12 to 19 years old has the metabolic syndrome and when a child is obese the odds of having the metabolic syndrome increases to one in three (de Ferranti et al., 2004). Many studies have found that an increase in visceral adipose tissue may be the

precursor to the development of metabolic abnormalities associated with the metabolic syndrome (Fox et al., 2007; Syme et al., 2008). Others have revealed that subcutaneous adipose tissue may play a role as well (Goel et al., 2010; Kelley et al., 2000) as deep subcutaneous adipose tissue has been shown to exhibit metabolic properties similar to visceral adipose tissue.

Some studies have shown that the metabolic syndrome may affect RMR however; controversy still remains as to whether the syndrome reduces (Buscemi et al., 2007) or increases it (Jacobson et al., 2006). Individuals with the metabolic syndrome may have a higher RMR due to hyperinsulinemia, which has been shown to increase sympathetic nervous system activity thereby leading to an increase in RMR (Després et al., 2008). However, others have suggested that those with the metabolic syndrome may have a "thrifty" gene, which is theorized as having an effect in decreasing muscle mitochondrial activity in individuals with the metabolic syndrome leading to a lowered RMR (York & Bouchard, 2000). The metabolic abnormalities associated with the metabolic syndrome may have an impact on RMR however; many of these factors have not been studied. Of these factors, visceral adipose tissue (Armellini et al., 2000; Leenen et al., 1992; Sharp et al., 2002), subcutaneous adipose tissue (Armellini et al., 2000), blood pressure (Bosy-Westphale et al., 2008; Luke et al., 2004; Snoodgrass et al., 2008) and insulin resistance (Armellini et al., 2000; Bosy-Westphale et al., 2008) have been shown to correlate with RMR. Whether waist circumference in relation to visceral or subcutaneous adipose tissue, triglyceride levels, HDL-cholesterol levels, blood pressure or fasting plasma glucose levels best predict RMR remains to be determined.

1.3 Objectives

The primary objective of the **H**ealthy **E**ating, **A**erobic and **R**esistance **T**raining in **Y**outh (**HEARTY**) randomized controlled trial is to evaluate the effect of resistance, aerobic, and combined aerobic and resistance training on body composition in sedentary, post-pubertal overweight or obese adolescents aged 14 to 18 years old. A substudy of the HEARTY trial will be performed where the main purpose is to compare the RMR of obese adolescents with the metabolic syndrome to those without the metabolic syndrome. A sub-objective to this sub-study will be to explore the association between adipose tissue distribution (total, visceral, subcutaneous, deep subcutaneous and superficial subcutaneous adipose tissue) and metabolic risk factors (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, fasting insulin, fasting glucose, 2-hour glucose, HOMA-IR and blood pressure) in obese adolescents, aged 14-18 years old.

1.4 Hypotheses

We hypothesize that obese adolescents with the metabolic syndrome will have a higher RMR compared to obese adolescents without the metabolic syndrome after matching for age, sex and body mass index (BMI).

For our second purpose, we hypothesize that visceral adipose tissue will independently predict more metabolic risk factors than will total body adipose tissue, total body subcutaneous adipose tissue, deep subcutaneous adipose issue and superficial subcutaneous adipose tissue.

1.5 Relevance

As the metabolic syndrome is associated with higher risk of developing cardiovascular disease, diabetes and all-cause mortality (Ford, 2005), the increase in prevalence amongst youth is of substantial concern. This syndrome may progress into adulthood causing an increase in the severity of the co-morbidities associated with the syndrome such as diabetes, cardiovascular disease and increased mortality (Katzmarzyk et al., 2001; Sun et al., 2008). Studying factors that may lead to the onset of the metabolic syndrome is critical for understanding the mechanisms that may be responsible for its development. An energy imbalance through a change in RMR or specific body fat distribution may help to explain why certain individuals may be predisposed to the metabolic syndrome.

This newly acquired information may be used to help alleviate or reduce the effects of associated co-morbidities through the alteration of RMR or through the reduction in certain fat depositions. It may provide clinicians, exercise specialists, physicians and educators with vital information to implement adequate obesity prevention programs or interventions to alter a potential energy imbalance that may be exhibited by those with the metabolic syndrome. This study may also provide insight into a mechanism whose modification could reduce the metabolic abnormalities associated with the metabolic syndrome or improve the condition of those affected by the syndrome.

1.6 Limitations and Delimitations

Data collection for the HEARTY study began in 2005 and is still ongoing. For the purpose of this sub-study, a current sample of baseline RMR and Magnetic

Resonance Imaging (MRI) data will be used therefore; results from a complete data set will not be presented.

The study sample is delimited to patients who meet all the inclusion criteria and may not be applicable to people who are lean and active, under the age of 14 years old or over the age of 18 years old. Pubertal maturation, growth and development are different in pre-pubertal and post-pubertal children and thus, body composition differs as well. In essence, results of this study will be limited to obese, sedentary adolescents.

2. LITERATURE REVIEW

2.1 Introduction to Childhood Obesity

During the past two decades the prevalence of youth obesity (defined as body mass index (BMI) above the 95th percentile for age and sex from BMI charts developed in 2004) has risen dramatically worldwide (Barnow et al., 2003; CDC, 2009; Shields et al., 2005). From 1999/2000, 10% of children were obese and by 2003/2004 this rate had increased to 13% (Lobstein & Jackson-Leach, 2007). This is of grave concern as childhood obesity has been associated with a wide range of serious health complications including orthopedic problems, metabolic disturbances, type 2 diabetes, sleep apnea, immune system dysfunctions, increased blood pressure and hypertension (Wabitsch, 2000). The complications associated with childhood obesity have direct implications on the severity of long-term health consequences associated with adulthood obesity as the obese child tends to become an obese adult (Guo et al., 2002; Whitaker et al., 1997). The related continuity of health complications into adulthood include cardiovascular disease (Field et al., 2001; Guh et al., 2009; Must et al., 1999), diabetes (Field et al., 2001; Guh et al., 2009; Must et al., 1999), asthma (Guh et al., 2009), hypertension (Field et al., 2001; Must et al., 1999), stroke (Field et al., 2001), gallbladder disease (Field et al., 2001; Guh et al., 2009; Must et al., 1999), osteoarthritis (Guh et al., 2009; Must et al., 1999), the metabolic syndrome (Weiss et al., 2004) and most types of cancer (Field et al., 2001; Guh et al., 2009; Lukanova et al., 2006;). Moreover, adults who were obese as children have an increased risk of morbidity and mortality (Must et al., 1999).

Although genetics have been shown to be a determinant of obesity (Frayling et al., 2007), trends seem to be more associated with a sedentary lifestyle and an increase in

the consumption of sweetened drinks, and energy dense foods that are low in fiber and high in sugar (Astrup et al., 2008). By maintaining this lifestyle, an energy imbalance may occur as a result of a decrease in the energy expended and an increase in energy consumed thus potentially leading to increased adiposity.

2.2 Metabolism

Metabolism is defined as the sum of the total processes occurring in a living organism (Brooks et al., 2005). These processes create energy required for internal and external work, but mostly produce heat. Energy balance occurs when the caloric consumption is equal to the total amount of energy expended by the body. A positive energy balance occurs when energy intake exceeds energy output, which may eventually result in obesity. A negative energy balance results in weight loss due to an increase in energy expenditure compared to caloric intake. Energy intake is the caloric or energy content of food that can be stored in the body in the form of fat, glycogen or protein, and can also be used to fuel the body for energy-requiring functions. The body is able to expend this energy by way of activity thermogenesis, the thermic effect of food and resting metabolic rate (RMR).

2.2.1 Activity Thermogenesis

Activity thermogenesis is the most variable component of total daily energy expenditure accounting for 15% of the energy expended in sedentary individuals to approximately 50% in highly active individuals (Dauncey, 1990; Livingstone et al, 1991). Activity thermogenesis can be subdivided into two categories: exercise-related activity thermogenesis and non-exercise activity thermogenesis. Exercise-related activity

thermogenesis is the energy expended through the use of skeletal muscles while doing planned physical activity (Levine, 2007). Non-exercise activity thermogenesis is the energy expended while doing unplanned activities such as sitting, standing, walking, talking, and shopping (Levine, 2007).

2.2.2 Thermic Effect of Food

In addition to activity thermogenesis, there is an increase in energy expenditure in response to food intake (Jequier & Schutz, 1988). This increase in metabolic rate after a meal is referred to as the thermic effect of food and constitutes to approximately 10% of energy expended (Schutz et al., 1984). It is the energy used to consume and break down food so it may be used as energy (Goran, 2000). The amount of energy used to break down the meal is largely influenced by energy content of a meal, but not by meal composition (Kinabo & Durnin, 1990).

2.2.3 Resting Metabolic Rate

Although the previous components of total energy expenditure consume a considerable amount of energy, resting metabolic rate (RMR) consumes a substantially larger proportion. On average, a human adult's RMR is approximately 1 kcal/kg/min (Goran, 2000) and accounts for approximately 60-75% of total daily energy expenditure (Brooks et al., 2005). The energy expended via RMR is used to sustain life by maintaining vital functions such as heartbeat, muscle contraction and function, and respiration (Brooks et al., 2005). It is generally measured after an 8-12 hour fast while the subject is awake and resting in a quiet, warm environment. RMR occurs throughout a 24-hour day and remains relatively constant within individuals over time (Goran, 2000).

Since it contributes a substantial proportion of daily energy expenditure, RMR may play a significant role in energy balance and weight regulation. It has been shown that a low RMR, after the effects of body composition are removed, may be a predisposing risk factor for the development of obesity (Ravussin et al., 1988; Weyer et al., 1999a).

2.3 Resting Metabolic Rate and Body Weight

The concept of energy balance holds that obesity is the result of energy intake exceeding that of energy expenditure. Studies comparing energy intake of obese and non-obese children have found inconsistent differences between groups (Epstein et al., 1991; Laessle et al., 1998). In fact, Laessle et al. (1998) found that obese children, aged 8-12 years old, consumed 940 kJ/day less than non-obese children. One possible interpretation for this difference in findings is overweight youth may maintain their weight or cannot easily lose weight because they have an increased metabolic efficiency via a reduced RMR. Another possible explanation could be due to the inaccuracy of self-reported food intake in children.

Ravussin et al. (1988) and Roberts et al. (1988) have suggested a significant relationship between RMR and body weight gain. For example, Ravussin et al. (1988) studied a group of Pima Indians, as they are known to have a greater predisposition to obesity. They demonstrated that among Pima Indians, those with a lower RMR, after accounting for differences in body composition, were found to be at a greater risk for body weight gain over a four-year period. It has also been demonstrated that children of obese parents compared to children of lean parents have a lower RMR (Griffiths et al., 1990; Roberts et al., 1988). These studies as well as those on families (Bogardus et al., 1986) and comparisons of mono- and dizygotic twins (Bouchard et al., 1989) attribute

this greater risk for body weight gain to a decrease in RMR, possibly due to genetics, rather than an increase in energy intake. Genetically determined reductions in RMR may be exacerbated in an environment that promotes food intake and physical inactivity thereby creating larger susceptibility to obesity.

Studies comparing obese and lean, children and adolescents have found obese youth have a higher absolute RMR (Bandini et al., 1990; Butte et al., 2007; DeLany et al., 1995; Fontvieille et al., 1992; Griffiths et al., 1990; Maffeis et al., 1993; Molnár & Schutz, 1997; Tounian et al., 1999; Treuth et al., 1998; van Mil et al., 2001) compared to age-matched non-obese youth. Tounian et al. (1999) observed that obese girls, aged 13-16 years old, had a RMR 16% higher compared to age-matched lean girls. This difference in RMR disappeared when adjustments for body composition differences were made (DeLany et al., 2004; Ekelund et al., 2002; Molnár & Schutz, 1997; Treuth et al., 1998). DeLany et al. (1995) studied RMR in forty-six boys and girls aged 10 years old. They divided them into tertiles based on the sum of the subscapular plus tricep skinfolds whereby the lowest tertile weighed 14kg less than the highest tertile. There were no significant differences in RMR across the three tertiles.

In Italian children (Maffeis et al., 1993) aged 8-18 years old, those who were obese had similar values of RMR to age-matched controls after correcting for differences in fat-free mass. Measurement of energy expenditure in a 24-hour metabolic chamber, total free-living energy expenditure tested using doubly-labeled water and body composition, measured by dual-energy X-ray absorptiometry, were compared in twelve overweight and twelve normal weight children (Treuth et al., 1998). RMR was significantly higher in obese girls however there were no differences after taking fat-free

mass into account. These studies show that RMR, determined by a variety of methods, is higher in overweight children. This effect can be attributed to an increase in metabolically active lean tissue (Bandini et al., 1990) or sympathetic nervous system activity (Ravussin & Tataranni 1996; Spraul et al., 1993) that accompanies fat gain.

2.4 Resting Metabolic Rate and Body Composition

Differences in body composition have often been examined in conjunction with RMR as it may result in between-group differences observed. Both fat-free mass and fat mass make a significant contribution to the variability in RMR between individuals. When comparing RMR between groups of individuals, these two components should be considered.

2.4.1 Fat-Free Mass

The main source of individual variability in RMR occurs predominantly in muscle and major organs of the body. Any mass that is not fat within the body is defined as fat-free mass. This component has been found to be the single most significant determinant of RMR accounting for 50 to 80% of the variation between individuals (Buscemi et al., 2007; Butte et al., 2007; Derumeaux-Burel et al., 2004; Goran et al., 1998; Kirkby et al., 2004; Lazzer et al., 2004; Leibel et al., 1995; Martin et al., 2007; Neuhauser-Berthold et al., 2000; Poehlman et al., 1997; van Mil et al., 2001). Since fat-free mass makes up a heterogeneous mixture of all components of the body that are not fat mass, the quality of the fat-free mass in terms of hydration and contribution of each individual organ directly influence RMR per kilogram of this tissue (Goran, 2000). Holliday (1986) and Forbes (1987) found that muscle and organ tissue combined constitute approximately 50% of the

total weight of fat-free mass. The other half of fat-free mass includes bone mass, extracellular fluid, blood plasma and other tissues.

Skeletal muscle and organs comprise the majority of RMR from fat-free mass. Although skeletal muscle makes up approximately 43% of body weight in an adult, it only constitutes 22% to 36% of RMR (Goran, 2000; Müller et al., 2002). The visceral organs and the brain encompass approximately only 5% of body weight. Despite its minor contribution to body weight, these organs account for 70% to 80% of RMR in relation to fat-free mass (Gallagher et al., 1998; Müller et al., 2002) indicating these tissues may be metabolically more active compared to skeletal muscle (Goran, 2000). The heterogeneous composition of fat-free mass may explain why RMR per kilogram of fat-free mass does not remain constant. With an increase in body weight, RMR per kg of fat-free mass decreases because of a disproportional increase of muscle mass (Weisier et al., 1992). Throughout development, the metabolic cost of each kilogram of fat-free mass decreases perhaps due to the increase in muscle mass-to-organ ratio within fat-free mass.

The relationship between RMR and fat-free mass across all ages is non-linear (Weisier et al., 1992). From birth to 2.5 years old RMR per kilogram of fat-free mass is estimated to be 79.2 kcal/kg, 36.0 kcal/kg in 4 to 7 year old children, 28.3 kcal/kg during adolescence, and 21.0 kcal/kg in adulthood (Weisier et al., 1992). The relationship between fat-free mass and RMR has therefore been investigated to explain differing rates of weight gain in various groups.

2.4.2 Fat Mass

RMR may also be influenced to a lesser degree by fat mass even though fat mass is generally thought to be metabolically inert (Bogardus et al., 1986). The role of fat

mass as a factor influencing RMR has been a matter of debate. Some studies have found a significant relationship between RMR and fat mass (Johnstone et al., 2005; Lazzer et al., 2007; Lührmann et al., 2001; Molnár & Schutz, 1997; Nelson et al., 1992; Sparti et al., 1997; Svendsen et al., 1993; Tataranni & Ravussin, 1995) while others have not been able to find this correlation (Bogardus et al., 1986; Segal et al., 1987). The contribution of fat mass to RMR is generally explained by the energy that adipose tissue requires, which is approximately 10 to 13 kcal/kg/day for RMR (Goran et al., 1994). The location of the adipose tissue may also have an effect on RMR depending on its location.

The metabolic activity of fat mass located in the abdomen, especially in the visceral region, has been found to be higher than that in the gluteal-femoral region (Arner, 1995; Hoffstedt et al., 1997; Jones et al., 1996a; Lührmann et al., 2001; Millet et al., 1998). Westrate et al. (1990) studied 32 premenopausal obese women and subdivided them into 3 groups (gluteal-femoral, intermediate and abdominal) according to their waist-to-hip ratio. After accounting for fat-free mass, fat mass and age, RMR was significantly higher in the abdominal group than in any other group. It may be concluded that adipose tissue located in the abdominal region may affect RMR. However, two types of adipose tissue exist in the abdominal region: visceral and subcutaneous adipose tissue.

2.4.2.1 Visceral Adipose Tissue

Visceral adipose tissue is located within the abdominal cavity and surrounds internal organs. Some studies have shown a relationship between abdominal visceral adipose tissue and RMR (Armellini et al., 2000; Leenen et al., 1992; Sharp et al., 2002) whereas others have not (Armellini et al., 1992; Macor et al., 1997; Nicklas et al., 1995). Armellini et al. (2000) conducted a study to determine if visceral adipose tissue,

determined by computed tomography (CT), was a significant predictor of RMR in 82 premenopausal women (18-52 years old), 27 postmenopausal (46-71 years old) obese women, and 21 men (18-70 years old). Their study revealed that those with a larger amount of visceral adipose tissue had a higher RMR. However, Macor et al. (1997) found contradictory findings whereby visceral adipose tissue did not correlate with RMR in 26 obese and 9 healthy premenopausal women. Discrepancies in results could be primarily due to sample size rather than method of measurement as these studies determined visceral adipose tissue mass by magnetic resonance imaging (MRI) or CT.

The higher RMR in the visceral region found by some studies may be attributed to the special metabolic characteristics of the abdomen. This tissue has an increased blood flow, responds better to norepinephrine, a lower sensitivity to insulin, an increased sympathetic nervous system activity and a high rate of lipolysis (Arner et al., 1995; Hoffstedt et al., 1997; Jones et al., 1996a; Millet et al., 1998). The lower sensitivity to insulin in visceral adipose tissue may be a predominant factor inducing the increased RMR in individuals with a larger amount of this tissue. Insulin resistance has been shown to increase RMR in diabetics (Misra & Vikram; 2003; Weyer et al., 1999a) however it has been speculated that this is primarily through its ability to elevate sympathetic nervous system function (Fontvieille et al., 1992b).

2.4.2.2 Subcutaneous Adipose Tissue

Although it has been found that visceral adipose tissue is more closely correlated to RMR than subcutaneous adipose tissue (Armellini et al., 2000; Leenen et al., 1992), it has been shown that subcutaneous adipose tissue is inversely related to RMR (Leenen et al., 1992). Leenen et al. (1992) found that visceral adipose tissue, determined by MRI,

was more significantly correlated with RMR in women however they did find a correlation with subcutaneous adipose tissue. No significant correlation was found in men for either visceral adipose tissue or subcutaneous adipose tissue. This suggests that there may be a difference in the metabolic properties of adipose tissue between the sexes.

Currently there exists little evidence regarding the correlation of the different subcutaneous adipose tissue layers on RMR. Subcutaneous adipose tissue can be divided into two layers through the identification of the fascia superficialis: the superficial and the deep subcutaneous layers. There are important morphological differences between these two layers. The fat lobules in the superficial layer are tightly packed whereas those in the deep layer are irregular and less organized (Markman & Barton, 1987). It has been suggested that deep subcutaneous adipose tissue behaves like visceral adipose tissue because it is strongly correlated with insulin resistance (Simonsen et al., 1994). Kelley et al. (2000) and Misra & Vikram (2003) conducted studies to determine if deep subcutaneous adipose tissue could provide insight into the relationship between obesity and metabolic abnormalities. They found that deep subcutaneous adipose tissue was a better predictor of insulin resistance than visceral adipose tissue. Some studies have also found this relationship (Smith et al., 2001; Toth et al., 2001) whereas others have not (Lovejoy et al., 2001; Ross et al., 2002b), creating controversy as to which adipose tissue layer may contribute to metabolic abnormalities. Since insulin resistance has been shown to increase RMR in diabetics (Misra & Vikram; 2003; Weyer et al., 1999a), it can be theorized that with increasing deep subcutaneous adipose tissue, like visceral adipose tissue, RMR may become more elevated.

Together fat-free mass and fat mass contribute significantly to differences in RMR observed between individuals. Each kilogram of fat-free mass exerts 5 times more of an effect on RMR than fat mass (Johnstone et al., 2005) thereby inducing a greater effect on RMR.

2.5 The Effect of the Sympathetic Nervous System on RMR

The sympathetic nervous system and the adrenal medulla are major regulators of body homeostasis. The sympathetic nervous system participates in the regulation of the cardiovascular system and blood pressure by controlling cardiac output and vascular resistance (Tataranni, 1997; Wilmore & Costill, 2004). It also regulates body temperature, digestive secretions and respiratory functions, dilates pupils, and affects the endocrine organs such as the pancreas and adipose tissue by affecting fuel mobilization (Tataranni, 1997; Wilmore & Costill, 2004). This system exerts its effects either directly by stimulating sympathetic nerve endings or indirectly through the circulation of catecholamines (Brooks et al., 2005; Tentolouris et al., 2006).

There is now ample evidence that the sympathetic nervous system may also play a vital role in the regulation of energy expenditure causing variability in RMR. Schwartz et al. (1988) reported clonidine, a central sympathetic inhibitory agent, causes a 6% decrease in RMR. It has also been shown that ß-adrenergic blockage with nadolol reduces RMR significantly by 7% without changes in thyroid hormone levels (Welle et al., 1991) by reducing sympathetic nervous system activity. Conversely, a single dose of sibutramine has been demonstrated to contribute to an increase in RMR by 3-5%, attributed to the increase in plasma epinephrine levels (Hansen et al., 1998) causing elevation in sympathetic nervous system activity.

Many animal studies suggest low sympathetic nervous system activity is associated with obesity. Early studies done by Knehans et al. (1983) and Levin et al. (1983) found sympathetic nervous system activity is low in genetically obese rodents resulting in decreased energy expenditure. In humans, there is growing evidence that obese subjects have increased sympathetic nervous system activity (Jones et al. 1996b; Poehlman et al. 1995; Sherrer et al. 1994; Spraul et al. 1993). By using microneurography, Spraul et al. (1993) showed muscle sympathetic nerve activity was increased in direct proportion to body fat in 19 Caucasian subjects. Sherrer et al. (1994) confirmed these results in a group of Swiss volunteers.

Furthermore, it has been shown higher sympathetic nervous system activity is related to adiposity development in the abdomen rather than peripheral regions (Jones et al., 1996b; Leonetti et al., 1991; Ng et al., 1993; Poehlman et al., 1997; Poehlman et al., 1995). Poehlman et al. (1995) examined norepinephrine kinetics in 69 younger (18-36 years old) and 69 older (55-80 years old) men to understand the role of total and central body fat in sympathetic nervous system activity. Their findings suggest total fat mass and central body fat accumulation is strongly related to higher levels of norepinephrine appearance. Norepinephrine is an indicator of sympathetic outflow therefore these results indicate that total and central body fat is more strongly associated with an increase in sympathetic nervous system activity.

Taken together, these results suggest an increase in total and central body fat is associated with higher sympathetic nervous system activity. A decrease in RMR may be mediated through a reduction in sympathetic nervous system activity however; this decrease does not exceed 6-12% (Tentouloris et al., 2006).

2.6 Other Factors Affecting RMR

2.6.1 Blood Pressure

Increased adiposity has been identified as a predictor of an increased risk of hypertension (Brown et al., 2000) however the underlying physiological mechanisms linking obesity to blood pressure are poorly understood. Researchers have strived to understand how an increased blood pressure may metabolically affect those who are obese. Some studies, examining the effect of blood pressure on RMR, have found that there exists a significant association between these two variables (Bosy-Westphale et al., 2008; Luke et al., 2004; Snodgrass et al., 2008) even when fat-free mass, fat mass, sex and age were adjusted for. RMR has been found to be 9% higher in 43 obese hypertensive patients when compared to 27 obese normotensive patients (Kunz et al., 2000). On the contrary, DeLuis et al. (2005) reported that no association exists between RMR and systolic blood pressure in 87 obese male and female non-diabetic patients. Differences in results cannot be attributed to experimental design as all studies performed RMR testing in the supine position after a 12-hour fast for a period of 30 minutes. The discrepancy in results may be due to differences in sample sizes where DeLuis et al. (2008) had a smaller sample size compared to other studies (Bosy-Westphale et al., 2008; Luke et al., 2004; Snodgrass et al., 2008).

The mechanism responsible for the relationship between blood pressure and RMR are currently unknown. Snodgrass et al. (2008) hypothesized that this relationship may be due to an increased sympathetic nervous system. This relationship may also be mediated by thyroid hormones. RMR and thyroid hormones are closely related through their effects on the rates of oxidative metabolism in most tissues (Guyton & Hall, 2006).

Developmental effects may also link blood pressure and RMR. Low birth weights have been found to be associated with higher blood pressure in adulthood (Adair & Dahly, 2005) and high sleeping metabolic rate (Weyer et al., 2000). This raises an interesting possibility that development due to birth weight may regulate metabolism.

2.6.2 Insulin Resistance

Obesity has been associated with an increased risk for the development of insulin resistance (Pratley et al., 2000). For this reason, fat-free mass and fat-mass must be taken into consideration when determining whether insulin resistance can be associated with RMR. Many studies use the homeostatic model assessment for insulin resistance (HOMA-IR), calculated from fasting insulin and glucose measures, as a method of determining insulin resistance.

A significant correlation between RMR and insulin resistance using HOMA-IR has been found in many non-diabetic adult studies (Armellini et al., 2000; Bosy-Westphale et al., 2008). These results are consistent with those of Blonk et al. (1994) who investigated the determinants of insulin sensitivity in adults with type 2 diabetes mellitus, using the euglycemic clamp technique. It was found that percent body fat and RMR were the main determinants of insulin sensitivity in these men and explained 44% of the variation in RMR. A higher than normal RMR was associated with insulin resistance in patients with liver cirrhosis regardless of diabetes (Perseghin et al., 2002). On the contrary DeLuis et al. (2005) found no correlation between RMR and insulin resistance in 87 obese non-diabetic patients. Their subjects had very low insulin resistance, which may have caused difficulty in determining any statistically significant change in RMR.

In young children the results are equivocal as demonstrated by Kirkby et al. (2004). They found a modest correlation between HOMA-IR and RMR in young girls however this significance was lost once adjusted for tissue mass. On the contrary, their sample of young boys displayed a small and statistically significant negative correlation even when adjusted for tissue mass. For boys both FM and RMR explained 11% of the variation in HOMA-IR. Increased protein turnover, futile cycling, gluconeogenesis and SNS activity have been the reasons to explain why insulin resistance may lead to a higher RMR (Weyer et al., 1999b), however these theories remain to be elucidated.

2.7 The Metabolic Syndrome

2.7.1 Prevalence and Health Risks Associated with the Metabolic Syndrome

An association between obesity, high fasting triglycerides, elevated fasting plasma insulin, impaired glucose tolerance, hypertension, and cardiovascular disease has been recognized for decades. These major risk factors tend to cluster together more than by chance in many individuals, which has been termed as the metabolic syndrome (Zimmet et al., 2007). Already, one quarter of the world's adult population has the metabolic syndrome (Cameron et al., 2004; Ford et al., 2002) and this condition is appearing with increased frequency in children and adolescents driven by the growing obesity epidemic in this young population (Cook et al., 2003; Cruz & Goran, 2004; Weiss et al., 2004). In fact, the overall prevalence of the metabolic syndrome among U.S. adolescents has increased from 4.2% in 1988-1992 (Cook et al., 2003) to 6.4% in 1999-2000 (Duncan et al., 2004) to 8.6% in 2001-2006 (Johnson et al., 2009). The syndrome, based on the International Diabetes Federation (IDF), affects approximately 32.1% of overweight adolescents (BMI $\geq 95^{th}$ percentile for age and sex), compared with 7.1% of

adolescents at risk for being overweight (BMI between the 85th and 95th percentile for age and sex) (Duncan et al., 2004). However, only 0.1% of those with a BMI below the 85th percentile for age and sex were found to display the metabolic syndrome phenotype (Amemiya et al., 2007). This suggests that the metabolic syndrome may only affect those with increased adiposity.

The increased prevalence of the metabolic syndrome is problematic as this syndrome is associated with many well-known health risks such as fatty liver, high cholesterol, gallstones, obstructive sleep apnea, gout, depression, musculoskeletal disease, polycystic ovarian syndrome and cardiovascular disease (Grundy, 2008). Those with the metabolic syndrome are twice as likely to die from cardiovascular complications as compared with those without the syndrome (Alexander et al., 2003; Eckel et al., 2005; McNeill et al., 2005; Tong et al., 2005). The increased cardiovascular risks associated with the syndrome have been found to proceed from childhood to adolescence, leading one to suspect the metabolic syndrome might also ensue into adulthood (Katzmarzyk et al., 2001). In fact childhood obesity predicts the development of the metabolic syndrome in adulthood (Vanhala et al., 1999).

2.7.2 The Metabolic Syndrome and RMR

Since the metabolic syndrome is strongly associated with overweight and obesity, an underlying cause such as an energy imbalance might exacerbate an individual's susceptibility to the syndrome. Currently, there are few studies relating the metabolic syndrome to a change in RMR. While Buscemi et al. (2007) reported a decreased RMR in those with the syndrome; Jacobson et al. (2006) observed that the RMR of those with the metabolic syndrome is 10% higher than those without. Differences could be due to

methods for the determination of body composition. Buscemi et al. (2007) found FM and FFM using bioelectrical impedance while Jacobson et al. (2006) used hydrodensitometry. Buscemi et al. (2007) suggested that lower mitochondrial levels of uncoupling proteins and genetic polymorphism in these proteins might explain the decreased RMR observed in those with the metabolic syndrome. On the other hand, Jacobson et al. (2006) theorized that specific genes relating to oxidative phosphorylation could explain the increased RMR in those with the metabolic syndrome.

It has also been proposed insulin resistance in those with the metabolic syndrome might be a survival mechanism for a concept they call "redox thriftiness" whereby insulin resistance is determined by the ability to resist oxidative stress (Nunn et al., 2010). One of the suspected causes of the metabolic syndrome is a lack of physical activity, which could reduce mitochondrial function leading to an increased tendency for inflammation. This inflammation may result in the inability to deal with consistently excessive calories thereby leading to mitochondrial overload (Bonnard et al., 2008). Through evolution, this "thriftiness" may have arisen to minimize energy expenditure by reducing mitochondrial density (Hudson et al., 2007). A reduction in mitochondrial activity through defects in mitochondrial oxidative phosphorylation may decrease energy expenditure to contribute to metabolic dysfunction in insulin resistance thereby inducing lipid accumulation (Auwerx, 2006; Petersen et al., 2004; Petersen et al., 2003).

Apart from environmental factors, the metabolic syndrome has been strongly associated with genetics. Many genes have been linked to an increased susceptibility to the development of the metabolic syndrome in certain individuals (Jacobson et al., 2006; Junyent et al., 2009; Rouskas et al., 2008; Scott et al., 2008; Wiedmann et al., 2008).

Certain genes are suggested to create an energy-sparing metabolism (York & Bouchard, 2000) while other researchers suggest that these genes may actually increase RMR (Jacobson et al., 2006). Although genetics may play a substantial role in the development of the metabolic syndrome, current findings cannot elucidate the exact effect that these genes may have on RMR of those with the syndrome.

Although Buscemi et al. (2007) found RMR is reduced in patients with the metabolic syndrome, studies on individuals with type 2 diabetes have found RMR is increased (Bitz et al., 2004). People with diabetes exhibit similar symptoms as those with the metabolic syndrome such as increased intra-abdominal obesity (Després, 2006a), hyperinsulinemia (Weyer et al., 2000) and increased fasting triglycerides (Wilson et al., 2005). The physiological mechanisms responsible for the increased RMR in individuals with type 2diabetes are not well understood however, several mechanisms have been proposed. Bitz et al. (2004) suggested that gluconeogenesis, a known energy-consuming metabolic pathway, is increased in type 2 diabetes and contributes to hyperglycemia. An increased sympathetic nervous system in those with the metabolic syndrome (Grassi et al., 2005; Huggett et al., 2004; Liao et al., 1998; Pikkujamsa et al., 1998) and those with diabetes (Ferraro et al., 1990) could also contribute to increased RMR.

2.8 The Relationship Between Metabolic Risk Factors and Body Fat Distribution

Abdominal obesity, determined by both subcutaneous and visceral adipose tissue, has been associated with an increased risk for metabolic abnormalities commonly observed in individuals with the metabolic syndrome (Kim & Park, 2008; Pontirolli et al., 2009). Many reports suggest that visceral adipose tissue is predictive of metabolic risk factors including an increase in fasting plasma triglycerides (Goel et al., 2010; Gutin et

al., 2007; Kim & Park, 2008; Owens et al., 2000; Pontirolli et al., 2009; Smith et al., 2001; Syme et al., 2008; Tanaka et al., 2004), fasting glucose (Caprio et al., 1995; Ross et al., 2002b) and fasting insulin (Kelley et al., 2000; Pontirolli et al., 2009; Syme et al., 2008), a decrease in HDL cholesterol (Goel et al., 2010; Gutin et al., 2007; Kim & Park, 2008; Owens et al., 2000; Pontirolli et al., 2009; Smith et al., 2001; Syme et al., 2008; Tanaka et al., 2004), and insulin sensitivity (Caprio et al., 1995; Kim & Park et al., 2008; Pontirolli et al., 2009; Ross et al., 2002a; Ross et al., 2002b).

Caprio and colleagues (1995) were amongst the first research team to explore the relationship between abdominal visceral adipose tissue and metabolic risk in adolescents using MRI to measure body fat distribution. In their group of obese adolescent girls, it was found that a significant positive relationship existed between visceral adipose tissue, and insulin secretion and sensitivity. An inverse relationship between HDL-cholesterol and visceral adipose tissue was also noted. In contrast, no significant correlation was found in the obese group between subcutaneous adipose tissue and these measures. The results of an investigation done by Kim & Park (2008) furthered the exploration into the risks of visceral obesity. They revealed that visceral adipose tissue is also predictive of increased blood pressure, HDL and LDL cholesterol as well as insulin resistance independently of total and subcutaneous adipose tissue in 175 obese adolescents, aged 9-19 years old confirming results found from previous investigations (Gutin et al., 2007; Owens et al., 2000). These results indicate that visceral adipose tissue is significantly related to risk factors for the metabolic syndrome while such association was not observed for total body and subcutaneous adipose tissue (Druet et al., 2008). The

metabolic syndrome was completely absent from adolescents with low visceral adipose tissue while 13.8% of the adolescent males and 8.3% of the females.

The anatomical location of visceral adipose tissue within the abdomen is believed to be the reason why visceral adipose tissue may be predictive of metabolic dysfunction. Visceral adipocytes are believed to be hyperlipolytic and are highly responsive to catecholamine stimulation and poorly responsive to lipolysis inhibition by insulin (Arner, 1995; Bergman et al., 2006; Bergman et al., 2001). The anatomical location of visceral adipoctyes allows an excess of free fatty acids, due to poor lipolysis inhibition of visceral adipose tissue, into the liver via portal circulation (Björntrop, 1990). Excess fat accumulation may ensue, affecting liver metabolism potentially inducing hypertriglyceridemia, hyperinsulinemia and glucose intolerance (Bergman et al., 2001; Björntrop, 1990).

Although it is known that visceral adipose tissue plays a significant role in metabolic abnormalities, a review of literature suggests that an excess of subcutaneous adipose tissue may also be problematic (Fox et al., 2007; Goel et al., 2010; Kelley et al., 2000; Koska et al., 2008; Pontirolli et al., 2009; Smith et al., 2001). In 100 male and female adults, Goel and colleagues (2010) found that both subcutaneous and visceral adipose tissue was positively correlated with hypertriglyceridemia. However, when comparing the two adipose tissue depots, they found that subcutaneous adipose tissue was more significantly correlated with insulin resistance compared to visceral adipose tissue.

Reports have also found that results differ when dividing subcutaneous adipose tissue into two separate compartments: superficial and deep layer. The superficial

subcutaneous adipose tissue is directly located under the skin whereas deep subcutaneous adipose tissue is located under the fascia superficialis. Evidence suggests that there may be a significant metabolic difference between deep versus superficial subcutaneous adipose tissue (Kelley et al., 2000; Miyazaki et al., 2002; Smith et al., 2001). Kelley et al. (2000) quantified deep and superficial subcutaneous adipose tissue in 15 lean and 32 obese men and women using computed tomography. They found that although visceral adipose tissue accounted for 45% of the variance in insulin sensitivity, deep subcutaneous adipose tissue still remained independently associated with insulin sensitivity as well (r=0.51). Both deep subcutaneous and visceral adipose tissues were both independently predictive of triglycerides, HDL-cholesterol, and blood pressure as well. Superficial subcutaneous adipose tissue did not correlate with the metabolic risk factors. Therefore, it was determined that deep subcutaneous adipose tissue mirrors the relationship found in visceral adipose tissue however, they were unable to elucidate the physiological differences between deep and superficial subcutaneous adipose tissue. If subcutaneous adipose tissue is considered as a single depot, the deleterious effects of deep subcutaneous adipose tissue may not be observed by the inclusion of the superficial depot.

2.9 Conclusion

With the increasing prevalence of obese children and adolescents and the known consequences of increased adiposity in adulthood, this generation may expect decreased adult health as compared to their parents' generation. Currently little is known about factors that may create a predisposition to the development of the metabolic syndrome. It is vital to understand risk factors associated with the metabolic syndrome in adolescents

since it is invariably associated with an increased risk of co-morbidities and mortality. A reduced RMR could be associated with development of the syndrome by predisposing certain individuals to gain excess adiposity within certain body fat depots. This may then alter their metabolic profile, exacerbating their susceptibility to the development of the metabolic syndrome. Collectively, the results from the present study could provide useful insight into how body fat depots may contribute to the abnormal metabolic profile observed in adolescents with the metabolic syndrome.

ARTICLE 1:

A comparison of resting metabolic rate in adolescents, aged 14-18 years old, with and without the metabolic syndrome: HEARTY study

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Short title: Metabolic syndrome and resting metabolic rate

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ABSTRACT

Background: The metabolic syndrome is associated with an accumulation of adipose tissue. There is a lack of consensus as to whether individuals with the metabolic syndrome have an altered resting metabolic rate (RMR) perhaps predisposing them to the syndrome. **Objectives:** To investigate whether obese adolescents, aged 14-18 years old, with the metabolic syndrome have a higher RMR compared to adolescents without the metabolic syndrome. **Methods:** 41 obese adolescents with the metabolic syndrome (10 males, 31 females, mean BMI= 35.0 ± 4.1 kg/m^2) were matched for BMI, age and sex with 41 obese adolescents without the metabolic syndrome (10 males, 31 females, mean BMI= 35.0 ± 4.0 kg/m^2). Sedentary obese adolescents, age 14-18 years old, were included in the study if they had a baseline BMI $\geq 95^{th}$ percentile for their age, sex and height, or $>85^{th}$ percentile + additional diabetes risk factors. RMR was measured using indirect calorimetry. Lean tissue, total adipose tissue, subcutaneous adipose tissue and visceral adipose tissue were quantified using Magnetic Resonance Imaging. **Results:** No significant differences in lean tissue, total adipose tissue and subcutaneous adipose tissue were observed between adolescents with and without the metabolic syndrome. However, adolescents with the metabolic syndrome had a greater amount of visceral adipose tissue than those without the metabolic syndrome. Absolute RMR or RMR relative to fat-free mass did not differ between groups. **Conclusion:** These results suggest that RMR may not be altered in those with the metabolic syndrome. An increased amount of visceral adipose tissue may be a significant determinant in the development of the syndrome.

Keywords: energy expenditure, metabolic risk factors, body composition, overweight, insulin resistance syndrome, adolescents

INTRODUCTION

The escalation of obesity in youth over the past 25 years (Shields et al., 2005) has led to an increase in metabolic abnormalities. Many obese children and adolescents suffer from hypertension, reduced high-density-lipoprotein (HDL) cholesterol, increased triglyceride concentration, and insulin resistance (l'Allemand et al., 2008; Reinhr et al., 2005). Abdominal obesity and the clustering of these major risk factors in many individuals suggest a common etiology that has been termed as the metabolic syndrome according to the International Diabetes Federation (IDF) (Zimmet et al., 2007). According to recent estimates, the metabolic syndrome affects 38% of adolescents (Duncan et al., 2004). The increasing prevalence of metabolic syndrome in youth is problematic as it has been associated with health risks such as fatty liver, gallstones, obstructive sleep apnea, gout, depression, musculoskeletal disease, polycystic ovarian syndrome and cardiovascular disease (Grundy, 2008). Adults with the metabolic syndrome are twice as likely to die from cardiovascular complications as compared to those without (Alexander et al., 2003; Eckel et al., 2005; McNeill et al., 2005; Tong et al., 2005).

Individuals with excess body weight often display characteristics associated with the metabolic syndrome (International Diabetes Federation, 2007). Excess adiposity may be caused by an energy imbalance whereby either there is an increase in energy consumption and/or a decrease in energy expended. Since the metabolic syndrome is invariably associated with increased adiposity, an energy imbalance may be an underlying cause that could exacerbate an individual's susceptibility to the syndrome. Energy expended at rest to maintain basic metabolic functions accounts for the largest

proportion of total daily energy expenditure and may therefore play a significant role in the regulation of energy balance by promoting weight gain, loss or maintenance. In fact resting metabolic rate (RMR) has been identified as a potential predictor of body weight gain (Ravussin & Gautier, 1999).

Since RMR accounts for the majority of an individual's total daily energy expenditure, it may play a pivotal role in the development of the metabolic syndrome. A study by Jacobson and colleagues (2006) demonstrated that RMR variability was associated with some genetic traits that overlapped regions previously linked to the metabolic syndrome. They also observed that there was a significant association between RMR and the metabolic syndrome in 426 siblings from 169 families. Those with the metabolic syndrome displayed a RMR that was 10% higher than their metabolically healthier siblings when using the International Diabetes Federation (IDF) criteria. On the contrary, Buscemi et al. (2007) found that obese adults with a BMI over 30 kg/m^2 with the metabolic syndrome displayed a decreased RMR compared to those without when using the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III). Conflicting results has lead to speculation as to whether RMR is associated with the development of the metabolic syndrome.

Therefore, the purpose of our investigation was to compare the RMR of 41 obese adolescents with the metabolic syndrome to 41 metabolically healthy individuals with similar body mass. We hypothesized that those with the metabolic syndrome will have a higher RMR than those without the metabolic syndrome even after controlling for fat-free mass.

METHODS

Design

This project was a sub-study of the larger ongoing Healthy Eating, Aerobic and Resistance Training in Youth (HEARTY) study. Overall the HEARTY study is a randomized, controlled trial with a parallel group design. Recruitment began in April 2005 and closed May 2010. The Ottawa Hospital Research Ethics Board approved of all the methods and procedures. All subjects provided written informed consent according to the Tri-Council Policy Statement Guidelines prior to participation in the study.

Participants

Sedentary, obese adolescents between the ages of 14-18 years old were recruited through family physician referrals as well as posters, school board advertisements, bus campaigns and radio station advertisements. To be eligible, potential participants had to be post-pubertal with a Tanner stage of IV or above. They also had to have a baseline body mass index (BMI) $\geq 95^{th}$ percentile for age and sex and/or $\geq 85^{th}$ percentile for age and sex plus additional diabetes risk factors. Diabetic risk factors include any of the following: fasting glucose $\geq$ 6.0mmol/L, 2h plasma glucose 7.8-11mmol/L after 75g oral glucose signifying impaired glucose tolerance, fasting triglycerides >1.7mmol/L, fasting plasma insulin >105mmol/L, HDL-C <0.9mmol/L, LDL-C >3.0mmol/L, total cholesterol/HDL-C $>90^{th}$ percentile or a first degree relative with type II diabetes and a waist circumference $\geq 75^{th}$ percentile for their age and gender.

Participants were excluded if they had participated in a regular exercise program in the previous 4 months or any aerobic sport more than twice per week for at least 20 minutes/session, had diabetes mellitus, had uncontrolled hypertension and/or were using

medications or herbal supplements that would alter their body composition. Potential participants with a body weight over 159 kg and/or a BMI over 45 kg/m^2 were also excluded from the study as their physical size exceeds that of the capability of the MRI machine.

Once participants met the inclusion criteria for the study, they completed a medical, drug and exercise history, and physical exam. Anthropometric measures of height, weight and waist circumference were determined. Weight was assessed in kilograms using a Health-O-Meter manual scale and height was measured in centimeters using a stadiometer. From these measurements, BMI was determined by calculating the participants weight (kg) divided by height squared (m^2). Waist circumference was measured as the midpoint between the top of the iliac crest and the last floating rib at the end of expiration. The research coordinator also evaluated a history of dieting, binge eating, eating disorders, weight fluctuations over time and time spent in sedentary activities.

Of the participants recruited for the larger ongoing HEARTY study, 82 participants who had completed baseline RMR and Magnetic Resonance Imaging (MRI) scan were used for this sub-study. Forty-one subjects with the metabolic syndrome (10 males, 31 females) were matched for BMI, age and sex with 41 subjects (10 males, 31 females) without the metabolic syndrome.

Body Composition Analysis By Magnetic Resonance Imaging

Whole body (45-48 images) MRI data were obtained using a 1.5-T General Electric scanner at CHEO (Ottawa, Ontario, Canada) or MRI+ (Gatineau, Québec, Canada) (Signa Excite 1.5T HD, General Electric, Peterborough, Ontario, Canada)_to

assess body composition and specifically total fat free mass. It has been shown that fat-free mass accounts for 50% to 80% of the variation in RMR between individuals (Buscemi et al., 2007; Butte et al., 2007; Derumeaux-Burel et al., 2004; Goran et al., 1998; Kirkby et al., 2004; Lazzer et al., 2004; Leibel et al., 1995; Martin et al., 2007; Neuhauser-Berthold et al., 2000; Poehlman et al., 1997; van Mil et al., 2001) therefore, by accounting for fat-free mass we are able to determine if disease state, not muscle accounts for differences observed in RMR.

Images were acquired using a T1-weighted spin-echo pulse sequence with a repetition time of 210ms and an echo time of 17ms with a 256x256 matrix (Ross et al., 1996). MRI data was transferred electronically to a stand-alone computer workstation for fat-free tissue analysis using Slice-O-Matic software (version 4.3, Tomovision, Montreal, Canada). Tissue area was computed automatically by Slice-O-Matic by summing the pixels and multiplying them by the individual pixel surface area. The volume (cm^3) of each tissue for the individual images was calculated by multiplying the respective tissue area (cm^2) by the slice thickness. The volume of each tissue for the spaces between two consecutive slices was then calculated with the following mathematical algorithm:

$$V = \sum_{i=1}^{N} A_i t + h/3 \sum_{i=2}^{N} (A_{i-1} + A_i + (A_{i-1}A_i)^{0.5})$$

Where: V is the total tissue volume
A is the tissue area
t is the slice thickness
h is the distance between consecutive slices
N is the number of slices

The volume (liters) was then converted to mass units (kg) by multiplying the volumes by the assumed constant density of the tissue (0.92 for fat mass and 1.04 for fat-free mass)

(Snyder et al., 1975). All measurements of body composition using the MRI are represented as mass in kilograms.

Metabolic Parameters

Components of the metabolic syndrome (systolic and diastolic blood pressure, fasting plasma glucose, triglycerides and HDL cholesterol levels) as well as other metabolic variables (total cholesterol, LDL cholesterol, fasting insulin and homeostasis model assessment score) were measured in all subjects. Systolic and diastolic blood pressure was measured using a random-zero mercury sphygmomanometer. Three readings of systolic and diastolic blood pressure were recorded, and the average of the 3 was used for analysis. If the first two measurements differed by more than 5 mm Hg, additional readings were obtained.

A fasting blood sample was drawn in the morning after a 12-hour fast by the research coordinator. A Beckman-Coulter LX20 enzymatic assay was conducted to determine plasma triglycerides, total cholesterol, LDL cholesterol and HDL cholesterol levels. Fasting insulin levels were determined using a Beckman-Coulter Access 2 Ultrasensitive kit. A 75 g glucose solution was given to the subject after a fasting blood sample was taken in order to perform the oral glucose tolerance test (OGTT). Blood was drawn 2 hours after the glucose ingestion to determine disposal levels.

The criteria proposed by the International Diabetes Federation (Zimmet et al., 2007) were us ed to identify participants with the metabolic syndrome. Participants between the ages of 14-16 years old were required to have a waist circumference>90^{th} percentile or the adult cutoff of $\geq$94 cm for men and $\geq$80 cm for women in order to be classified as having the metabolic syndrome. If participants over the age of 16 years old

had a waist circumference of ≥94cm for boys and ≥80cm for girls and also possessed two or more of the following criteria: raised triglycerides>1.7 mmol/L (>150 mg/dL), reduced HDL-cholesterol <1.03 mmol/L (<40 mg/dL), raised blood pressure with systolic blood pressure >130 mmHg and diastolic blood pressure>85mmHg, fasting plasma glucose ≥5.6 mmol/L (≥100 mg/dL), or known type 2 diabetes (Table 1) they were considered as having the metabolic syndrome for this study.

In addition to waist circumference criteria, those over the age of 16 years old had to also have two or more of the following criteria to be considered as having the metabolic syndrome: raised triglycerides >1.7 mmol/L (>150 mg/dL), reduced HDL-cholesterol <1.03 mmol/L (<40 mg/dL) in males and <1.29 mmol/L (<50 mg/dL) in females, raised blood pressure with systolic blood pressure >130 mmHg and diastolic blood pressure >85mmHg, fasting plasma glucose ≥5.6 mmol/L (≥100 mg/dL), or previously diagnosed type 2 diabetes. Criteria used to determine the identification of the metabolic syndrome is shown in the Appendices.

Resting Metabolic Rate

RMR was measured between 7 am and 10 am following a 12 hour fast. Participants were asked to sleep at least 8 hours the night before and not partake in strenuous physical activity for 48 hours. Upon arrival at the University of Ottawa laboratory, participants relaxed in the supine position in a semi-darkened, and temperature controlled room for 30 minutes to ensure all measurements were taken in resting conditions. After the rest period, a mouthpiece and nose clip were placed on the participant in order to commence indirect calorimetry. Participants were instructed to refrain from moving, talking or sleeping during data collection. Oxygen consumption was

measured using an automated gas analysis system (AMETEK ModelS S-3A/1 and CD 3A; Applied Electrochemistry, Pittsburg, PA, USA) for a 20-minute data collection period. The system was calibrated before each test according to manufacturer specifications to ensure reliability. Oxygen consumption (VO_2), carbon dioxide output (VCO_2), ventilation (V_E), the respiratory quotient (RQ), tidal volume and respirations values were collect every 30 seconds. The mean values from 5-15 minutes for variables to calculate RMR was used for data analysis as this was found to be the time frame that most consistently reached a steady state. It is the time period during which VO_2, V_E and RER did not vary by more than 10% (Horner et al., 2001). Respiratory exchange ratio (RER) and VO_2 (mL O_2/kg/min) were used to calculate RMR. RER is the ratio of the volume of carbon dioxide eliminated from the lungs per minute to the volume of oxygen taken into the lungs during the same time. RMR (kcal/day) was calculated using the following equation:

$$E_{TOT} = \dot{V}O_2 \cdot \left[\frac{(RER - 0.7)}{0.3} \cdot e_g + \frac{(1 - RER)}{0.3} \cdot e_f \right]$$

where:

eg = caloric equivalent in kilocalorie per litre of O_2 for carbohydrates (5.047 kcal·LO_2);

ef = caloric equivalent in kilocalorie per litre of O_2 for fats (4.686 kcal·LO_2); and,

RER = the respiratory exchange ratio.

Resting heart rate was also measured in 30 second average intervals using a Polar transmitter and recorded continuously with a Polar Advanced Interface (Polar Electro Oy, Finland).

Pedometer logs

Habitual physical activity was assessed using Step Count Pedometers. Methods to determine physical activity patterns in children are controversial (Ruiz, Rizzo & Hurtig-Wennlof, 2006). Pedometers are regarded as valid tools to assess the quantity of physical activity (Sirard & Pate, 2001; Trost, 2001). Through self-report, participants were asked to write down the number of steps taken as the beginning and end of their day for a period of 7 consecutive days. The information gathered from the pedometer logs was used to compare the physical activity levels between adolescents with and without the metabolic syndrome in the HEARTY study.

STATISTICAL ANALYSIS

An independent samples t-test was performed to determine if there were mean differences in absolute resting metabolic rate, resting metabolic rate normalized for lean tissue, body fat indices, dietary intake and physical activity between those with the metabolic syndrome and those without the metabolic syndrome matching for BMI, sex and age. All data were analyzed using SPSS software v.16.0 with significance set at $p<0.05$.

RESULTS

The mean baseline subject characteristics are presented in Table 1. There were no differences in absolute RMR, RMR normalized for lean tissue, absolute oxygen consumption, oxygen consumption relative to body mass, respiratory rate, tidal volume, ventilation, respiratory exchange ratio and resting heart rate between those with the metabolic syndrome and those without the metabolic syndrome in our sample ($p>0.05$)

(Table 2). Those with the metabolic syndrome did not differ significantly in regards to waist circumference, waist to hip ratio, total adipose tissue, subcutaneous adipose tissue, lean tissue, and percent body fat (Table 1). However, results revealed a significant differences where patients with the metabolic syndrome having greater visceral adipose tissue (mean = 1.49 kacl/day, standard deviation= 0.55452) than those without the metabolic syndrome (mean= 1.2151 kcal/day, standard deviation= 0.45890) (Table 1).

The dietary intake for the sample is presented in Table 3. Because of the lack of completed dietary logs, a sub-sample of data (N=76) was used to determine dietary intake in patients with the metabolic syndrome (N=38) and in patients without (N=38). Total caloric intake (p=0.183), intake of protein (p=0.077), intake of carbohydrate (p=0.244) and fat intake (p=0.476) did not differ between patients with and without the metabolic syndrome.

A sub-sample of data was also used to determine physical activity patterns monitored via pedometer logs. Total weekly step count did not differ between the subsample for participants with the metabolic syndrome (51528 ± 22330 steps, N=15) and participants without the metabolic syndrome (58655 ± 40056 steps, N=29) (p=0.527) (Table 4).

DISCUSSION

In this study, we compared the resting metabolic rate of obese, sedentary adolescents with the metabolic syndrome to age, sex and BMI matched obese, sedentary adolescents without the metabolic syndrome. We did not observe differences in resting metabolic rate between the two groups even when normalized for lean tissue. However, our results demonstrate that participants with the metabolic syndrome had a larger

proportion of visceral adipose tissue compared to their metabolically healthy counterparts.

Currently there exist few studies examining whole-body composition of adolescents using MRI technology. No research has been conducted on adolescents regarding differences in resting metabolic rate between those with and without the metabolic syndrome. Therefore we compared our findings to the studies conducted in adult populations. By comparing our findings to those done in adults, we found that our results are not in agreement with some of the previous reports. A study performed on 40 obese adult males and females (30-60 years old) reported that those with the metabolic syndrome, determined using the National Cholesterol Education Panel, Adult Treatment Panel III criteria, had a lower resting metabolic rate compared to those without the syndrome (Buscemi et al., 2007). The differing results may be attributed to the method of quantifying fat-free mass and fat mass or the criteria used to identify the metabolic syndrome. Buscemi and colleagues (2007) used bioelectrical impedance analysis to determine body composition, which has been shown to overestimate muscle, lean tissue or fat-free mass in individuals with a large amount of adipose tissue (Baumgartner et al., 1998). On the other hand, MRI is considered the gold standard for the quantitative assessment of body composition and is renowned for its reproducibility and precision (Jones et al., 2009; Seidell et al., 1990).

Differences in fat-free mass between the control and the metabolic syndrome group may have also contributed to the differences in RMR in the study by Buscemi et al. (2007) as well as that by Jacobson et al. (2006). Jacobson and colleagues (2006) found that siblings with the metabolic syndrome had an RMR that was 10% higher than their

metabolically healthy siblings. However, they did not control for differences between-groups for age and fat-free mass. Fat-free mass has been found to account for 50 to 80% of the variation between individuals for RMR (Butte et al., 2007; Derumeaux-Burel et al., 2004; Kirkby et al., 2004; Lazzer et al., 2004; Martin et al., 2007; Neuhauser-Berthold et al., 2000; van Mil et al., 2001). In the present study, participants were matched for age, sex and BMI to account for differences in weight, height, fat mass and fat-free mass.

Since there is currently a lack of information of the metabolic syndrome, we must compare our data to those studies done on adults with diabetes. This was done because individuals with the metabolic syndrome may also show an abnormal glycaemia level but not as drastically abnormal as individuals with diabetes. Studies on patients with type 2 diabetes have reported a 5-8% higher RMR than controls without diabetes (Bitz et al., 2004; Fontvieille et al., 1992; Franssila-Kallunki et al., 1992; Weyer et al., 1999b). They attributed this elevation to be the result of deteriorating glycemic control during the development of type 2 diabetes (Weyer et al., 1999b). A number of studies in participants with uncontrolled diabetes have cited a relationship between glycaemic level or glucose tolerance and RMR (Gougeon et al., 2002; Huang et al., 2004; Martin et al., 2004) after controlling for body composition, sex, age and race. In most studies, however, it is only when differences in fat-free mass are adjusted for that RMR appears higher, with no difference in absolute metabolic rate otherwise being observed between those with and without diabetes (Bitz et al., 2004; Fontvieille et al., 1992; Huang et al., 2004).

It has been found however, that glycaemia itself may determine an increase in RMR as high as 5% when fasting plasma glucose is 10mmol/l or higher (Gougeon et al., 2002). When fasting plasma glucose was lowered by 5.9mmol/L from a fasting plasma

glucose level of 10.2mmol/L ± 0.2, RMR was reduced by 5% (Mãkimattila et al., 1999) indicating that hyperglycemia may have an important impact on RMR. Collectively however, these studies do not provide an adequate argument to suggest a glycaemic threshold whereby RMR is increased. In a study done by Gougeon and colleagues (2002) which involved a large glycaemic range, the addition of fasting glycaemia to the regression analysis only added 3% to the variance explained in RMR. It is plausible that RMR is increased in individuals with poorly controlled diabetes and that such an increase cannot be seen in those who are mildly hyperglycaemic like those with the metabolic syndrome.

The metabolic syndrome may be a precursor to the development of type 2 diabetes and the long-term effects of hyperglycaemia may not be apparent in this population as of yet. Increased energy costs during hyperglycamia via an increased gluconeogenesis, protein turnover and sympathetic nervous system activity (Fontvieille et al., 1992) as a result of diabetes over a prolonged period may be more likely to occur in patients with uncontrolled hyperglycaemia as opposed to those who are yet to be diagnosed with type 2 diabetes. In fact, it has been found that fat accumulation in elderly individuals with insulin resistance and offspring with type 2 diabetes is associated with a 30-40% decreased mitochondrial activity which may have caused a lower RMR in these subjects (Petersen et al., 2004; Petersen et al., 2003).

The increased visceral adiposity observed in our cohort of obese adolescents with the metabolic syndrome may potentially explain their unhealthy metabolic profile. Imaging studies have shown that excess adipose tissue located in the viscera is the key correlate to metabolic dysfunction in overweight and obese individuals (Després, 2006b;

Després et al., 1990; Goodpaster et al., 2003; Matsuzawa et al., 1995; Pouliot et al., 1992; Ross et al., 2002a; Ross et al., 2002b). The hyperlipolytic state of visceral adipose tissue, which is resistant to insulin, may contribute to an increase in free fatty acids in the liver thereby contributing to the metabolic dysfunction (Mathieu et al., 2009). The anatomical location of visceral adipocytes is ideal for fatty acids to be directly released into portal circulation to the liver (Björntrop, 1990; Després et al., 2008). The increased free fatty acids in the liver can impair hepatic metabolic processes leading to hyperinsulinemia, glucose intolerance and hypertriglyceridemia (Bergman et al., 2001; Björntrop, 1990).

The function of adipose tissue may also explain why excess visceral adipose tissue may be involved in the development of the metabolic syndrome. Adipose tissue has been established as an endocrine organ producing a variety of steroids, cytokines and adipokines (Després et al., 2008; Kershaw & Flier, 2004; Scherer, 2006). These compounds contribute to the insulin resistant, proinflammatory and hypertensive state of visceral obesity (Després et al., 2008).

It may well be that some individuals are more susceptible to increased visceral adiposity. Studies have suggested that hyperplasia expand subcutaneous adipose tissue in certain individuals more than others (Adams et al., 1997; Tchoukalova et al., 2008). Excess visceral adipose tissue is partially a marker of the inability of subcutaneous adipose tissue to act as a metabolic "sink" because it may become hypertrophied, dysfunctional or insulin resistant (Després et al., 2008). Individuals who are not able to store excess energy in the subcutaneous adipose tissue depot could potentially accumulate adipose tissue in the liver, heart, skeletal muscle and pancreas. It could well be that the subcutaneous adipocytes of obese adolescents with the metabolic syndrome in

our study are unable to expand as adequately as those without the metabolic syndrome. The excess fat in these adolescents is then stored in the viscera, leaving them unprotected against metabolic dysfunction.

Limitations

It is important to note that all patients without the metabolic syndrome had similar waist circumferences to those with the metabolic syndrome. The lack of differences in dietary intake and physical activity between both groups could help explain the lack of difference in RMR in adolescents with and without the metabolic syndrome. In our study, we did account for sex, age and body composition related differences in RMR by matching our participants however; additional variability such as genetic differences, and individual day-to-day variability (Donahoo et al., 2004) are more difficult to account for. Our lack of findings could also be attributed to the appearance of the criteria for the metabolic syndrome in our healthy sample. Some participants without the metabolic syndrome had elevated blood pressure or triglyceride levels, or lowered HDL cholesterol. It has been observed that blood pressure, in particular, is significantly associated with RMR (Bosy-Westphale et al., 2008; Luke et al., 2004; Snodgrass et al., 2008). Kunz et al. (2000) found that RMR was 9% higher in obese hypertensive patients compared to obese normotensive patients.

Conclusion

In summary, we show that obese adolescents aged 14-18 years old with the metabolic syndrome do not have an altered RMR compared to those without the metabolic syndrome when matched for age, sex and BMI. We also showed that

adolescents with the metabolic syndrome have a larger amount of visceral adipose tissue compared to the participants that were categorized as metabolically healthy. Although our subjects without the metabolic syndrome did not fit the diagnosis, some of them still exhibited at least one metabolic abnormality if not more. Therefore future studies should focus on comparing adolescents who have a completely healthy metabolic profile to those with the metabolic syndrome. Also, other mechanisms that may increase an individual's susceptibility to the metabolic syndrome should be explored. Strategies targeted at reducing visceral adipose tissue should be studied in order improve the metabolic profile of obese adolescents with the metabolic syndrome.

ACKNOWLEGEMENTS

The HEARTY trial was supported by a grant from the Canadian Institutes of Health Research (MCT-71979). Ms. Pamela Martino also holds an Ontario Graduate Scholarship and Ms. Angela Alberga holds a Doctoral Student Research Award from the Canadian Diabetes Association. Dr. Sigal holds a Health Senior Scholar award from the Alberta Heritage Foundation for Medical Research. He was previously supported by an OHRI Research Chair in Lifestyle Research. Dr. Kenny holds a University of Ottawa Research Chair.

Table 1. Characteristic of HEARTY participants with and without the Metabolic Syndrome

	MetS (N=41)	**nonMetS (N=41)**
Age (years)	15.6 ± 1.2	15.6 ± 1.2
Weight (kg)	100.9 ± 15.7	98.6 ± 15.3
Height (cm)	169.7 ± 8.1	168.2 ± 6.1
BMI (kg/m^2)	35.0 ± 4.1	34. 9 ± 4.0
WC (cm)	98.4 ± 10.1	96.6 ± 11.0
WHR	0.83 ± 0.06	0.81 ± 0.06
Percent body fat	47.5 ± 5.5	47.4 ± 5.0
Total AT (kg)	48.1 ± 10.2	46.9 ± 9.8
SAT (kg)	40.9 ± 9.4	40.2 ± 8.8
VAT (kg)	1.5 ± 0.6*	1.2 ± 0.5*
LT (kg)	51.0 ± 8.2	49.3 ± 7.9
Fasting glucose	5.15 ± 0.53	4.99 ± 0.34
2-hour glucose	6.09 ± 1.22	5.32 ± 1.01
Fasting insulin	128.1 ± 64.284	84.4 ± 40.1
Triglycerides	1.92 ± 0.76	1.01 ± 0.39
HbA1c	0.52 ± 0.003	0.52 ± 0.002
Systolic blood pressure	119.9 ± 12.3	109.1 ± 8.6
Diastolic blood pressure	77.0 ± 7.8	71.9 ± 7.7

Values are given as means ± SD.
Abbreviations: HEARTY, Healthy Eating, Aerobic and Resistance Training in Youths; MetS, Metabolic Syndrome; nonMetS, without the Metabolic Syndrome; BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; AT, adipose tissue; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; LT, lean tissue. *, indicates significant difference between those with and without the MetS, $p<0.05$

Table 2. RMR variables of HEARTY participants with and without the Metabolic Syndrome

	MetS (N=41)	**nonMetS (N=41)**
Absolute RMR (kcal/day)	2052 ± 379	2002 ± 393
Relative RMR (kcal/kgLT/day)	40 ± 5	41 ± 6
Absolute $V0_2$ (mLO_2/min)	304 ± 57	293 ± 61
Relative VO_2 (mLO_2/kg/min)	3.0 ± 0.4	3.0 ± 0.4
RR (breaths/min)	16 ± 4	16 ± 5
VT (mL)	485 ± 167	493 ± 187
V_E (mL/min)	7 ± 2	8 ± 2
RER	0.79 ± 0.06	0.80 ± 0.09
RHR (beats/min)	71 ± 9	69 ± 9

Values are given as means ± SD.
Abbreviations: HEARTY, Healthy Eating, Aerobic and Resistance Training in Youths; MetS, Metabolic Syndrome; nonMetS, without the Metabolic Syndrome; RMR, resting metabolic rate; LT, lean tissue; $VC0_2$, volume of carbon dioxide elimination; VO_2, volume of oxygen consumption; RR, respiratory rate; VT, tidal volume; VE, minute ventilation; RER, respiratory exchange ratio; RHR, resting heart rate. *, indicates significant difference between those with and without the Metabolic Syndrome,$p<0.05$.

Table 3. Dietary intake and physical activity patterns of HEARTY participants with and without the Metabolic Syndrome

	MetS (N=41)	**nonMetS (N=41)**
Total energy intake (kcal)	2194 ± 578	2027 ± 503
Protein intake (g)	89 ± 30	78 ± 18
Carbohydrate intake (g)	285 ± 87	262 ± 79
Fat intake (g)	81 ± 24	77± 22
Number of pedometer steps per week	50589 ± 21487	57200 ± 39546

Values are given as means ± SD.
Abbreviations: HEARTY, Healthy Eating, Aerobic and Resistance Training in Youths; MetS, Metabolic Syndrome; nonMetS, without the Metabolic Syndrome. *, indicates significant difference between those with and without the Metabolic Syndrome, $p<0.05$

ARTICLE 2:

Relationship of abdominal fat distribution to metabolic risk factors in sedentary, obese adolescents, aged 14-18 years old: HEARTY study

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Short title: Fat distribution and metabolic risk factors

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ABSTRACT

Background: Abdominal adipose tissue, especially in the visceral region has been strongly related to increased metabolic risk. However, the relationship between deep and superficial subcutaneous abdominal adipose tissue and altered metabolic profile warrants further investigation in obese adolescents. **Objectives:** To examine the relationship between abdominal fat distribution and metabolic risk factors in overweight and obese adolescents. **Methods:** 105 adolescents (26 males, 79 females, mean BMI= 34.5 ± 4.0 kg/m^2) who volunteered to participate in the Healthy Eating, Aerobic and Resistance Training in Youth (HEARTY) randomized controlled trial study. Sedentary obese adolescents aged 14-18 years old were included in the study if they had a baseline BMI $\geq 95^{th}$ percentile for their age, sex and height, or were consider overweight $\geq 85^{th}$ BMI percentile + additional diabetes risk factors. Lean tissue and adipose tissue variables were quantified using Magnetic Resonance Imaging. Blood pressure, fasting plasma glucose, 2-hour plasma glucose, triglycerides, fasting insulin, homeostasis model assessment (HOMA-IR) score, and total, HDL and LDL cholesterol were measured. **Results:** Blood pressure, fasting insulin and HOMA-IR score were significantly correlated with total body, visceral, total body subcutaneous and superficial subcutaneous adipose tissue. Triglycerides were significantly correlated with total body and visceral adipose tissue. Multiple regression analysis revealed that visceral adipose tissue was independently associated with total cholesterol, LDL cholesterol, 2-hour glucose and triglycerides, while deep subcutaneous adipose tissue independently associated with blood pressure. **Conclusion:** Although both subcutaneous adipose tissue and visceral adipose tissue were

correlated with metabolic risk factors, visceral adipose tissue remained more predictive of an adverse metabolic profile.

Keywords: visceral fat, total body fat, subcutaneous fat, teenagers, MRI, cholesterol, triglycerides, glucose, insulin resistance

INTRODUCTION

It has long been recognized that obesity is related to several metabolic disturbances including insulin resistance, impaired insulin secretion, hypertension, dyslipidemia, the metabolic syndrome and cardiovascular disease (Kim & Park, 2008; Pontirolli et al., 2009). The regional distribution of adipose tissue is important in understanding the relation of obesity to increased metabolic risk. The metabolic risks associated with obesity are more closely correlated to android (abdominal) obesity compared to gynoid (gluteo-femoral) obesity (Poirier & Després, 2003). The differences in disease risk due to the accumulation of certain adipose tissue layers may be attributed to differences in endocrine function as adipose tissue has been established as an endocrine organ producing a variety of steroids, cytokines and adipokines (Kershaw & Flier, 2004; Scherer, 2006).

Complications of obesity have been associated with an increase in visceral adipose tissue with an associated rise in portal vein free fatty acid levels due to poor lipolysis inhibition (Arner, 1995; Bergman et al., 2006; Bergman et al., 2001). Increased free fatty acid levels have been shown to affect liver metabolism potentially inducing hypertriglyceridemia, hyperinsulinemia and glucose intolerance (Bergman et al., 2001; Björntrop, 1990). However, recent studies have found that subcutaneous adipose tissue may also be associated with abnormal triglyceride, HDL cholesterol, total cholesterol, systolic blood pressure, diastolic blood pressure and blood glucose levels as well as insulin resistance (Fox et al., 2007; Goel et al., 2010; Kelley et al., 2000; Kim & Park, 2008; Koska et al., 2008; Pontirolli et al., 2009; Smith et al., 2001).

It has been observed that subcutaneous adipose tissue can be further subdivided into a superficial and deep layer by locating the fascia superficialis as the dividing factor. Studies have found that a difference in metabolic function may exist between these two layers (Kelley et al., 2000; Miyazaki et al., 2002; Smith et al., 2001). In a study of 199 adult men and women, Smith and colleagues (2001) found that superficial subcutaneous adipose tissue correlated significantly with HDL cholesterol whereas deep subcutaneous adipose tissue correlated with fasting insulin independent of all other adipose tissue depots.

Imaging studies evaluating how fat distribution may affect the metabolic profile of adolescents are limited (Caprio et al., 1995; Druet et al., 2008; Gutin et al., 2007; Kim & Park; 2008; Owens et al., 2000). Moreover, to our knowledge no studies have examined this relationship with respect to the deep and superficial subcutaneous fat distribution in overweight and obese adolescents. Therefore, the purpose of our investigation was to examine the association between abdominal fat distribution (visceral, total subcutaneous, deep subcutaneous and superficial subcutaneous adipocytes) and metabolic risk factors (total, HDL, and LDL cholesterol, blood pressure, triglycerides, fasting insulin, insulin resistance and glucose metabolism) in overweight and obese adolescents, aged 14-18 years old. The majority of studies done on adults have found that visceral adipose tissue is strongly associated with triglyceride, cholesterol, fasting blood glucose and fasting insulin levels. Therefore we hypothesize that visceral adipose tissue will be a stronger correlate of metabolic risk factors than will any other fat tissue depot.

METHODS

Design

The sample of this sub-study comes from participants between the ages of 14-18 years old (N= 105; 26 males, 79 females) who completed baseline Magnetic Resonance Imaging (MRI) testing for the larger ongoing Healthy Eating, Aerobic and Resistance Training in Youth (HEARTY) study. Recruitment began in April 2005 and recently closed at the end of May 2010. Overall the HEARTY study is a randomized, controlled trial with a parallel group design. Several recruitment methods were employed to attract potential participants such as family physician referrals, posters and school board advertisements, bus campaigns and radio station advertisements. The Ottawa Hospital Research Ethic Board approved of all the methods and procedures.

Participants

The research coordinator at the Riverside Hospital (Ottawa, Ontario, Canada) screened sedentary, overweight or obese, post-pubertal male and female adolescents to determine eligibility. To be eligible, potential participants were considered if they were Tanner stage IV or above in pubertal maturity. They were also required to have a body mass index (BMI) $\geq 95^{th}$ percentile for age and sex or a BMI $\geq 85^{th}$ percentile for age and sex plus any of the following: fasting glucose $\geq$ 6.0 mmol/L, 2h plasma glucose 7.8-11 mmol/L after 75g oral glucose signifying impaired glucose tolerance, fasting triglycerides >1.7mmol/L, fasting plasma insulin >105 mmol/L, HDL-C <0.9 mmol/L, LDL-C >3.0 mmol/L, total cholesterol/HDL-C $>90^{th}$ percentile or a first degree relative with type II diabetes and a waist circumference $\geq 75^{th}$ percentile for their age and gender.

They were excluded from the study if they had diabetes mellitus, used any performance-enhancing medications or herbal supplements that could alter their body composition and/or had uncontrolled hypertension. Potential participants with a body weight over 159kg and/or a BMI over 45 kg/m^2 were also excluded from the study as their physical size exceeds that of the capability of the MRI machine.

Anthropometric Variables

Body mass was measured on a Health-O-Meter manual scale to the nearest 0.1kg with the subjects dressed in light clothing. Standing height was measured to the nearest 0.1cm using a stadiometer. BMI was calculated by dividing the participant's weight in kilograms by the square of the participant's height in meters. Waist circumference was measured as the midpoint between the top of the iliac crest and the last floating rib at the end of expiration.

Tissue Measurement By Magnetic Resonance Imaging

Whole body (45-48 images) MRI data was obtained using a 1.5-T General Electric scanner (Signa Excite 1.5T HD, General Electric, Peterborough, Ontario, Canada). Images were acquired using a T1-weighted spin-echo pulse sequence with a repetition time of 210 ms and an echo time of 17 ms with a 256 x 256 matrix (Ross et al., 1996). After removal of all metal objects, participants were placed in the supine position on their stomachs with their arms extended over their head. A sagittal localizing image was used to center transverse sections on the line through the space between L_4 and L_5. Using L_4-L_5 as the point of origin, subjects went through the MRI scanner feet first until the scanner reached the L_4-L_5 region. Thereafter subjects were repositioned with their

arms entering the scanner first until L_4-L_5 was reached once again. Images acquired within the abdomen were 10 mm thick and spaced by 40 mm. Respiratory gating was used to combat motion-induced artifacts and to reduce the blurring of fat boundaries in the anterior region of the abdomen. Appendicular images taken were 5 mm thick and spaced by 15 mm.

MRI data was then transferred electronically to a stand-alone computer workstation for analysis using Slice-O-Matic software (version 4.3, Tomovision, Montreal, Canada). Subcutaneous adipose tissue was first analyzed in all the scans by using a watershed algorithm that distinguishes between gray-level regions of the image. Subcutaneous adipose tissue was delineated as the tissue located outside of the outermost boundary of the muscle wall. Abdominal subcutaneous adipose tissue was subdivided into superficial and deep areas by identifying the fascia superficialis that demarcates these two fat depots at the L_4-L_5 region. Visceral adipose tissue was defined as the adipose tissue contained within the boundaries of the rectus abdominus, internal obliques, quadratus lumborum and long back muscles excluding lean tissue within the viscera.

Tissue area was computed automatically by Slice-O-Matic by summing the pixels and multiplying them by the individual pixel surface area. The volume (cm^3) of each tissue for the individual images was calculated by multiplying the respective tissue area (cm^2) by the slice thickness. The volume of each tissue for the spaces between two consecutive slices was then calculated with the following mathematical algorithm:

$$V = \sum_{I=1}^{N} A_I t + h \sum_{I=2}^{N} (A_{I-1} + A_I + (A_{I-1} A_I)^{0.5})$$

Where: V is the total tissue volume
A is the tissue area
t is the slice thickness
h is the distance between consecutive slices
N is the number of slices

The volume (liters) was then converted to mass units (kg) by multiplying the volumes by the assumed constant density of the tissue (0.92 for fat mass and 1.04 for fat-free mass). Body fat percentage was derived by dividing fat mass (kg) by total body weight and expressing this result as a percent. All measurements of body composition using the MRI are represented as mass in kilograms.

Measurement of Metabolic Parameters

Metabolic parameters were measured in all participants by the research coordinator at the Riverside Hospital (Ottawa, Ontario, Canada). Systolic and diastolic blood pressure was measured using a random-zero mercury sphygmomanometer. Three readings of systolic and diastolic blood pressure were recorded, and the average of the 3 was used for analysis.

A fasting blood sample was drawn in the morning after participants had undergone a 12-hour fast. Plasma triglycerides, total cholesterol, LDL cholesterol and HDL cholesterol levels were measured using a Beckman-Coulter LX20 enzymatic assay. A Beckman-Coulter Access 2 Ultrasensitive kit was used to determine blood glucose levels. Participants were given a 75g glucose solution for the oral glucose tolerance test

(OGTT). Blood was drawn 2 hours after glucose ingestion to determine blood glucose disposal levels.

For analysis of insulin resistance, the homeostasis model assessment index (HOMA-IR) was used and calculated from measures of fasting insulin and glucose (Matthew et al., 1985) using the following equation:

*HOMA-IR= fasting insulin concentration (U/mL) * fasting glucose concentration (mmol/L)/22.5*

STATISTICAL ANALYSIS

All variables were checked for normality of distribution using scatterplots and Shapiro-Wilk test before analyses. Data are presented as group means ± SD. Relationships between body composition and metabolic variables were determined using Pearson product moment correlation coefficient blood pressure variables, total cholesterol, HDL cholesterol, fasting glucose and 2-hour glucose. Spearman product moment correlation was performed for those variables that were not normally distributed such as triglyceride, fasting insulin, LDL cholesterol and HOMA-IR levels. Independent predictions were determined using multiple regression stepwise analysis. Significant outliers were not included in the regression model. All statistical measurements were analyzed using SPSS software v.16.0 with a significance set at $p<0.05$. Subject characteristics, correlations between adipose tissue measures and metabolic risk factors and the multiple regression analysis will be presented in the results section. The beta value, standard deviation and mean for the multiple regression values are shown in the results. All others values are depicted in tables.

RESULTS

Subject Characteristics

Subject characteristics for 105 female and male adolescents are presented in Table 1. The adolescents were obese (mean BMI= 34.5 ± 4.0 kg/m^2) and abdominally obese (mean waist circumference= 97.2, ±10.3 cm). The groups were characterized by a wide variation in total, visceral and subcutaneous adipose tissue (Table 1). In addition there was a wide range variation in fasting glucose, 2-hour glucose, fasting insulin, cholesterol, blood pressure and triglyceride values (Table 2).

Correlation Between Adipose Tissue Measures and Metabolic Variables

Pearson product moment correlations between adipose tissue measures and metabolic variables are presented in Table 3. A correlation coefficient of $r = \pm 0.1$, $r = \pm 0.3$, $r = \pm 0.5$ corresponded to small, medium, and large effect sizes, respectively (Yockey, 2008).

Total adipose tissue was positively correlated with SBP, DBP, MAP, triglycerides, 2-hour glucose, fasting insulin, and HOMA-IR. There were no significant relationships observed between total adipose tissue and HDL cholesterol, LDL cholesterol, total cholesterol, and fasting glucose.

Visceral adipose tissue was positively correlated with SBP, DBP, MAP, triglycerides, fasting insulin, total cholesterol, 2-hour glucose, and HOMA-IR. A significant negative correlation was observed between visceral adipose tissue and HDL cholesterol (Figure 1). There were no significant relationships observed between visceral adipose tissue and fasting glucose or LDL cholesterol.

Total subcutaneous adipose tissue was positively correlated with SBP, DBP, MAP, 2-hour glucose, fasting insulin and HOMA-IR. No significant relationship existed between total subcutaneous adipose tissue and fasting glucose, triglycerides or cholesterol measures.

Superficial subcutaneous adipose tissue was positively correlated with SBP, DBP, MAP, fasting insulin and HOMA-IR. There were no significant relationships observed between superficial subcutaneous adipose tissue and triglycerides, cholesterol variables, fasting glucose and 2-hour glucose values.

Deep subcutaneous adipose tissue was positively correlated with fasting insulin. No significant relationship between deep subcutaneous adipose tissue and SBP, DBP, MAP, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, fasting glucose, 2-hour glucose or HOMA-IR were observed.

Multiple Regression Analysis Between Adipose Tissue Measures and Metabolic Variables

A multiple regression analysis was conducted to predict metabolic variables from total adipose tissue, visceral adipose tissue and subcutaneous adipose tissue (total, superficial and deep) quantity. According to the analysis, quantity of visceral adipose tissue was a significant correlate of total cholesterol (ß=0.50, $t(103)=2.37$, $p<0.05$), LDL cholesterol (ß=0.43, $t(103)=2.02$, $p<0.05$), 2-hour glucose (ß=0.43, $t(103)=2.02$, $p<0.05$), and triglycerides (ß=0.56, $t(103)=2.81$, $p<0.01$) however, it did not predict fasting insulin (Figure 1). Total subcutaneous adipose tissue (ß= -2.76, $t(103)= -2.42$, $p<0.01$) and total adipose tissue (ß=3.16, $t(103)=2.65$, $p<0.01$) significantly predicted insulin. Deep subcutaneous adipose tissue predicted DBP (ß= -0.33, $t(103)= -2.33$, $p<0.05$), SBP (ß= -

0.314, $t(103)= -2.19$, $p<0.05$), and MAP(ß= -0.37, $t(103)= -2.71$, $p<0.01$). Adipose tissue variables did not significantly predict HOMA-IR or HDL cholesterol.

DISCUSSION

Our findings demonstrate that visceral adipose tissue alone was a significant correlate of total cholesterol, LDL cholesterol, glucose uptake and triglycerides after controlling for total adipose tissue, total subcutaneous adipose tissue, deep subcutaneous adipose tissue and superficial subcutaneous adipose tissue. Subcutaneous adipose tissue alone significantly predicted fasting insulin levels while deep subcutaneous adipose tissue significantly predicted measures of blood pressure.

These results suggest that visceral adipose tissue is a strong marker of abnormal metabolic profile independent of subcutaneous adipose tissue. Even after investigating the effects of deep and superficial subcutaneous adipose tissue on metabolic profile, visceral adipose tissue remains the main fat depot that is associated with metabolic abnormalities in obese adolescents. These observations confirm and strengthen previous observations from others, even those studies that did not account for deep and superficial subcutaneous adipose tissue. Previous reports suggest that visceral adipose tissue in Caucasian adults (Pontirolli et al., 2009; Smith et al., 2001; Tanaka et al., 2004), East Indians (Goel et al., 2010) and adolescents (Kim & Park, 2008; Gutin et al., 2007; Owens et al., 2000; Syme et al., 2008) is a significant correlate of triglycerides and cholesterol.

The location of adipose tissue within the abdomen is believed to affect metabolic function in different ways due to their distinct anatomic and metabolic characteristics. Free fatty acids released through lipolysis in visceral adipose tissue have direct access to the liver because it is close to the portal vein. An excess of free fatty acids may interfere

with liver metabolism inducing hypertriglyceridemia and glucose intolerance (Bergman et al., 2001; Björntrop, 1990). The higher lipolytic rate in visceral fat compared to subcutaneous fat may be attributed to anatomical variation in the action of hormones such as catecholamines and insulin (Arner, 1995; Bergman et al., 2006; Bergman et al., 2001). Visceral adipocytes are highly responsive to catecholamine stimulation and poorly responsive to lipolysis inhibition by insulin (Arner, 1995; Bergman et al., 2006; Bergman et al., 2001) whereas the opposite is true for superficial subcutaneous adipocytes. The accumulation of visceral adipose tissue may also induce changes in the concentration of adipokines such as adiponectin, leptin and resistin, thereby causing chronic inflammation which may be related to the development of the metabolic syndrome and type 2 diabetes (Matzuzawa et al., 1999).

It has also been proposed that visceral adipose tissue exerts a significant effect on glucose metabolism (Randle, 1964). In the liver, the increased plasma free fatty acids could lead to a decrease in hepatic extraction of insulin, increasing peripheral insulin concentrations. Also, high peripheral free fatty acid levels could decrease glucose oxidation and utilization by the Randle cycle due to the increased rate of lipid oxidation (Randle, 1964). Previous study findings support the hypothesis that visceral adipose tissue is predictive of glucose metabolism in both adults (Pontirolli et al., 2009; Ross et al., 2002a; Ross et al., 2002b) and adolescents (Caprio et al., 1995; Kim & Park et al., 2008). In contrast, we did not find that visceral adiposity was a correlate of insulin resistance using HOMA-IR.

Discrepancies in techniques for determining insulin resistance may have contributed to these differing results. We used HOMA-IR to determine insulin resistance

however Caprio et al. (1995) and Ross et al. (2002a, 2002b) used the "gold standard" technique (Ferannini & Mari, 1998) for the determination of insulin resistance. In our study however, HOMA-IR was used instead of the euglycemic clamp technique, as it would have been prohibitively burdensome and expensive. Although HOMA-IR may not be the "gold standard" for the measure of insulin resistance, Emoto et al. (1999) found a correlation of r=0.72 while Bonora et al. (2000) found a correlation of r=0.82 between HOMA-IR and the hyperinsulinemic euglycemic clamp technique (Bonora et al., 2000) suggesting a satisfactory assessment of insulin resistance in children and adolescents (Keskin et al., 2005; Stumvoll et al., 2000).

Another plausible reason to explain why our results differ from those of Pontirolli et al. (2009) and Ross et al. (2002a, 2002b) may be the age of our sample. Visceral and subcutaneous fat distribution may be different during puberty compared to adulthood, which may lead to differences in tissue metabolism in adolescents compared to adults (Frudelund-Larsen et al., 2007; Lundgren et al., 2007). During puberty, growth spurts have a significant effect on visceral adipose tissue whereby an increase in this tissue must occur to adjust for the increase in subcutaneous adipose tissue to enable growth in adolescents (Huang et al, 2001). Also, the length of time the participants have been overweight or obese may account for some differences in results. In adults, years of being obese allow for the contribution of adipokines to insulin resistance (Bergman et al., 2006). Since our sample is younger than previous reports, they may not be displaying this alteration in adipokines as that seen in adults.

Previous studies have suggested subdividing abdominal subcutaneous adipose tissue into superficial and deep compartments to help explain differences in results

between reports (Ross et al., 2002a). Studies have found that deep abdominal subcutaneous adipose tissue is a stronger correlate of insulin, triglyceride and cholesterol values compared to other fat depots (Kelley et al., 2000; Miyazaki et al., 2002; Smith et al., 2001). Monzon and colleagues (2002) confirmed these findings in biopsies of deep and superficial subcutaneous adipocytes from 8 male and female patients undergoing elective abdominal surgery for cholecystectomies or hernia repairs. They found that deep subcutaneous adipoctyes were lipolytically more active than superficial subcutaneous adipocytes. In swine, Mersmann & Leymaster (1984) observed that the deep layer of subcutaneous adipose tissue accumulates faster than the more superficial layer and arises from cells similar to those found in visceral fat. It has been hypothesized that these differences could be due to differentiating roles of the adipose tissue layers. Mersmann & Leymaster (1984) theorized that the superficial subcutaneous adipose tissue may play more of a "thermo-insulatory" role, whereas the deep subcutaneous adipose tissue may play more of a "metabolic" role

To examine the difference in subcutaneous tissue types, we subdivided it into deep and superficial layers at the L_4-L_5 region. However, using Spearman and Pearson's regression correlation, we did not find that deep or superficial subcutaneous adipose tissue were strong correlates of cholesterol, insulin, glucose tolerance or triglyceride values. Differences in results could pertain to inter-rater reliability in distinguishing the fascia superficialis. In our sample, as in others, fascial discontinuity can occur due to MRI artifacts known as volume averaging rather than an anatomic interruption of the fascia superficialis (Smith et al., 2001). This can occur when the fascial plane is not perpendicular to the scan axis resulting in a loss of visualization of the fascia.

Visceral adipose tissue accumulation but not subcutaneous adipose tissue has also been related to increased sympathetic activation in adult males (Alvarez et al., 2004; Alvarez et al., 2002). Sympathoactivation is thought to be the mechanism underlying obesity-related hypertension (Esler et al., 2006). Increased sympathetic outflow to the kidneys and vasculature can increase sodium and water reabsorption and peripheral resistance thereby increasing blood pressure. It has been suggested that hyperinsulinemia may play a role in sympathoactivation (Rahmouni et al., 2005). In our study, however, deep subcutaneous adipose tissue was a significant correlate of blood pressure and subcutaneous adipose tissue was a significant correlate of insulin values rather than visceral adipose tissue. These results do not support the involvement of insulin in high visceral adipose tissue-associated sympathoactivation and blood pressure changes previously observed in males.

Limitations

A limitation of the present study was its cross-sectional design, which made it difficult to determine causality with the observed relationships. However, the evidence from our study and others (Goel et al., 2010; Gutin et al., 2007; Kim & Park, 2008; Owens et al., 2000; Pontirolli et al., 2009; Smith et al., 2001; Syme et al., 2008; Tanaka et al., 2004) indicates that visceral fat plays an important role in the development of many metabolic abnormalities including glucose intolerance, high cholesterol, dyslipidemia and hypertriglyceridemia. In addition, all the participants included in the study were considered overweight or obese based on their BMI percentile, which may have influenced our results and the applicability of our findings to a leaner population.

Conclusion

Our findings suggest that even during adolescence, visceral obesity, not subcutaneous obesity, appears to be the most important determinant of metabolic abnormalities in overweight and obese individuals aged 14-18 years old. Further subdivisions of abdominal subcutaneous adipose tissue did not provide any additional insight into the relationship between abdominal obesity and metabolic abnormalities in our sample. These observations reinforce the importance of treatment strategies designed to reduce visceral adipose tissue in obese adolescents. A longitudinal study considering physiological changes of hormone metabolism during puberty could provide insight on the influence of sex hormones on adipose tissue during this adolescent period of growth and maturation. Future research should also focus how visceral adipose tissue metabolism may change over a period of time, beginning in prepubescent children.

ACKNOWLEGEMENTS

The HEARTY trial was supported by a grant from the Canadian Institutes of Health Research (MCT-71979). Ms. Pamela Martino also holds an Ontario Graduate Scholarship and Ms. Angela Alberga holds a Doctoral Student Research Award from the Canadian Diabetes Association. Dr. Sigal holds a Health Senior Scholar award from the Alberta Heritage Foundation for Medical Research. He was previously supported by an OHRI Research Chair in Lifestyle Research. Dr. Kenny holds a University of Ottawa Research Chair.

Table 1. Characteristics of HEARTY participants

	Mean ± SD (N=105)	**Range**
Anthropometric measurements		
Age (yrs)	15.5 ± 1.3	14-18
Weight (kg)	97.7 ± 15.5	67.2-133.5
Height (cm)	168.3 ± 7.1	155.0-194.0
Waist Circumference (cm)	97.1 ± 10.3	77.5-124.0
BMI (kg/m^2)	34.5 ± 4.0	25.6-45.8
MRI measurements		
Total AT (kg)	46.4 ± 9.5	27.7-71.8
SAT (kg)	39.5 ± 8.5	22.3-61.5
Superficial SAT (kg)	0.87 ± 0.28	0.37-1.60
Deep SAT (kg)	0.80 ± 0.23	0.26-1.42
VAT (kg)	1.35 ± 0.53	0.54-2.84

Abbreviations: HEARTY, Healthy Eating Aerobic and Resistance Training in Youths; Values are means ± SD. BMI, body mass index; MRI, magnetic resonance imaging; AT, adipose tissue; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

Table 2. Metabolic characteristics of HEARTY subjects

	Mean ± SD (N=105)	**Range**
SBP (mm Hg)	114 ± 12	90-138
DBP (mm Hg)	74 ± 8	50-90
MAP (mm Hg)	86 ± 8	64-104
Triglycerides (mmol/L)	1.37 ± 0.67	0.33-3.18
Total cholesterol (mmol/L)	4.24 ± 0.77	2.90-6.40
HDL cholesterol (mmol/L)	1.08 ± 0.03	0.55-1.69
LDL cholesterol (mmol/L)	2.54 ± 0.66	1.30-4.40
Fasting glucose (mmol/L)	5.07 ± 0.42	4.2-6
2 hour glucose (mmol/L)	5.64 ± 1.12	3.00-8.50
Fasting insulin (mmol/L)	104.0 ± 57.8	34.0-288.0
HOMA-IR	3.85± 2.34	1.21-12.24

Abbreviations: HEARTY, Healthy Eating Aerobic and Resistance Training in Youths; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; OGTT, oral glucose tolerance test; HOMA-IR, homeostasis model assessment for insulin resistance.

Table 3. Pearson and Spearman correlation coefficients between fat distribution and metabolic variables

	Total AT	**SAT**	**sSAT**	**dSAT**	**VAT**
SBP (mL/Hg)	0.36†	0.35†	0.30†	0.14	0.36†
DBP (mL/Hg)	0.39†	0.38†	0.32†	0.16	0.37†
MAP (mL/Hg)	0.43†	0.42†	0.36†	0.17	0.37†
Triglycerides (mmol/L)	0.23*	0.19	0.08	0.15	0.45†
Total cholesterol (mmol/L)	-0.01	-0.04	-0.03	-0.08	0.20*
HDL cholesterol (mmol/L)	-0.13	-0.09	-0.005	-0.09	-0.30†
LDL cholesterol (mmol/L)	-0.09	-0.11	-0.14	-0.11	0.15
Fasting glucose (mmol/L)	-0.02	-0.01	0.02	-0.09	-0.002
2 hour glucose (mmol/L)	0.20*	0.20*	0.17	0.14	0.20*
Fasting insulin (mmol/L)	0.39†	0.34†	0.32†	0.22*	0.41†
HOMA-IR	0.35†	0.32†	0.30†	0.18	0.37†

Abbreviations: HEARTY, Healthy Eating Aerobic and Resistance Training in Youths; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; OGTT, oral glucose tolerance test; HOMA-IR, homeostasis model assessment for insulin resistance. *, indicates significance at $p<0.05$; †, indicates significance at $p<0.01$.

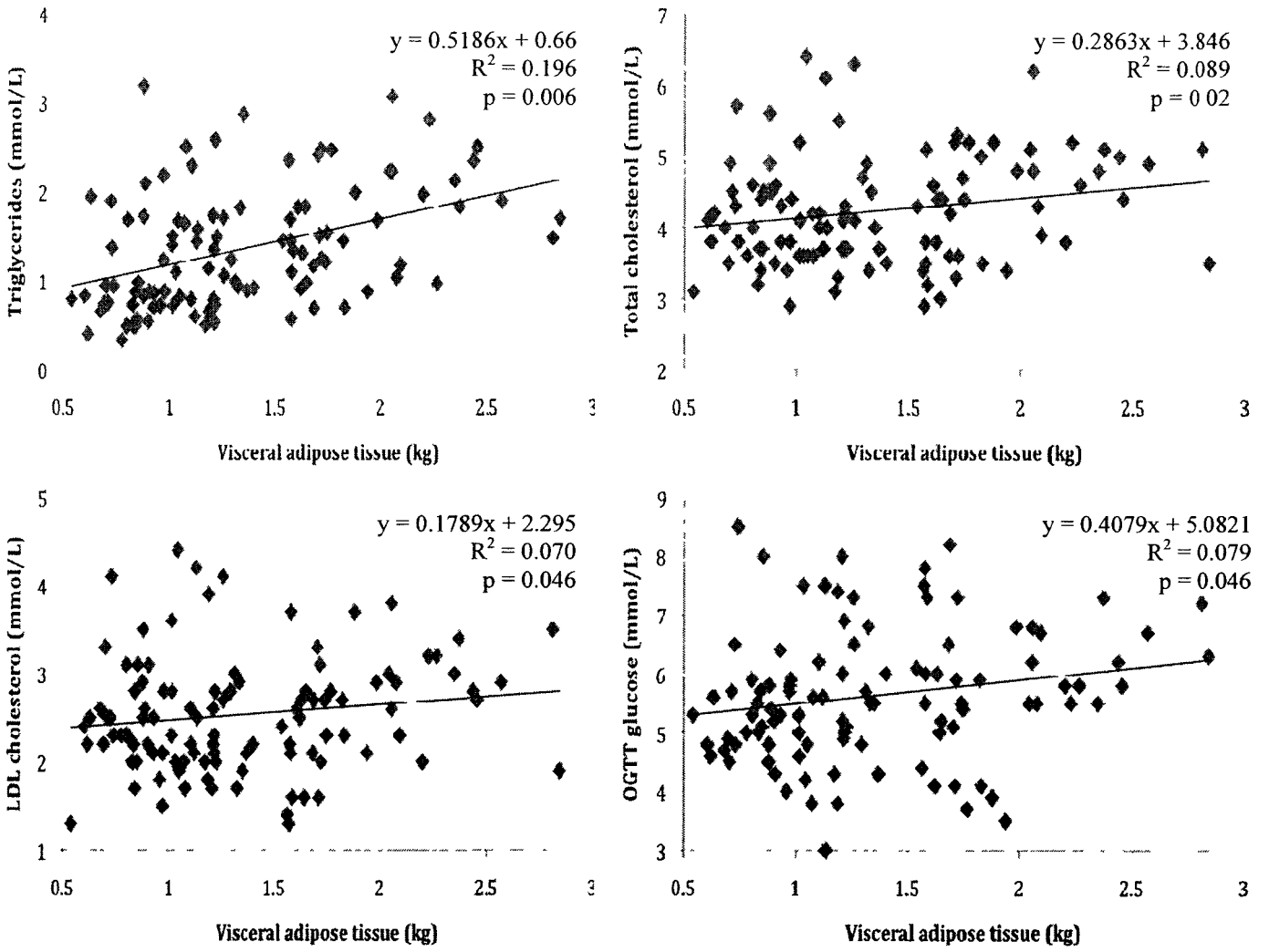

Figure 1. Spearman correlation between visceral adipose tissue and triglycerides, total cholesterol, LDL cholesterol, OGTT glucose, insulin, and HOMA-IR. Regression lines are shown for those metabolic variables that significantly predicted by visceral adipose tissue.

4. GENERAL CONCLUSIONS

The primary purpose of the first article was to compare the resting metabolic rate of obese adolescents, aged 14-18 years old, with and without the metabolic syndrome matched for age, sex and BMI. A second objective was to determine if adolescents with the metabolic syndrome would exhibit differences in body composition compared to those without the metabolic syndrome. The results of our study demonstrate that obese adolescents with the metabolic syndrome do not exhibit a difference in resting metabolic rate compared to those without. However, they did have a greater amount of visceral adipose tissue compared to their metabolically healthy counterparts. This important finding should be incorporated into the design for future studies exploring how different exercise programs may change the body fat composition in obese adolescents. Future studies should also focus on comparing the metabolic profile of adolescents who do not exhibit any criteria of the metabolic syndrome to those with the metabolic syndrome.

The objective of the second article was to explore the relationship between body adipose tissue compartments and metabolic risk factors in our sample of obese adolescents. It was found that visceral adipose tissue independently predicts triglyceride, total cholesterol, LDL cholesterol and 2-hour glucose levels. The results of this study clearly indicate that during the early stages of obesity visceral adipose tissue, not subcutaneous or total adipose tissue is the most important determinant of metabolic abnormalities in obese adolescents. Although exercise is beneficial for everyone, it is clear that exercise interventions and obesity prevention programs should be geared towards obese adolescents with high amounts of visceral fat as well as those who are obese or overweight. A review by Wajchenberg (2000) emphasizes the positive

influence of physical activity on metabolic risk factors and the amount of visceral fat. Studies have shown that physical exercise with (Barbeau et al., 2007; Park & Lee, 2004) and without (Gutin & Owens, 1999) dietary intervention show favourable changes in visceral adipose tissue and metabolic profile. Our results support the recommendation by Suliga (2009), which underlines the need for more effective interventions with obese youth to decrease visceral adipose tissue. The intensity and type of physical activity that is most efficient for visceral adipose tissue reduction needs to be explored.

The results obtained from this research project provide useful insight into how body fat depots differ in adolescents with the metabolic syndrome and which of these depots contributes to an unhealthy metabolic profile. While our cross-sectional study cannot preclude the most appropriate exercise program that will cater to the needs of obese male and female adolescents, we can deduce that interventions are a worthwhile consideration to decrease adipose tissue in order to ameliorate the metabolic profile of this vulnerable population.

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APPENDIX

International Diabetes Federation Definition for the Metabolic Syndrome in children and adolescents

Age	Waist circumference	Triglycerides	HDL-cholesterol	Blood pressure	Fasting Plasma Glucose
6-<10 years old	>90th percentile	Metabolic syndrome cannot be diagnosed, but further measurements should be made if there is a family history of metabolic syndrome, type II diabetes, dyslipidemia, cardiovascular disease, hypertension and/or obesity			
10-<16 years old	>90th percentile/adult cutoff if lower	≥1.7mmol/L (≥150mg/dL)	<1.03mmol/L (<40mg/dL)	Systolic≥130mmHg Diastolic≥85mmHg	≥5.6mmol/L (≥100mg/dL) Or known type 2 diabetes
>16 years old	Men: ≥94cm Women: ≥80cm	≥1.7mmol/L (≥150mg/dL)	Men:<1.03mmol/L (<40mg/dL) Women:<1.29mmol/L (<50mg/dL)	Systolic≥130mmHg Diastolic≥85mmHg	≥5.6mmol/L (≥100mg/dL) Or previously diagnosed type 2 diabetes

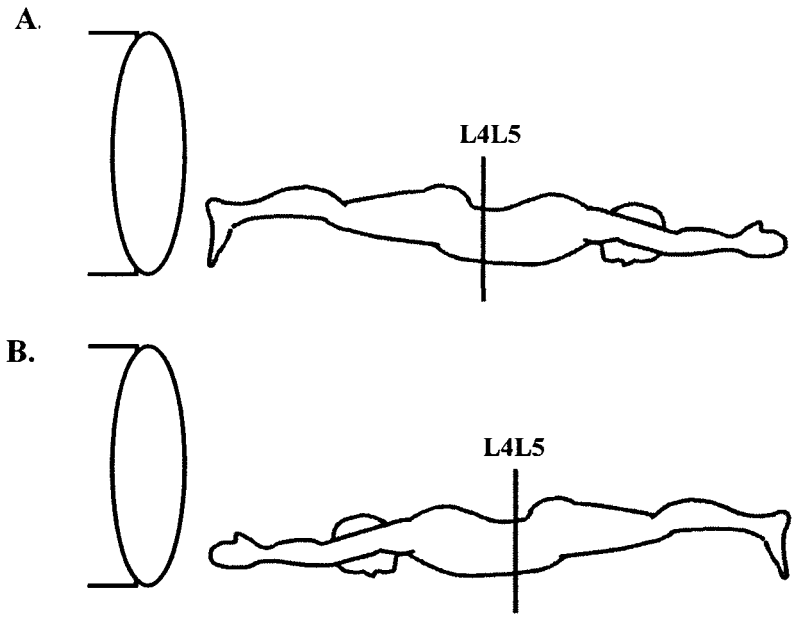
International Diabetes Federation (2007)

Waist Circumference Percentile Regression Values in North America for all Children and Adolescents According to Sex

	Percentile for boys					Percentile for girls				
Age (years)	10th	25th	50th	75th	90th	10th	25th	50th	75th	90th
2	43.2	45.0	47.1	48.8	50.8	43.8	45.0	47.1	49.5	52.2
3	44.9	46.9	49.1	51.3	54.2	45.4	46.7	49.1	51.9	55.3
4	46.6	48.7	51.1	53.9	57.6	46.9	48.4	51.1	54.3	58.3
5	48.4	50.6	53.2	56.4	61.0	48.5	50.1	53.0	56.7	61.4
6	50.1	52.4	55.2	59.0	64.4	50.1	51.8	55.0	59.1	64.4
7	51.8	54.3	57.2	61.5	67.8	51.6	53.5	56.9	61.5	67.5
8	53.5	56.1	59.3	64.1	71.2	53.2	55.2	58.9	63.9	70.5
9	55.3	58.0	61.3	66.6	74.6	54.8	56.9	60.8	66.3	73.6
10	57.0	59.8	63.3	69.2	78.0	56.3	58.6	62.8	68.7	76.6
11	58.7	61.7	65.4	71.7	81.4	57.9	60.3	64.8	71.1	79.7
12	60.5	63.5	67.4	74.3	84.8	59.5	62.0	66.7	73.5	82.7
13	62.2	65.4	69.5	76.8	88.2	61.0	63.7	68.7	75.9	85.8
14	63.9	67.2	71.5	79.4	91.6	62.6	65.4	70.6	78.3	88.8
15	65.6	69.1	73.5	81.9	95.0	64.2	67.1	72.6	80.7	91.9
16	67.4	70.9	75.6	84.5	98.4	65.7	68.8	74.6	83.1	94.9
17	69.1	72.8	77.6	87.0	101.8	67.3	70.5	76.5	85.5	98.0
18	70.8	74.6	79.6	89.6	105.2	68.9	72.2	78.5	87.9	101.0

International Diabetes Federation (2007)

Positioning of Subject in the MRI scanner



Subject was first positioned with feet entering the MRI scanner (A.) and then repositioned with the hands going into the MRI scanner (B.).