1-1-2013

Associations Between Resting Metabolic Rate and Energy Storage in Young Adults

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ASSOCIATIONS BETWEEN RESTING METABOLIC RATE AND ENERGY STORAGE IN YOUNG ADULTS

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Submitted in Partial Fulfillment of the Requirements

For the Degree of Doctor of Philosophy in

Exercise Science

The Norman J. Arnold School of Public Health

University of South Carolina

2013

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DEDICATION

To my loyal friend Tobie, who is always eager for a long walk

ACKNOWLEDGEMENTS

I am extremely thankful to have been supported in my life by many caring and thoughtful people. My words will never convey the full scope of my appreciation, but I hope each of you sees your strengths in me as a person and a professional.

A sincere thank you to Dr. Blair- Not only have you given me professional opportunities that I could have never imagined, but the kindness from you and Jane towards me and my family will never be forgotten.

Thank you to Drs. Hand, Wang, and Moran for your guidance and individual expertise throughout my dissertation process.

I am extremely thankful to Steven Hooker and Sara Wilcox- Steve for giving me my first opportunity at USC as a PhD student and Sara for kindly giving me an academic home when I was advisor-less.

I have been extremely fortunate to been mentored by several talented researchers throughout my short career- Warren Franke, Benjamin Levine, and Tim Church. My accomplishments would not be possible without your leadership and every day I use the lessons you taught me.

I am grateful to the Energy Balance staff for their tireless work on the project. Literally dozens of people have worked to make this study successful and I hope they are as proud of the project as I am. Specific thanks are extended to Amanda Paluch, Beth Lach, Patrick Crowley, Sarah Schumacher, and Madison Demello. Big thanks to the best

recruitment team in the country- Ellen Wingard, Leanna Ross, Lauren Seilor, and Nichole Gribben.

My family has been a rock to me throughout my life- I always know you are here for me. Thank you to my parents for never questioning my decisions to move to some far off place. Thank you, Katie, for your love and support.

Finally, thank you to my wonderful friends. You will never fully understand how happy I am to have gone through this time in South Carolina with each of you. Whether it was debates around the second floor cubicles, coffee breaks at Cool Beans, or running from Columbia to Charleston, your friendships made the tough days better and the good days great.

ABSTRACT

At the most basic level obesity is the result of a chronic imbalance between energy intake and energy expenditure. However, the exact etiology is considerably more complex and may involve a variety of physiological and behavioral factors. Metabolic disturbances, including reduced fat oxidation as measured by the respiratory quotient (RQ) and reduced resting metabolic rate (RMR), have been identified as possible predictors of changes in body weight and body composition. RMR represents the largest component (60-80%) of caloric expenditure that contributes to total daily energy expenditure in humans and has high inter-person variability (±25%) but not within individuals (<±5%). The cause of this variability between individuals and the exact role of RMR and RQ in determining body weight and body composition are uncertain.

This dissertation consists of three studies that were designed to 1) Identify correlates of RMR among behavioral and physiological variables in a cohort of young adult men and women; 2) Examine racial differences in RMR, body weight, and body composition among young adult women; and 3) Explore the longitudinal effects of RMR, RQ, physical activity, and dietary intake on subsequent changes in body weight and body composition in young adults followed for nine months.

Three manuscripts were composed by analyzing data collected from the Energy Balance Study, an observational research study involving young adults (N=430). We measured RQ and RMR using indirect calorimetry, along with body weight and body

composition using dual energy X-ray absorptiometery, energy expenditure and time spent in physical activity using an arm-based activity monitor, and energy intake using interviewer-administered dietary recalls.

The results of study 1 found fit individuals had a higher RMR compared to unfit individuals after controlling for differences in body composition between the groups. However, the decrease in RMR from low levels fitness compared to moderate or high levels of fitness was modest and represented approximately 3% of RMR or 47 kcal/day. Time spent in moderate to vigorous physical activity was also significantly related to RMR, but this influence was also small and had little predictive value over adjustments for body composition.

Study 2 confirmed previous research study finding young adult African-American women have a lower RMR compared to their white peers after statistical adjustments for differences in body composition (1400.3±9.1 kcal/day vs. 1299.8±18.9 kcal/day, $P<0.0001$). African-American women had higher levels of fat mass compared to white women which resulted in elevated RMR beyond the differences in fat free mass prior to statistical adjustment. Additionally, cardiorespiratory fitness was significantly positively associated with RMR, but time spent in moderate to very vigorous physical activity was not.

Finally, individuals with a high RQ gained significantly more body weight (1.55±0.23 vs. 0.83±0.18 kg, $P=0.0040$) and fat mass (1.19±0.23 vs. 0.60±0.18 kg, $P=0.0150$) over a 9 month period compared to those with a low or moderate RQ, independent of changes in energy intake, energy expenditure, macronutrient composition

of the diet, and physical activity. Additionally, a low RMR was not associated with gains in body weight or fat mass over the same period.

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List of Abbreviations

BMI ..... Body mass index

BP ..... Blood pressure

CRF ..... Cardiorespiratory fitness

DXA ..... Dual-energy absorptiometry

ECG ..... Electrocardiogram

EPOC ..... Excess post-exercise oxygen consumption

FPV ..... Food portion visual

GXT ..... Graded exercise test

HR ..... Heart rate

NDSR ..... Nutrition data system for research

PAEE ..... Physical activity energy expenditure

REE ..... Resting energy expenditure

RMR ..... Resting metabolic rate

RQ ..... Respiratory quotient

TDEE ..... Total daily energy expenditure

TEF ..... Thermic effect of food

VO_2 ..... Volume of oxygen

VCO_2 ..... Volume of carbon dioxide

CHAPTER 1

OVERALL INTRODUCTION

Resting metabolic rate (RMR), the amount of calories burned from normal physiological functions (E.g. respiration, brain activity), represents the largest component (60-80%) of total daily energy expenditure (TDEE) in humans. Because of this, the relationship of RMR to body weight and body composition is of great interest given the current levels of overweight and obesity. While RMR is remarkably stable within individuals (less than ±5% day-to-day variability), RMR has high inter-person variability (±25%). The cause of this variability and the role of energy expended from RMR on changes in body weight and body composition over time is uncertain and controversial. This dissertation consists of three studies that have been developed to better understand 1) the relationship of RMR with behavioral (levels of physical activity, dietary intake) and physiological variables (gender, age, fitness level, body composition) in a group of healthy young adults, 2) the racial differences in RMR among young adult women, and 3) the longitudinal effects of RMR, physical activity energy expenditure, and dietary intake on changes in body weight and body composition over 6 months in a group of healthy young adults.

Statement of the Problem

The average adult in the United States consumes approximately 1 million calories each year. Despite this large intake of energy, most healthy adults are able to achieve a balance of energy expenditure primarily through mechanisms required to sustain life

(resting metabolic rate), digest food (thermic effect of food), and perform activities (thermic effect of physical activity). It is a chronic mismatch over time in this energy balance which results in changes of body energy stores. Because the largest contributor to energy expenditure is resting metabolic rate, small changes in it could result in a large number of calories over time. RMR was once thought to be determined only by body size and thus easily estimated using prediction equations. However, it is now accepted that RMR is highly variable, with differences as much as 25% between individuals. Given the high volume of energy expended and the large variability between individuals, it is important to better understand the determinants of RMR and the subsequent effect on body weight and composition.

SCOPE OF THE STUDY

The overall goal of this dissertation is to 1) identify variables which explain the inter-individual variation of RMR, 2) determine if differences exist in RMR between AA and W women after adjustment for potentially confounding variables, and 3) explore the role of RMR, RQ, energy expenditure, and energy intake on subsequent changes in body weight and energy storage.

SPECIFIC AIMS AND RESEARCH QUESTIONS

The specific aims and research questions that will be explored in this dissertation are as follows:

Specific Aim 1: Identify correlates of RMR among behavioral and physiological variables in a cohort of young adult men and women.

Research Question 1.1 What is the nature of associations between RMR and behavioral variables associated with physical activity?

Research Question 1.2 What is the nature of the associations between RMR and behavioral variables associated with dietary intake?

Research Question 1.3 What is the nature of the associations between RMR and physiological variables?

Specific Aim 2: Examine racial differences in RMR, body weight, and body composition among young adult women.

Research Question 2.1 Do African-American women have a lower RMR compared to Caucasian women?

Research Question 2.2 Are there differences in body weight, body composition, and body composition distributions among African-American and Caucasian women?

Research Question 2.3 Are certain behavioral and physiological variables associated with diet and physical activity different among African-American and Caucasian women?

Specific Aim 3: Explore the longitudinal effects of RMR, RQ, physical activity, and dietary intake on subsequent changes in body weight and body composition in young adults followed for nine months.

Research Question 3.1 What is the longitudinal trend of RMR and RQ on body weight and body composition trajectory in young adults?

Research Question 3.2 Is volume or intensity of physical activity associated with changes in body weight and body composition?

Research Question 3.3 Are there gender differences in the relationships described in research questions 3.1 and 3.2?

CHAPTER 2

LITERATURE REVIEW

Components of energy expenditure

There are three major components of energy expenditure: basal metabolic rate, thermic effect of a meal, and thermic effect of physical activity. Basal metabolic rate is the 'minimal level of energy expended to sustain life' in a neutrally temperate environment while in a post-absorptive state and includes the energy cost of physiological functions such as muscle contractions, respiration, and brain function (Goran, 2000; Henry, 2005). Measurement of basal metabolic rate can be complicated task, so measurement of resting metabolic rate (RMR) is typically utilized instead, with the only methodological difference being basal metabolic rate is measured shortly after waking (<45 minutes) but before arousal (Ravussin, Lillioja, Anderson, Christin, & Bogardus, 1986), while RMR is measured after arousal (Goran, 2000). Because the values are similar (RMR is approximately 3% higher), the two terms are frequently used interchangeably (Goran, 2000) and for simplicity RMR will be used for the remainder of this text. RMR constitutes between 60 to 80% of total daily energy expenditure (TDEE)(Goran, 2000). Meal-induced thermogenesis, also referred to as the thermic effect of food (TEF), is the energy that is expended to digest, metabolize, and store ingested macronutrients (Goran, 2000). TEF constitutes approximately 6-10% of TDEE (Goran, 2000; Ravussin et al., 1986). Thermic effect of exercise, or as it is more widely called, physical activity energy expenditure (PAEE), describes the increase in metabolic rate that

is caused by contraction of skeletal muscles (Goran, 2000). PAEE constitutes between 10 to 30% of total energy expenditure. Additionally, two minor components of energy expenditure also exist. The energy cost of growth contributes to energy expenditure, but is inconsequential beyond the first months of life (Goran, 2000). Energy expenditure from exposure to environmental temperatures or diet is referred to as adaptive thermogenesis, but also rarely occurs outside of the initial months of life, extreme temperature changes (humans have broad thermoneutral zone with relatively small changes in metabolic rate occurring over relatively wide temperature changes, primarily due to behavioral responses such as changes in clothing), or prolonged caloric restriction or overfeeding (Doucet et al., 2001; Goran, 2000; Lowell & Spiegelman, 2000).

Resting Metabolic Rate

Overview RMR is the largest contributor of TDEE, representing between 60-80% of calories burned (Goran, 2000; Ravussin et al., 1986; Shetty, 2005), with sedentary individuals displaying a higher relative contribution due to lower levels of PAEE. As mentioned previously, basal metabolic rate is the minimum level of energy expended to sustain life and is typically measured shortly after sleep but before arousal (Henry, 2005). Due to the difficulty of measuring basal metabolic rate in a laboratory setting, RMR is generally used instead and is only slightly higher (3%) due to the metabolic cost of arousal (Goran, 2000; Ravussin et al., 1986). RMR is typically obtained soon after arousal, after 12 hours of fasting, and before any major physical activity (Ravussin et al., 1986).

The concept of measuring the burning of fuel in the body was originally described by the classic experiments by Lavoisier, often referred to as the father of modern

chemistry, in the late 1700s (Goran, 2000). The term *Grundumsatz* or basal metabolism was first coined by Magnus-Levy in 1899 and his work gained popularity due to the emphasis placed on standardizing measurements, such as an absence of gross muscular activity, a post-absorptive state, minimal emotional disturbance, wakefulness, normal nutritive condition, an absence of disease or infection, and a thermo-neutral environment (Henry, 2005). In the first half of the 20th century RMR was most commonly measured to diagnose clinical diseases, particularly hypo- and hyperthyroidism. It is now more frequently used to understand the etiology of obesity and to calculate food energy requirements, particularly since the publication of a joint report by the Food and Agricultural Organization, World Health Organization, and United Nations University (Food and Agriculture Organization; Food and Agriculture Organization of the United Nations., United Nations University., & World Health Organization., 2004).

Assessment Despite the attempts to standardize RMR methodology by Magnus-Levy over 100 years ago, interpretation of the existing literature can be a challenging task due to the variety of measurement protocols, participant populations, and analysis techniques utilized. For example, a review of literature summarizing 10 studies exploring racial differences found RMR as low as 1267 kcals/day to as high as 1899 kcals/day, depending on if the RMR measurements were made fasted or on a controlled diet, if the setting was an inpatient unit or outpatient laboratory, if the method for assessing body composition was dual-energy X-ray absortiometry, skinfolds, or underwater weighing, whether the participants included children, adolescents, adults, or post-menopausal women, and if the population was normal weight, overweight, obese, or matched on body composition (Gannon, DiPietro, & Poehlman, 2000). Additionally, there are evolving

concepts for the proper adjustment RMR for body size, with emphasis shifting from the whole-body to individual tissues and organs to better reflect metabolically active mass (Gannon et al., 2000; Heymsfield et al., 2012). These irregularities in the existing literature represent both challenges in interpretation and opportunities for new discoveries.

The origins of RMR measurement date back to the experiments of Lavoisier in the late 1700s after he observed a candle would burn only in the presence of oxygen, similar to how living organisms also required oxygen for life to produce heat as they combust food (Goran, 2000). The first direct calorimeter involved a small animal placed in a sealed chamber that was then surrounded by ice. As the animal expended energy and produced body heat, Lavoisier would measure the melted ice water and then calculate the amount of heat required to melt the ice.

RMR can be quantified by directly measuring the amount of heat produced by the human body through metabolism direct calorimetry, as used by Lavoisier, or indirectly by measuring carbon dioxide production (indirect calorimetry). Measurement of energy expenditure via direct calorimetry while a participant rests in a specialized chamber is technically complex, time intensive, and expensive, so indirect calorimetry is typically used in research settings (Branson & Johannigman, 2004; Manini, 2010; Ravussin et al., 1986). Indirect calorimetry measures RMR not through heat production but instead assessment of respiratory gases using a mouthpiece or ventilated hood. This method is based on the volume of oxygen consumed and carbon dioxide produced from the combustion of carbohydrate, protein, fat, and alcohol. Two types of indirect calorimetry systems exist; open and closed circuit. Closed circuit systems were commonly used in the

early 20th century, and involved determining oxygen consumption by measuring the absorption of carbon dioxide within the system (e.g. by soda lime) (Branson & Johannigman, 2004; Henry, 2005). Open circuit systems are most commonly used today, with RMR measured by calculating the difference between oxygen consumption and carbon dioxide production collected via a mouthpiece or ventilated hood and sent to a mixing chamber (Branson & Johannigman, 2004). Closed circuit systems are prone to measurement error and have been shown to produce spuriously higher RMR values compared to open circuit system (Henry, 2005), so caution is advised when directly comparing values from these two different systems (E.g. historical trends in RMR). Regardless of the method used, RMR should be measured under conditions of immobility, fasted (>12 hours after a meal), and at least 24 hours since the last bout of strenuous activity in an environment with a temperature between 26 to 30 degrees centigrade (Henry, 2005; Shetty, 2005).

As the name implies, RMR represents the rate of oxygen consumed over a period of time, typically one minute or one hour. It can be difficult contextually to interpret RMR in this form, so it is often more useful to express RMR as energy expenditure in terms of kilocalories per day. When expressed as kilocalories per day the term resting energy expenditure (REE) is often used interchangeably with RMR. For simplicity, RMR will be used throughout this document. Once the volume of oxygen (VO_2) consumed and carbon dioxide (VCO_2) produced are known, RMR can easily be calculated by application of the Weir equation (Weir, 1949):

where VO_2 and VCO_2 are expressed as L/min and 1440 is equal to the number of minutes in 24 hours. Also of interest when measuring RMR is respiratory quotient (RQ), which represents the oxidation of substrates such as carbohydrates and lipids. RQ is calculated by dividing VCO_2 by VO_2, with a value of 0.7 indicating pure lipid oxidation and 1.0 indicating pure carbohydrate oxidation (Branson & Johannigman, 2004).

Factors that influence resting metabolic rate

Fat free mass Body size is the primary determinant of RMR, a concept first systematically observed beginning in the 19th century by the French scientist Rameux (Heymsfield et al., 2012; Lusk, 1909). While initial hypotheses focused on the role of body surface area, it is now well-established that the fat free mass (FFM) component of the human body is the principal predictor of RMR (Heymsfield et al., 2012). FFM, which is predominantly composed of metabolically active tissues such as skeletal muscle and internal organs, explains 60-80% of the variation in RMR between individuals (Ravussin & Bogardus, 1989; Ravussin et al., 1986). This wide variance between individuals is due to heterogeneity of the non-fat components of the human body (i.e. FFM is not a single mass of metabolically identical tissue) (Heymsfield et al., 2002). For example, four organs (the brain, liver, heart, and kidneys) constitute <6% of body mass but are responsible for approximately 60% of RMR (Gallagher et al., 1998; Heymsfield et al., 2012; Keys & Brozek, 1953), with a metabolic rate of 357.6 kcal/kg/day (Holliday, 1971). Meanwhile, skeletal muscle represents approximately 40-50% of body mass in adults, but only approximately 18-36% of RMR (Gallagher et al., 1998; Goran, 2000), and has a metabolic rate of 17.6 kcal/kg/day (Holliday, 1971). Disproportional relative levels of skeletal muscle will result lower than expected levels of RMR due to the

discrepancy in size of other metabolically more active tissues such as the brain, liver, kidneys, and heart (Heymsfield et al., 2002). These and other components of FFM are not universally constant among humans, but vary in mass between individuals with body size (Gallagher, Allen, Wang, Heymsfield, & Krasnow, 2000), age (Gallagher et al., 2000), sex (Hayes et al., 2002), and race (Gallagher et al., 2006; A. Jones, Jr. et al., 2004).

Wiensier et al. combined RMR and FFM values from several studies involving individuals ranging in age from infants to adults to describe the contribution of metabolically active tissue across life (1992). During early life when internal organs comprise the highest relative amount of FFM, RMR from FFM is 79.0 kcal/kg/day. As skeletal muscle becomes the predominant component of FFM in adolescence and into adulthood, RMR resulting from FFM drops to 28.3 kcal/kg/day and 20.9 kcal/kg/day, respectively. This non-linear relationship between RMR and FFM in which the slope of the regression line decreases as FFM increases is critical to understanding the relationship between metabolism and body composition.

Fat mass Fat mass (FM) is also independently related to RMR despite often being incorrectly considered metabolically inert (Dionne, Despres, Bouchard, & Tremblay, 1999; Ferraro et al., 1992; M. P. St-Onge, 2005; X. Wang, You, Lenchik, & Nicklas, 2010). The contribution of FM to RMR is estimated to be between 5 kcal/kg/day (Elia, 1992) to 10-13 kcal/kg/day (Goran, Kaskoun, & Johnson, 1994; M. P. St-Onge, 2005), explaining between 1-10% of the variability in young and middle aged adults (Nelson, Weinsier, Long, & Schutz, 1992; Sparti, DeLany, de la Bretonne, Sander, & Bray, 1997; Tataranni & Ravussin, 1995) and 2-3% in older adults (Lhrmann, Herbert, & Neuhuser-Berthold, 2001). This range of variance across populations is due to the differences in FM

distribution across sexes and ages. Much like FFM, FM is not a metabolically homogenous substance; rather, there are regional variations in the physiological characteristics of adipose tissue. Specifically, abdominal fat has a higher metabolic rate than FM located in the gluteal-femoral region (Lhrmann et al., 2001; Weststrate et al., 1990). This is likely due to the increased blood flow, greater responsiveness to norepinephrine, lower sensitivity to the antilipolytic effect of insulin, and higher rate of lipolysis of visceral fat compared to subcutaneous fat (Arner, 1995; Arner, Engfeldt, & Lithell, 1981; Hoffstedt, Arner, Hellers, & Lonnqvist, 1997; P. P. Jones, Snitker, Skinner, & Ravussin, 1996; Millet, Barbe, Lafontan, Berlan, & Galitzky, 1998).

Sex RMR is also influenced by sex, though few studies have directly explored the topic. Many studies have attributed sex differences in RMR to differences in FFM, though several with proper statistical analyses have shown an independent relationship. The largest study to examine sex differences in RMR was conducted on 328 males and 194 females ranging in age from 17-81(Arciero, Goran, & Poehlman, 1993). After adjustments for FFM, FM, and CRF, males had a 3% (50 kcal/day) higher RMR compared to females. This difference persisted when the groups were divided into pre- (4% difference) and postmenopausal (5%) age categories. No difference was detected between the pre- and postmenopausal women, indicating gender differences were not related to menopausal status. Ferraro et al. (1992) found a similar lower RMR in females (44 kcal/day), though the difference was not statistically significant. However, the large standard deviation (314 kcals) and range of RMR among females (1038-2435 kcals) suggest possible measurement error, perhaps due to a relatively short measurement period (9-15 minutes). The mechanisms for a gender difference in RMR is unknown, though

hypotheses include differences in body cell mass operations (i.e. Na^{+}-K^{+}-ATPase activity), eating patterns, and skeletal muscle metabolism (Arciero et al., 1993; Poehlman, Toth, & Webb, 1993).

Age RMR decreases at a rate of 1-2% per decade of life beginning at age 20 (Elia, Ritz, & Stubbs, 2000) via numerous mechanisms (Manini, 2010). Declines in metabolically active FFM are responsible for most of the age-related decreases in RMR, but do not completely explain it (Krems, Luhrmann, Strassburg, Hartmann, & Neuhauser-Berthold, 2005; M. P. St-Onge & Gallagher, 2010). Instead, the likely cause of the declining RMR during aging is the combination of heterogeneous decreases in size and metabolic rate of the various tissues which make up FFM. For example, the brain, bones, and kidneys decline in mass at relatively the same rate between the ages of 20-80 years (approximately 10-20%), but the spleen decreases much quicker (approximately 38%) while the heart increases in mass (approximately 10%) (Gallagher et al., 1997; He et al., 2009; Rico, Revilla, Villa, & Alvarez de Buergo, 1993). Likewise, there is evidence to suggest extracellular components that are not involved in metabolize activities increase with age, thus decreasing the overall specific metabolic rate within an organ (Z. Wang, Heshka, Heymsfield, Shen, & Gallagher, 2005).

Physical Activity It has been suggested 'the factor that causes by far the most dramatic effect on metabolic rate is strenuous exercise' (Guyton, 1997). Physical activity may have an effect on RMR via two distinct pathways: 1) the growth of FFM (i.e. skeletal muscle), and 2) the effect on physiological processes that influence RMR (Speakman & Selman, 2003). The physiological effects of physical activity on RMR may

have both short (<48 hours post exercise) and long term (>48 hours post exercise) effects, while changes in FFM occur over much longer periods of time (> 4 weeks).

It has been known for nearly a century that physical activity will produce acute elevations in RMR (Edwards, Thorndike, & Dill, 1935; Margaria, Edwards, & Dill, 1933) referred to as excess post-exercise oxygen consumption (EPOC); what is unclear is the exact duration of this elevation. Previous research has shown energy expenditure returning to baseline levels as quickly as 20 minutes post-exercise (Sedlock, Fissinger, & Melby, 1989) or as long as 48 hours post-exercise (Dolezal, Potteiger, Jacobsen, & Benedict, 2000; Edwards et al., 1935; Margaria et al., 1933) depending on the bout characteristics (type, intensity, and duration of the activity). In general, aerobic exercise at an intensity <70% of maximal capacity and <50 minutes do not have a prolonged effect (> 3 hours) on metabolic rate (LaForgia, Withers, & Gore, 2006; Poehlman, Melby, & Goran, 1991).

The majority of cross-sectional studies indicate a 5-20% elevated RMR among individuals who participate in regular activity compared to sedentary controls, and cardiorespiratory fitness (CRF) is highly correlated with RMR ($r=0.42$, $P<0.001$) (Arciero et al., 1993; Ballor & Poehlman, 1992; Burke, Bullough, & Melby, 1993; Hill, Heymsfield, McMannus, & DiGirolamo, 1984; Poehlman et al., 1992; Poehlman, Melby, & Badylak, 1988; Poehlman, Melby, Badylak, & Calles, 1989; Ravussin & Bogardus, 1989; Schulz, Nyomba, Alger, Anderson, & Ravussin, 1991; Sjodin et al., 1996; Tremblay, Coveney, Despres, Nadeau, & Prud'homme, 1992; Tremblay, Despres, & Bouchard, 1985; Tremblay et al., 1986; Tremblay et al., 1990; van Pelt, Dinneno, Seals, & Jones, 2001). An excellent example of this relationship is described by van Pelt et al.

(2001). Four groups of males where measured for RMR, CRF, and weekly exercise volume; those who were young and active, young and sedentary, old and active, and old and sedentary. The authors found RMR declined with age, but this decline was a result of age-related declines in weekly exercise and energy intake, and older individuals who maintained their exercise volume and dietary intake had a similar RMR compared to younger physically active men.

The results from exercise interventions are not as clear due to wide variations in the sample size, the sample population, characteristics of the intervention, and time of RMR measurement following the last exercise bout. Several have shown increases in RMR (Berke, Gardner, Goran, & Poehlman, 1992; Goran & Poehlman, 1992; Lawson, Webster, Pacy, & Garrow, 1987; Poehlman, McAuliffe, Van Houten, & Danforth, 1990), while others have not (Bingham, Goldberg, Coward, Prentice, & Cummings, 1989; Blaak, Westerterp, Bar-Or, Wouters, & Saris, 1992; Broeder, Burrhus, Svanevik, & Wilmore, 1992b; Bullough, Gillette, Harris, & Melby, 1995; Westerterp, Meijer, Janssen, Saris, & Ten Hoor, 1992). Perhaps the study which best addressed whether physical activity may alter RMR was conducted by Byrne and Wilmore (2001). Participants were randomized into three groups for 9 weeks: 1) resistance training only, 2) resistance training plus aerobic training, and 3) control (no exercise). Importantly, RMR measurements were made 72 hours after the last bout of exercise, eliminating the acute effects of exercise on metabolic rate. Both exercise groups gained FFM as a result of the intervention (1.9 kg each). RMR increased in the resistance training group (44 kcals/day), but decreased in the combined group (53 kcal/day). When expressed as RMR/FFM, the resistance training group was not different post-intervention, and the combined group was

lower by 2.2 kcal/kg/day post-intervention. It is unclear the reason for the decline in RMR despite the increase in FFM in the combined group, but given that this group performed exercise each day of the week compared to four days/week of the resistance training group, perhaps compensatory responses to high exercise may be responsible.

Insight into this compensation may be found in the study of energy flux (Bell et al., 2004; Bullough et al., 1995). Undereating has been shown to result in lower RMR (Poehlman et al., 1991; J. L. Thompson, Manore, Skinner, Ravussin, & Spraul, 1995; Warwick & Garrow, 1981), while overeating increases RMR (Mole, 1990). If highly active individuals match their EI to TDEE, they would be considered in energy balance at a *high energy flux* state (i.e. high EI and high TDEE), while sedentary individuals matching their EI to TDEE would be considered in energy balance at a *low energy flux* state (i.e. low EI and low TDEE). If exercise is stopped for 24-36 hours per the standard RMR measurement protocol (thus reducing TDEE), yet active individuals maintain a high level of EI they would be in a positive energy balance) and RMR may be elevated (Sjodin et al., 1996; Soares, Piers, & Shetty, 1989). Likewise, if highly active individuals are not matching their TDEE with EI (i.e. in a negative energy balance) their RMR measurement may be decreased (Bullough et al., 1995).

Indeed, a cross-sectional study involving trained and untrained young adults exploring differences at least 48 hours after the last bout of exercise and consuming a weight-maintenance diet (i.e. low energy flux state) found no difference in RMR between the two groups (Schulz et al., 1991). This finding was replicated in a separate study, which also found elevations in RMR only when individuals were in a high energy flux state (Bullough et al., 1995). The mechanisms responsible for an increase RMR in high

flux conditions are purely speculative and have not been well-explored, but include likely include regulation of the sympathetic nervous system (SNS) (Bell et al., 2004; Bell et al., 2001; Bullough et al., 1995; Ravussin, 1995). Reduced SNS activity has been identified in low energy flux state as expressed as decreases in norepinephrine (Cannon et al., 1991; Haahr et al., 1991) and muscle sympathetic nerve activity (Bell et al., 2004). Leptin, a hormone which regulates metabolism, is positively related to MSNA (Monroe, Van Pelt, Schiller, Seals, & Jones, 2000; Snitker, Pratley, Nicolson, Tataranni, & Ravussin, 1997), and leptin levels are associated with low energy flux states (Bell et al., 2004). Other mechanisms potentially responsible include changes in muscle cell structure (Hather, Tesch, Buchanan, & Dudley, 1991), immune systems responses (Cannon et al., 1991; Haahr et al., 1991), neuroendocrine function (Herring, Mole, Meredith, & Stern, 1992; Luger et al., 1987), and substrate cycling (Bahr, 1992; Wolfe, Klein, Carraro, & Weber, 1990).

Other physiological mechanisms responsible for increases in RMR related to CRF irrespective of energy flux are unclear. However, studies in both animals (Borsheim, Knardahl, Hostmark, & Bahr, 1998) and humans (Tremblay et al., 1992) suggest involvement of the sympathetic nervous system , particularly the β3 adrenoreceptors (Speakman & Selman, 2003). This relationship was assessed by giving trained and sedentary individuals a β-blocker or placebo following a bout of exercise. In the placebo condition, trained individuals had higher post-exercise (15-minutes) RMR values compared to controls. However, this difference disappeared when participants were given the β-blocker, suggesting the sympathetic nervous system plays a role in the effect of exercise on RMR, an idea supported by others (Spraul et al., 1993).

Race Current research is equivocal in regards to differences in RMR among adults due to variations in physiological variables (E.g. FFM) between races which makes direct comparisons difficult. A recent review of the literature found 10/15 studies examined reported lower levels of RMR between African Americans (AA) compared to Whites (W) ranging from 81-274 kcal/day (Gannon et al., 2000). The primary cause of this discrepancy appears to be due to higher levels of FFM in AA populations resulting from greater skeletal muscle and bone mineral density (Carpenter et al., 1998; Forman, Miller, Szymanski, & Fernhall, 1998; Foster, Wadden, & Vogt, 1997; Jakicic & Wing, 1998; A. Jones, Jr. et al., 2004; Ortiz et al., 1992; Wagner & Heyward, 2000). Theoretically, since the metabolic activity of bone and, to a lesser extent, skeletal muscle, is low compared to internal organs (Elia, 1992; Gallagher et al., 1998; Holliday, Potter, Jarrah, & Bearg, 1967), analyses that do not independently account for these variables in FFM may spuriously overestimate FFM and thus indicate a lower RMR in AA based on their metabolic size (Gannon et al., 2000). In practice, few studies have accounted for differences in bone mineral content or skeletal muscle mass, but those which have reported similar RMR values with and without adjustments (Jakicic & Wing, 1998; Morrison, Alfaro, Khoury, Thornton, & Daniels, 1996; Yanovski, Reynolds, Boyle, & Yanovski, 1997), though two of these studies were in children and the assessment of RMR was after a three hour fast, not twelve hours as is the standard. Perhaps the most methodologically sound study to date found the difference in RMR between AA and Cauc increased from 119 kcal/day to 182 kcal/day when adjusted for bone mineral content (Jakicic & Wing, 1998).

In addition to differences on bone mineral content and skeletal muscle, there may also be differences in the mass of high metabolically active organs between races (N. M. Byrne et al., 2003; Gallagher et al., 2006; Hunter, Weinsier, Darnell, Zuckerman, & Goran, 2000; A. Jones, Jr. et al., 2004). Perhaps the most thorough, though small, study to date used both magnetic resonance imaging and dual-energy X-ray absorptiometry to partition the body mass into four compartments: adipose tissue, skeletal muscle, bone, and residual mass (A. Jones, Jr. et al., 2004). Residual mass in this context represents the highly metabolically active organs of the brain, liver, kidneys, heart, gastrointestinal tract, and other organs/tissues. Among the sample of women matched for age, weight, and height, AA's (n=22) had higher skeletal muscle (1.52±2.48 kg, $P<0.01$) and skeletal muscle + bone mass (1.72±2.66 kg, $P< 0.01$) and lower residual mass (1.05±4.96 kg, NS) compared to W women (n=22). Additionally, measured RMR was also lower among AA women by 38 kcals/day, though the difference was not significant, potentially due to the small sample size. A similar study with FFM, FM, and regional lean tissue measured by DXA found a lower RMR (120 kcal/day) among AA women cared to W women, but no difference after adjustment for trunk lean tissue (Hunter et al., 2000). The findings from these studies and others (N. M. Byrne et al., 2003; Gallagher et al., 2006; Yanovski et al., 1997) suggest smaller mass of metabolically active organs mediate the low RMR observed in AA women.

Menstrual Cycle As with many of the variables described earlier, the exact role of menstrual cycle on RMR remains unclear. Previous studies have shown RMR may differ between 5-10% from the luteal to follicular phase of the menstrual cycle, with a low point occurring 1 week prior to ovulation followed by a rise before the following

menstrual period (Hessemer & Bruck, 1985; Rubinstein, 1937; Solomon, Kurzer, & Calloway, 1982; Webb, 1986), while others have not (Blunt & Dye, 1921; Jakicic & Wing, 1998; Weststrate, 1993; Williams, 1943). The studies that have found variations have attributed the changes to progesterone secretion (Hessemer & Bruck, 1985; Rubinstein, 1937), though the exact mechanisms are unclear (McNeil, Bruce, Ross, & James, 1988).

Other biological factors In addition to the mechanisms described previously, a host of other physiological and genetic factors have been posited to explain the individual variations in RMR (Speakman & Selman, 2003). This list includes: circulating leptin (Toth, Sites, & Poehlman, 1999) and ribosomal protein L3 levels (Allan, Nielsen, & Pomp, 2000), thyroid status (Freake & Oppenheimer, 1995) and circulating thyroid hormones (tri-iodothyronine and di-iodothyronine) levels (Moreno et al., 2002), thyroid receptor deficiencies (retinoic acid X-γ) (Brown et al., 2000), protein turnover (Badaloo, Singhal, Forrester, Serjeant, & Jackson, 1996), mitochondrial proton leak (Rolfe & Brand, 1996), and polymorphisms of uncoupling proteins 2 and 3 (Astrup, Toubro, et al., 1999; Barbe et al., 1998; Bouchard, Perusse, Chagnon, Warden, & Ricquier, 1997).

Role of resting metabolic rate and respiratory quotient in obesity

Given the large percentage of TDEE resulting from RMR, the relationship of it with body weight and body composition is of great interest (Katzmarzyk, Perusse, Tremblay, & Bouchard, 2000). However, the role RMR plays in the weight gain is not clear, both in terms of absolute involvement and also the direction of the relationship. For example, some prospective studies suggest RMR is predictive of subsequent weight gain (Astrup, Gotzsche, et al., 1999; Leibel, Rosenbaum, & Hirsch, 1995; Ravussin et al.,

1986; Ravussin et al., 1988; Zurlo et al., 1990) while others do not (Katzmarzyk et al., 2000; Marra, Scalfi, Covino, Esposito-Del Puente, & Contaldo, 1998; Seidell, Muller, Sorkin, & Andres, 1992). Even among those that suggest RMR is a factor in weight gain, some suggest low RMR is the predictor (Ravussin et al., 1988) while others state high RMR is the predictor (Leibel et al., 1995; Ravussin et al., 1986).

Among the Pima Indians, low baseline RMR predicts subsequent weight gain (Ravussin et al., 1988). Perhaps the most advanced examination terms of assessment methods and statistical analyses, Ravussin et al. found negative relationship between baseline RMR and weight gain (e.g. participants in the lowest tertile of RMR had the highest incidence of weight gain) over 4 years. After adjustments for FFM, FM, age, and sex, RMR was 70 kcal/day lower in those who gained at least 10 kg compared to those who did not. Results were similar when RQ was examined; 24-hour adjusted RQ was an independent predictor of gains in both body weight ($P<0.001$) and fat mass ($P=0.004$), suggesting weight gain is a result of reduced rates of fat oxidation (Zurlo et al., 1990).

These results are in direct contrast to the Quebec Family Study, which found no association between RMR or RQ on changes in body weight or fat during a 5.5 year follow-up (Katzmarzyk et al., 2000). The correlations were low between measures of body weight/fatness (E.g. weight, BMI, or sum of skinfolds) and RMR (R^2= -0.03 to 0.16, not significant) or RQ (R^2= -0.05 to 0.12, not significant). Neither RMR nor RQ were significant predictors of increases in body weight or fatness from Cox regressions.

Other prospective studies also found similar conflicting results. The Baltimore Longitudinal Study on Aging is the largest study to examine the role of RMR and RQ on

weight gain in men (N=775) over 10.3 years of follow-up (Seidell et al., 1992). After multivariate adjustments for age, BMI, FFM, and RQ were all significantly associated with weight change at $P<0.05$, but RMR was significantly associated only at $P<0.10$. However, those with an RQ of >0.85 (individuals with low rates of fat oxidation) were 2.42 times more likely to gain at least 5 kg compared to those with an RQ of <0.76 (individuals with high rates of fat oxidation). A study of Italian women (N=58) found similar results, with those who gained >3kg over a three year follow-up period having an RQ of 0.91 vs. 0.84 of those who did not (Marra et al., 1998). Based on these studies, it is oxidation of energy stores not RMR that is predictive of weight gain.

The variations in findings are likely due to many factors. The studies that have shown the relationships between RMR and weight gain have consisted of young adult populations, and this age group is more likely to gain weight (Sheehan, DuBrava, DeChello, & Fang, 2003). For example, the mean age of the Pima Indians was 26 years with 11.9% of participants gaining at least 10 kg over four years of follow-up, while the mean age of the BLSA was 49 years and weight gain was 0.07 kg over a mean follow-up of 10.3 years and the mean age in the Quebec Family Study was 39 years and mean weight gain was 2.8 kg for men and 3.5 kg for women over 5.5 years. There are also methodological differences across studies. For example, RMR was assessed in the Pima Indians over a 24-hour period in a respiratory chamber, while RMR in the BLSA was measured using multiple techniques over a 19 year period. Also, baseline values in the BLSA were not reported; instead mean RMR values from all visits over the 19 year follow-up were used for data analysis which may dilute the findings. Additionally, body composition assessment techniques varied in quality from hydrostatic weighing in the

Pima Indians, skinfolds in BLSA and QFS, and not reported in the study from Italy, which makes direct comparisons of adjusted RMR values difficult.

Also adding to the confusion of determining the relationship of RMR on weight gain is the fact that RMR increases as body weight increases. For example, among Pima Indians, which have the largest gains in weight based on low RMR values, RMR values *after* weight gain are similar to those who did not gain weight (Ravussin et al., 1988). A tightly controlled study which involved measuring RMR during normal weight, a 10% weight gain, a return to baseline weight, a 10% weight loss, and finally a 20% weight loss among a single cohort, found compensatory matching based on weight status (Leibel et al., 1995). For example, weight gain resulted in increases in RMR, weight loss resulted in decreases in RMR, and a return to baseline weight resulted in a return to baseline RMR. These findings make it difficult to disassociate the role of RMR on weight gain if weight stability is not assured. In other words, is RMR a reflection of actual metabolic status, or is it a marker for biological regulation of an energy imbalance (Ravussin & Gautier, 1999)?

CHAPTER 3

GENERAL METHODOLOGY

Participants and enrollment process The three studies which form this dissertation will be completed using data collected in The Energy Balance Study, a prospective observational study following young adults for 24 months (Hand et al., 2013). All participants were recruited from the Columbia, South Carolina area between June 2011 and July 2012. Flow of contact with participants is described in Appendix 1. Interested individuals completed an online screener on the study website (http://energybalance.sc.edu/) followed by a telephone interview to exclude participants not meeting inclusion criteria. All participants were required to have a BMI ≥20 and ≤35 kg/m^2 and age ≥21 and ≤35 years, and recruitment was designed for an equal distribution of male and female participants across the age categories of ≥21 and <28, and ≥28 and ≤35. Exclusion criteria included use of medications to lose weight, started or stopped smoking in the previous 6 months, or planned weight loss surgery. Further, individuals were excluded for resting blood pressure (BP) exceeding 150 mmHg systolic and/or 90 mmHg diastolic, an ambulatory blood glucose level of greater than 145 mg/dl, or those currently diagnosed with/or taking medications for a major chronic health condition. Individuals with a history of depression, anxiety, or panic were excluded, as were those taking selective serotonin inhibitors for any reason. All women were eumenorrheic, and

those who were planning to begin or stop birth control during the duration of the study also were excluded.

Study design The Energy Balance Study involved of a period of baseline measurements consisting of three laboratory visits, followed by repeated measurements every three months for one year. Baseline visit #1 consisted of a thorough review of medical history and demographic information, completion of an extensive battery of demographic, psychometric, and activity recall questionnaires, followed by dietary assessment training to prepare them for interviewer administered dietary recalls later in the study. Baseline visit #2 included measurements of resting BP, height, weight, waist and hip circumference, body composition via dual-energy X-ray absortiometry (DXA) full body scan, as well as determination of CRF via a maximal fitness test using a modified Bruce protocol with 12-lead electrocardiogram (ECG) and BP measurements. Baseline visit #3 included measurements of RMR, height, weight, waist and hip circumferences, and a blood draw. Additionally, a 10 day assessment of energy expenditure and three random 24-hour dietary recalls began at the conclusion of Baseline visit #3. All measurements were repeated every three months for the following year, except for RMR which was measured every six months and CRF which was not repeated. All study protocols were approved by the University of South Carolina Institutional Review Board, and informed consent was obtained from each participant prior to data collection.

Anthropometrics The DXA provided data on bone mineral density, FM, and FFM, for both individual body segments (head, arms, legs, trunk, ribs, pelvis, spine for bone mineral density; arms, legs, and trunk for FM and FFM) and the whole body. The

scan was completed with a Lunar DPX system (version 3.6; Lunar Radiation Corp, Madison, WI). All anthropomorphic measurements were performed with the participant dressed in surgical scrubs and in bare feet. Body mass index (BMI; kg/m^2) was calculated from the average of three height and weight measurements using a traditional standiometer and electronic scale and recorded to the nearest 0.1 centimeter and 0.1kg, respectively. Hip and waist circumferences were measured with a calibrated, spring-loaded tape measure. Waist circumference was determined at the point midway between the costal margin and iliac crest in the mid-axillary line approximately 2 inches above the umbilicus. Hip circumference was measured at the widest point around the greater trochanter. Circumferences recorded were the average of three measurements and were rounded to the nearest 0.1 cm.

Cardiorespiratory fitness Fitness testing was conducted on a treadmill (Trackmaster 425, Carefusion, Newton, Kansas) with respiratory gases sampled using a True Max 2400 Metabolic Measurement Cart (ParvoMedics, Salt Lake City, Utah). The metabolic cart was calibrated prior to each test using known gas concentrations and volumes as recommended by the manufacturer. A trained exercise physiologist prepared eligible subjects for the graded exercise test (GXT), and a standard 12-lead ECG was performed. Subjects sat quietly for examination of the real-time resting ECG, heart rate (HR), and BP. All subjects exercised to volitional fatigue, followed by continued walking at a slow pace until HR and BP returned to near baseline levels. HR, BP, and treadmill total time were recorded at each stage of the protocol.

The Modified Bruce GXT begins at a speed of 1.7 mph at 0% grade for 3 minutes then progresses to 1.7 mph at 5% grade for 3 minutes. After this stage, the protocol is

identical to that of the Bruce Protocol. The Modified Bruce Protocol was used due to its lower initial intensity for this generally deconditioned population. Previous research has shown a high correlation between the Modified Bruce and Bruce protocols for HR responses (r=0.97) and peak VO_2 measurements (r=0.72) (McInnis & Balady, 1994).

Resting Metabolic Rate RMR was measured via indirect calorimetry using a ventilated hood and an open-circuit system, True Max 2400 Metabolic Measurement Cart (ParvoMedics, Salt Lake City, Utah), over a 30 minute period with data collection beginning after a 15 minute resting period. The metabolic cart was calibrated prior to each test using known gas concentrations and volumes as recommended by the manufacturer. Participants arrived for a morning visit following in a 12 hour dietary fasting state and at least 24 hours after the last bout of structured exercise. RMR was calculated from O_2 consumption and CO_2 production as measured continuously during the testing period with a constant airflow rate into the hood (Branson & Johannigman, 2004; Weir, 1949, 1990). Airflow rate was based approximately on body weight with the goal of maintaining the fraction of end tidal CO_2 between 1.0 and 1.2% (L/min), and the flow rate was set to not exceed 33 L/min for a person weighing 68 kg or 40 L/min for a 91 kg person. The ratio of the volume of carbon dioxide produced to the volume of oxygen consumed was used to compute the RQ for each minute. Participants remained quiet and still through the entire RMR procedure. The room was maintained in low light, noise was kept at a minimum, and the temperature remained between 26 to 30 degrees centigrade (Branson & Johannigman, 2004; Henry, 2005). Participants were kept awake with continuous monitoring.

Energy Expenditure Energy expenditure was measured using the SenseWear Mini Armband (BodyMedia Inc. Pittsburgh, PA), an arm-based activity monitor. This portable, multi-sensor device, worn on the upper arm, incorporates tri-axial accelerometry, heat flux, galvanic skin response, skin temperature, and near-body ambient temperature. These measures are entered in combination with demographic information into an algorithm to estimate TDEE, PAEE, and sleep. The armband has been shown to be a valid device to measure energy expenditure and activity (Johannsen et al., 2010; M. St-Onge, Mignault, Allison, & Rabasa-Lhoret, 2007; Welk, McClain, Eisenmann, & Wickel, 2007). Participants were trained for approximately 20 minutes on the care and use of the armband activity monitor. The individuals started wearing the monitor immediately and were asked to continue use of the monitor except during periods when the monitor could get wet. For most individuals, this only included periods of showering or bathing. The participants wore the armband for 10 days and recorded their activities during any period of that time that the armband was not worn. Participants were deemed compliant if they completed 7 days of wear (including two weekend days) with at least 23 hours of wear time on each of the days.

Energy Intake Energy intake was measured using interviewer-administered 24 hour dietary recalls. The Nutrient Data System for Research software (NDSR Version 2012), licensed from the Nutrition Coordinating Center (NCC) at the University of Minnesota, was utilized to conduct the dietary interviews. NDSR is considered the state-of-the-art research software for conducting dietary recalls (F. E. Thompson & Subar, 2013). The food database includes over 19,000 foods, is updated yearly, and provides nutrient composition information for over 120 nutrients. The quality of a dietary recalls

depends both on the ability of the subject to remember which foods were consumed (Novotny et al., 2001) as well as the skill of the interviewer in eliciting complete and accurate information (F. E. Thompson & Subar, 2013). In this study, the dietary recalls were collected by a team of experienced (> 6 years using NDSR), registered dietitians specifically trained in using the NCC protocol. This protocol employs the multi-pass approach which utilizes prompting to reduce omissions, and standardizes the interview methodology across interviewers (Dwyer, Ellwood, Leader, Moshfegh, & Johnson, 2001). Portion estimation is facilitated with the use by the subject of a validated, 2-dimensional, food portion visual (FPV) that is an integral part of the NDSR software (Posner et al., 1992) and which we have used successfully in multiple studies in adults and adolescents. Prior to data collection, study participants undergo a brief training (10-15 minutes) on how to use the FPV to estimate portion sizes of commonly eaten foods. The training incorporates life-sized plates, glasses and utensils and food models, in a hands-on experiential interchange (Wilcox, Sharpe, Parra-Medina, Granner, & Hutto, 2011). Interviews are assigned on randomly selected, non-consecutive days, and cold calls are made to the study subject to minimize preparation that could bias recall (Hebert et al., 2002). The sampling window was set at 14 days to be adequately large to allow multiple attempts on multiple days to maximize as much as possible, the likelihood of completing an interview.

STUDY 1 METHODOLOGY

Purpose This study will address Aim #1: Identify correlates of RMR among behavioral and physiological variables in a cohort of young adult men and women.

Research Questions

Research Question 1.1 What is the nature of associations between RMR and behavioral variables associated with physical activity?

Research Question 1.2 What is the nature of the associations between RMR and behavioral variables associated with dietary intake?

Research Question 1.3 What is the nature of the associations between RMR and physiological variables?

Study Design

This study will utilize a cross-sectional design.

Study Population

The current analyses will include males and females between the ages of 21 to 35 years who completed all baseline measurements for The Energy Balance Study (n=430).

Study Measurements

For study #1, all participants completed the following measurements utilizing the methodology described earlier: height, weight, waist circumference, hip circumference, CRF, RMR, energy expenditure via the SenseWear Mini Armband, and energy intake via interviewer administered dietary recalls.

Statistical Analyses

Participant characteristics will be based on demographic and physiological measurements using means and standard deviations. Significance will be tested using t-tests for continuous variables and chi-square for categorical variables. Univariate

correlations and regression analysis will be performed to determine relationships between variables. The dependent variable will be RMR expressed as L/min. All computations were performed using SAS 9.2 (Cary, N.C.).

STUDY 2 METHODOLOGY

Purpose This study will address Aim #2: Identify the racial differences in RMR among young adult women.

Specific Aim 2: Examine racial differences in RMR, body weight, and body composition among young adult women.

Research Question 2.1 Do African-American women have a lower RMR compared to Caucasian women?

Research Question 2.2 Are there differences in body weight, body composition, and body composition distribution among African-American and Caucasian women?

Research Question 2.3 Are certain behavioral and physiological variables associated with diet and physical activity different among African-American and Caucasian women?

Study Design

This study will utilize a cross-sectional design.

Study Population

The current analyses will include AA and W females between the ages of 21 to 35 years who completed all baseline measurements for The Energy Balance Study (N=120).

Study Measurements

For study #2, all participants completed the following measurements utilizing the methodology described earlier: height, weight, waist circumference, hip circumference, CRF, RMR, energy expenditure via the SenseWear Mini Armband, and energy intake via interviewer administered dietary recalls.

Statistical Analyses

Participant characteristics will be based on demographic and physiological measurements using means and standard deviations. Significance will be tested using t-tests for continuous variables and chi-square for categorical variables. Univariate correlations and regression analysis will be performed to determine relationships between variables. The dependent variable will be RMR expressed as L/min. All computations were performed using SAS 9.2 (Cary, N.C.).

STUDY 3 METHODOLOGY

Purpose This study will address Aim #3: the longitudinal effects of RMR, physical activity energy expenditure, and dietary intake on changes in body weight and body composition over 6 months in a group of healthy young adults.

Specific Aim 3: Explore the longitudinal effects of RMR, respiratory quotient, and physical activity on subsequent changes in body weight and body composition in young adults followed for nine months.

Research Question 3.1 What is the longitudinal trend of RMR and RQ on body weight and body composition trajectory in young adults?

Research Question 3.2 Is volume or intensity of physical activity associated with changes in body weight and body composition?

Research Question 3.3 Are there gender differences in the relationships described in research questions 3.1 and 3.2?

Study Design

This study will utilize a longitudinal observational design.

Study Population

The current analyses will include males and females between the ages of 21 to 35 years who completed all baseline and nine month measurements for The Energy Balance Study.

Study Measurements

For study #3, all participants completed the following measurements utilizing the methodology described earlier: height, weight, waist circumference, hip circumference, CRF, RMR, energy expenditure via the SenseWear Mini Armband, and energy intake via interviewer administered dietary recalls.

Statistical Analyses

Participant characteristics will be based on demographic and physiological measurements using means and standard deviations. Significance will be tested using t-

tests for continuous variables and chi-square for categorical variables. A linear mixed models (LMM) regression random intercept growth model will be used to analyze the longitudinal data (West, Welch, & Galecki, 2007). An advantage of LMM approach is it allows for unbalance, unequally spaced observations over time making it ideal to analyze longitudinal data. The dependent variable will be change in body weight and body composition over 6 months expressed in kg. All computations were performed using SAS 9.2 (Cary, N.C.).

CHAPTER 4

MANUSCRIPT 1: CARDIORESPIRATORY FITNESS IS ASSOCIATED WITH ELEVATIONS IN RESTING METABOLIC RATE AMONG YOUNG ADULTS

Abstract

Introduction: Previous research suggests resting metabolic rate (RMR) is elevated in endurance athletes with high levels of cardiorespiratory fitness (CRF) compared to sedentary individuals. It is less clear whether moderate levels of fitness or regular physical activity are associated with similar elevations in RMR.

Objective: The purpose of the present study is to examine the role of CRF and objectively measured physical activity in explaining interpersonal variance of RMR among a cohort of young adults across a broad range of activity, fitness, and adiposity levels.

Methods: RMR, body composition, CRF, total daily energy expenditure, and energy intake were measured in 285 fit and 138 unfit young adults.

Results: Unfit participants had higher body weight, body mass index, fat mass, and body fat percentage compared to fit participants. There were no differences in RMR expressed as kcals/day between the groups. However, after statistical adjustment for differences in body composition between the groups, fit individuals had a higher RMR compared to unfit individuals by 45 kcals/day.

Conclusion: The primary finding of the present study is fit individuals have a higher RMR compared to those who are unfit. Differences in body composition, specifically skeletal muscle mass, residual mass, and fat mass, explained a large portion of the variability in RMR between fit and unfit individuals. Additionally, while time spent in moderate to vigorous physical activity was also significantly related to RMR and varied

considerably between the groups, the influence was small and had little predictive value over adjustments for body composition.

Introduction

Obesity, at the most basic level, is the end product of a chronic imbalance of energy intake and energy expenditure over an extended period of time. There is no consensus on the exact cause of the current levels of obesity seen in much of the developed world, but most of the research focuses on excess energy intake or deficient physical activity energy expenditure. However, resting metabolic rate (RMR) represents the largest contribution of total energy expenditure (60-80%) and has been hypothesized as potential predictor of weight gain, with some analyses finding a low RMR predictive (Griffiths, Payne, Stunkard, Rivers, & Cox, 1990; Ravussin et al., 1988), while others have not (Katzmarzyk et al., 2000), and others somewhat ambiguous (Seidell et al., 1992).

While RMR is relatively stable within individuals (<5% day-to-day variation), variability between individuals is much higher (±25%) (Murgatroyd, Davies, & Prentice, 1987). RMR is primarily determined by fat-free mass (FFM, approximately 63%), fat mass (FM, 6.7%), and age (1.7%) leaving over 25% of the variability unexplained (Johnstone, Murison, Duncan, Rance, & Speakman, 2005; Nelson et al., 1992; Weinsier et al., 1992). Identifying other variables responsible for the variation in RMR is important in order to understand energy balance and the etiology of obesity (Scrimshaw, 1996).

It has been suggested 'the factor that causes by far the most dramatic effect on metabolic rate is strenuous exercise' (Guyton, 1997). The majority of studies indicate a 5-

20% higher RMR among individuals who participate in regular activity compared to sedentary controls, and cardiorespiratory fitness (CRF) is highly correlated with RMR ($r=0.42$, $P<0.001$) (Arciero et al., 1993; Broeder, Burrhus, Svanevik, & Wilmore, 1992a; Ravussin & Bogardus, 1989; Tremblay et al., 1985). However, most studies have examined the role of physical activity on RMR in the context of highly fit individuals (e.g. endurance runners) studied cross-sectionally (Schulz et al., 1991; Sjodin et al., 1996) or in unfit individuals following a short-term (< 4 months) exercise intervention (Tremblay et al., 1986; Tremblay et al., 1990). It is unclear if moderate amounts of physical activity or fitness explain any of the interpersonal variance found in RMR.

Given recent technical advancements, it is now possible to assess objectively assess daily physical activity with great specificity, in terms of both absolute total energy expenditure and by time spent at a given level of intensity (e.g. moderate intensity activity) (Johannsen et al., 2010). Pattern recognition monitors integrate information from multiple sensors to provide highly sensitive and valid assessment of both structured exercise and complex lifestyle tasks, such as carrying loads, walking up grades, and non-ambulatory activities (Johannsen et al., 2010; Welk et al., 2007). These technical advances now permit the repeated assessments of physical activity energy expenditure among large samples of individuals over extended periods of time.

The purpose of the present study is to examine the role of CRF and objectively measured physical activity in explaining interpersonal variance of RMR among a cohort of young adults across a broad range of activity, fitness, and adiposity levels.

Methods

Participants and enrollment process The design and rationale for this study have been described in detail previously (Hand et al., 2013). All participants were recruited between June 2011 and July 2012, and were required to have a BMI $\geq$20 and $\leq$35 kg/m^2 and age $\geq$21 and $\leq$35 years. Exclusion criteria included use of medications to lose weight, started or stopped smoking in the previous 6 months, or planned weight loss surgery. Further, individuals were excluded for resting blood pressure (BP) exceeding 150 mmHg systolic and/or 90 mmHg diastolic, an ambulatory blood glucose level of greater than 145 mg/dl, or those currently diagnosed with/or taking medications for a major chronic health condition. Individuals with a history of depression, anxiety, or panic were excluded, as were those taking selective serotonin inhibitors for any reason. All women were eumenorrheic, and those who were planning to begin or stop birth control during the duration of the study also were excluded. All study protocols were approved by the University of South Carolina Institutional Review Board, and informed consent was obtained from each participant prior to data collection.

Anthropometrics A dual energy X-ray absorptiometer (DXA) provided data on bone mineral density, fat mass (FM), and FFM, both overall and for various body regions (arms, legs, etc.). The scan was completed with a Lunar DPX system (version 3.6; Lunar Radiation Corp, Madison, WI). All anthropomorphic measurements were performed with the participant dressed in surgical scrubs and in bare feet. Body mass index (BMI; kg/m^2) was calculated from the average of three height and weight measurements using a traditional stadiometer and electronic scale and recorded to the nearest 0.1 centimeter and 0.1kg, respectively.

Skeletal muscle mass was calculated from appendicular lean soft tissue (ALST) mass using the following linear regression equation:

Skeletal muscle mass= (1.13 x ALST) - (0.02 x age) + (0.61 x sex) + 0.97

where sex= 0 for females and 1 for males (Kim, Wang, Heymsfield, Baumgartner, & Gallagher, 2002). This equation was developed and validated with groups of 321 and 93, respectively, ethnically diverse men and women using magnetic resonance imaging (MRI) and DXA. Correlations between skeletal mass derived from the equation and MRI were high (R^2= 0.96, P< 0.0001) during the validation study (Kim et al., 2002). Residual mass, including brain, liver, kidneys, heart gastrointestinal tract, and other organs and tissues, was then calculated using the following equation:

Residual mass= body weight – fat mass – skeletal mass – bone mass (A. Jones, Jr. et al., 2004).

Cardiorespiratory fitness Fitness testing was conducted on a treadmill (Trackmaster 425, Carefusion, Newton, Kansas) with respiratory gases sampled using a TrueOne 2400 Metabolic Measurement Cart (ParvoMedics, Salt Lake City, Utah) using a Modified Bruce protocol. The metabolic cart was calibrated prior to each test using known gas concentrations and volumes as recommended by the manufacturer. A trained exercise physiologist prepared eligible participants for the graded exercise test (GXT), and a standard 12-lead ECG was performed. Participants sat quietly for examination of the real-time resting ECG, heart rate (HR), and BP. All participants exercised to volitional fatigue, followed by continued walking at a slow pace until HR and BP returned to near baseline levels. HR, BP, and treadmill total time were recorded at each

stage of the protocol. Given no widely accepted criteria exist to categorize fitness levels, participants were classified as 'unfit' if they were in the bottom tertile for CRF (mL/kg/min) among the entire cohort for each gender or 'fit' if they were in the upper two tertiles by gender. These cutoff points closely match a large a widely cited population-based fitness classification system (Sui, LaMonte, & Blair, 2007a, 2007b), with the 'unfit' group from the present study indicating 'low fitness' and the 'fit' group indicating 'moderate' and 'high' fitness levels.

Resting Metabolic Rate RMR was measured via indirect calorimetry using a ventilated hood and an open-circuit system, True Max 2400 Metabolic Measurement Cart (ParvoMedics, Salt Lake City, Utah), over a 30 minute period with data collection beginning after a 15 minute resting period. The metabolic cart was calibrated prior to each test using known gas concentrations and volumes as recommended by the manufacturer. Participants arrived for a morning visit following in a 12 hour dietary fasting state and at least 24 hours after the last bout of structured exercise. RMR was calculated from O_2 consumption and CO_2 production as measured continuously during the testing period with a constant airflow rate into the hood (Branson & Johannigman, 2004; Weir, 1949, 1990). Airflow rate was based approximately on body weight with the goal of maintaining the fraction of end tidal CO_2 between 1.0 and 1.2% (L/min), and the flow rate was set to not exceed 33 L/min for a person weighing 68 kg or 40 L/min for a 91 kg person. Participants remained quiet and still through the entire RMR procedure. The room was maintained in low light, noise was kept at a minimum, and the temperature remained between 26 to 30 degrees centigrade (Branson & Johannigman, 2004; Henry, 2005). Participants were kept awake with continuous monitoring.

Energy Expenditure Total daily energy expenditure (TDEE) was estimated using the SenseWear Mini Armband (BodyMedia Inc. Pittsburgh, PA), an arm-based activity monitor. This portable, multi-sensor device, worn on the upper arm, incorporates tri-axial accelerometry, heat flux, galvanic skin response, skin temperature, and near-body ambient temperature. Data is processed using software which calculates energy expenditure using complex pattern recognition algorithms consisting of 'activity classification' and 'energy expenditure estimation.' A Naïve Bays classifier matches the armband data to an activity class that best describes the current bout (e.g walking, running stationary bike, road bike, rest resistance, other activity). Each activity class has a linear regression model which maps the sensor values and body parameters to energy expenditure (Johannsen et al., 2010). The armband has been shown to be a valid device to measure energy expenditure and activity (Johannsen et al., 2010; M. St-Onge et al., 2007; Welk et al., 2007). Participants were trained for approximately 20 minutes on the use and care of the armband activity monitor. The individuals began wearing the monitor immediately and were asked to continue use of the monitor except during periods when the monitor could get wet. For most individuals, this only included periods of showering or bathing. The participants wore the armband for 10 days and recorded their activities during any period of that time that the armband was not worn. Participants were deemed compliant if they completed 7 days of wear (including two weekend days) with at least 23 hours of wear time on each of the days.

Energy Intake Energy intake was measured using interviewer-administered 24 hour dietary recalls. The Nutrient Data System for Research software (NDSR Version 2012), licensed from the Nutrition Coordinating Center (NCC) at the University of

Minnesota, was utilized to conduct the dietary interviews. NDSR is considered the state-of-the-art research software for conducting dietary recalls (F. E. Thompson & Subar, 2013). The food database includes over 19,000 foods, is updated yearly, and provides nutrient composition information for over 120 nutrients. The quality of a dietary recalls depends both on the ability of the subject to remember which foods were consumed (Novotny et al., 2001) as well as the skill of the interviewer in eliciting complete and accurate information (F. E. Thompson & Subar, 2013). In this study, the dietary recalls were collected by a team of experienced (> 6 years using NDSR) registered dietitians specifically trained in using the NCC protocol. This protocol employs the multi-pass approach, which utilizes prompting to reduce omissions, and standardizes the interview methodology across interviewers (Dwyer et al., 2001). Portion estimation is facilitated with the use by the subject of a validated, 2-dimensional, food portion visual (FPV) that is an integral part of the NDSR software (Posner et al., 1992) and which we have used successfully in multiple studies in adults and adolescents. Prior to data collection, study participants undergo a brief training (10-15 minutes) on how to use the FPV to estimate portion sizes of commonly eaten foods. The training incorporates life-sized plates, glasses and utensils and food models, in a hands-on experiential interchange (Wilcox et al., 2011). Interviews are assigned on randomly selected, non-consecutive days, and cold calls are made to the study subject to minimize preparation that could bias recall (Hebert et al., 2002). The sampling window was set at 14 days to be adequately large to allow multiple attempts on multiple days to maximize as much as possible, the likelihood of completing an interview.

Statistical analysis Participant characteristics are based on demographic and physiological measurements using means and standard deviations. Differences between fit and unfit groups were tested using t-tests for continuous variables . Univariate correlations and regression analysis was performed to determine relationships between variables. The dependent variable was RMR expressed as kcal/day. Analysis of covariance was used to compare the dependent variable between fit and unfit groups with adjustment of covariates. All computations were performed using SAS 9.2 (Cary, N.C.).

Results

Participant demographics are presented in Table 4.1 overall and by fitness level. Fit participants had higher levels of oxygen consumption compared to the unfit participants, both absolue (3.09±0.86 vs. 2.46±0.77 L/min, P<0.0001) and relative to body weight (3.08±3.0 vs. 2.73±2.7 mL/kg/min, P<0.0001). Unfit participants (defined as those in the bottom tertile for CRF in mL/kg/min by gender) had higher body weight, BMI, fat mass, and body fat percentage compared to fit participants (defined as those in the top two tertiles for CRF in mL/kg/min by gender). Table 4.2 displays body composition compartmentalized by tissue. There were no differences in skeletal muscle mass, residual mass, or bone mass between the groups when expressed in kg. However, unfit participants were lower in each variable compared to fit participants when expressed relative to body weight. There were no differences in RMR expressed as kcals/day between the groups (1524.8±262.1), but fit participants had higher RMR when expressed relative to body weight (3.08±3.0 vs. 2.73±2.7, P<0.0001, Table 4.3).

Table 4.4 describes energy expenditure and energy intake information for participants. Compliance with the armband was excellent, with mean wear time of 23 hours and 14 minutes per day (no difference between groups). There were no differences in TDEE between the groups when expressed as kcal/day, but Fit individuals had a high energy expenditure when expressed per kg of body weight (38.9±5.2 vs. 33.1±4.3 kcal/kg/day, P<0.0001). Unfit participants spent more time each day in sedentary activities compared to fit participants and less in moderate, vigorous, or very vigorous activities. Compliance with dietary recalls was excellent as well, with 2.8 recalls completed per participant (no difference between groups). Fit participants reported consuming more kcals/day than unfit participants, with a lower percent of their kcals from carbohydrates, more from alcohol, and no difference in fat and protein.

Table 4.5 displays the Pearson product-moment correlation coefficients among RMR (kcals/day) and physiological and physical activity variables in the full cohort and by group. As expected, fat free mass (r=0.85, P<0.0001), skeletal mass (r=0.82, P<0.0001), and residual mass (r=0.84, P<0.0001) were most highly correlated with RMR and by group. CRF, in L/min and mL/kg/min, was significantly correlated with RMR in each group, though time spent in physical activity was not. The relationship between fat free mass and RMR is displayed in Figure 4.1. The slope of the regression line between RMR and FFM is the same for unfit and fit groups (P=0.7882), but the Y-intercept is higher for the unfit group (532.1±63.13 vs. 518.5±34.5, P=0.0294) suggesting a modestly higher RMR at a given FFM.

As described earlier, unadjusted RMR was higher in unfit participants compared to fit participants. However, this is misleading given the large differences in body size

and composition between the groups. To statistically adjust for these differences, linear regression models were created to adjust RMR and the results are presented in Table 4.6. The base model adjusted RMR by the variables race, age, and gender which have been described previously in the literature as being predictive of RMR, and accounted for 45% of the variability in the present study. The four body compartments (fat mass, skeletal muscle mass, residual mass, and bone mass) were added in subsequent models. The model which included race, age, gender, and skeletal muscle mass explained 69% of the variance in RMR, and adding residual mass explained 74%. After these statistical adjustments, RMR remained higher in the unfit group (1554.1±11.7 vs. 1510.6±8.1, P=0.0027). The addition of FM explained 79% of RMR, and the adjusted mean RMR was now significantly higher for the fit group compared to the unfit group (1539.4±7.9 vs. 1494.7±12.3, P=0.0054). Adding bone mass to the model did not significantly improve the model. The RMR values adjusted for skeletal, residual, fat, and bone mass are displayed in Figure 4.2.

To determine the influence of aerobic capacity on RMR, CRF (L/min) was added to the model described previously. After statistical adjustment for all of the other covariates in the model, CRF was significantly related to RMR (P=0.0261) though it did not improve the model (R^2=0.79), and the adjusted RMR was no longer different between fit and unfit groups (P=0.3949). This approach was repeated to determine the influence of moderate to vigorous physical activity on RMR, with time spent in physical activity replacing CRF in the model. Despite the non-significant univariate correlation described in Table 4.5, time spent in moderate to vigorous physical activity was statistically related to RMR, though the significance was small, after adjustment for the body composition

covariates in the model (β coefficient= 0.2427, F value= 5.30, P=0.0218), and the adjusted RMR value was statistically higher in the fit group (1537.8±7.9 vs. 1498.0±12.4, P=0.0137). The individual components of energy expenditure related to physical intensity (time spent in sedentary, light, moderate, vigorous, and very vigorous activity) were also entered into the model both separately and together, but none were statistically related to RMR (results not shown). Neither energy intake nor any of the diet composition variables were statistically related to RMR (results not shown).

Discussion

The primary finding of the present study is that fit individuals have a higher RMR compared to those who are unfit. Differences in body composition, specifically skeletal muscle mass, residual mass, and fat mass, explained a large portion of the variability in RMR between fit and unfit individuals. Additionally, while time spent in moderate to vigorous physical activity was also significantly related to RMR and varied considerably between the groups, the influence was small and had little predictive value beyond adjustments for body composition.

Previous research suggests a 5-20% elevated RMR among those who are highly fit or participate in regular physical activity (Arciero et al., 1993; Burke et al., 1993; Hill et al., 1984; Ravussin & Bogardus, 1989; Schulz et al., 1991; Sjodin et al., 1996; Tremblay et al., 1992; Tremblay et al., 1985; Tremblay et al., 1986; Tremblay et al., 1990; van Pelt et al., 2001). However, nearly all of these previous studies have explored differences between highly fit (e.g. endurance athletes) and sedentary individuals, and none have explored patterns of regular physical activity using accelerometer-based

monitors. By objectively measuring activity intensity we are able to specifically examine regular physical activity over an extended period of time independent of cardiorespiratory fitness. Since fitness is partially determined by genetic factors (Bouchard et al., 2011), this approach allows the examination specifically of physical activity.

In the current study unfit individuals had higher RMR in kcals/day compared to fit individuals prior to adjustment for the differences in body composition between the groups. After accounting for higher levels of fat mass (both absolute and relative to body weight) and lower levels of skeletal muscle mass, residual mass, and bone mass (all relative to body weight) compared to fit individuals, unfit individuals had a lower RMR by approximately 45 kcals/day or 2.7%. After further statistical adjustment for CRF, this difference disappeared. This difference between fitness groups is modest and less than other researchers have found, likely due to the population studied. The fit participants in the present study were not elite endurance athletes as often studied (Broeder et al., 1992a), but instead their mean CRF was at approximately the 80th percentile of a widely cited population-based fitness classification system (Sui et al., 2007a, 2007b) (males= 48.6±5.7 mL/kg/min, females= 37.0±5.8 mL/kg/min). Thus, this study extended the finding by showing that even those who are not endurance athletes, relatively fit individuals had greater RMR than unfit individuals after adjustment for body composition.

Physical activity may have an effect on RMR via two distinct pathways: 1) the growth of FFM (i.e. skeletal muscle), and 2) the effect on physiological processes that influence RMR (Speakman & Selman, 2003). Since the results of the linear modeling in Table 4.6 shows CRF predicts RMR independent of FFM, this relationship is mediated

by physiological processes. The mechanisms have not been well-explored, but likely include regulation of the sympathetic nervous system (SNS) (Bell et al., 2004; Bell et al., 2001; Bullough et al., 1995; Ravussin, 1995), changes in muscle cell structure (Hather et al., 1991), immune systems responses (Cannon et al., 1991; Haahr et al., 1991), neuroendocrine function (Herring et al., 1992; Luger et al., 1987), and substrate cycling (Bahr, 1992; Wolfe et al., 1990).

Given the complexities of normalizing RMR to body size (Heymsfield et al., 2002; Heymsfield, Gallagher, Mayer, Beetsch, & Pietrobelli, 2007; Heymsfield et al., 2012) the appropriate analysis technique is linear modeling in which differences in body composition are statistically accounted for and adjusted mean RMR values are calculated (Ravussin & Bogardus, 1989). For example, Figure 4.1 indicates unfit individuals have a higher RMR compared to fit individuals at each level of FFM. However, this difference is spurious due to the differences between the groups for each body compartment. We thus attempted to segment the body into tissues based on metabolic rate using DXA and validated regression equations, then statistically adjusted absolute RMR (kcals/day) by each compartment (skeletal muscle, residual, fat, and bone mass, each in kg) using general linear modeling. The result is adjusted mean values indicating a statistically significantly elevated RMR in fit individuals compared to their unfit peers (Table 4.6, Figure 4.2).

There is evidence to suggest that lower levels of RMR result in weight gain over time (Astrup, Gotzsche, et al., 1999; Leibel et al., 1995; Ravussin et al., 1986; Ravussin et al., 1988; Zurlo et al., 1990), though the literature is equivocal on the topic (Katzmarzyk et al., 2000; Marra et al., 1998; Seidell et al., 1992). The hypothesis for this

theory is that lower levels of expected RMR, the largest contributor to TDEE, result in lower levels of TDEE, and individuals with a lower than expected TDEE are more likely to be in energy imbalance (Ravussin et al., 1988). In the present study those with lower levels of RMR (the unfit group) did have significantly higher levels of fat mass compared to age matched peers (35.0±9.4% vs 24.8±10.2%, respectively). However, given the cross-sectional nature of the study design we cannot determine causation. Future research should explore the role of the RMR on weight gain.

The strength of the present study includes a large sample size with nearly equal numbers of men and women with a wide range of fitness levels. Our body composition measurement technique (DXA) allows for the quantification of body mass into segments by metabolic properties (lean tissue mass, adipose tissue, bone mass),. The use of validated multi-sensor technology allowed us to accurately assess energy expenditure cumulatively over a 24 hour period and by intensity of activity. To our knowledge, this is the first examination of the relationship between objectively determined physical activity intensity and RMR. This study is limited by the cross-sectional design which does not allow the determination of causal pathways. The present study also does not allow exploration of potential mechanisms for the role physical activity may play in altering RMR.

In conclusion, after controlling for differences in body composition fit individuals had a higher RMR compared to unfit individuals. Time spent in moderate to vigorous physical activity was also significantly related to RMR, but this influence was small and had little predictive value over adjustments for body composition.

Acknowledgements The authors thank the participants of the present study for volunteering their time and effort. In addition, we are appreciative of the staff for their efforts collecting and managing the data and all other aspects of the study. This study was supported by an unrestricted grant from the Coca-Cola Company.

Table 4.1. Participant characteristics overall and by fitness level.

	All (N=423) (mean±SD)	**Unfit** (n=138) (mean±SD)	**Fit** (n=285) (mean±SD)	**P value** between group differences
Female (%)	51.3	51.5	51.2	0.9660
Age (years)	27.6±3.8	28.3±3.8	27.3±3.7	0.0075
Height (cm)	171.1±9.4	170.4±9.2	171.3±9.5	0.3520
Weight (kg)	75.2±13.9	82.3±14.9	72.0±12.1	<0.0001
Body Mass Index (kg/m^2)	25.6±3.9	28.1±4.1	24.4±3.1	<0.0001
Fat mass (kg)	21.3±10.0	28.8±9.7	17.7±7.9	<0.0001
Fat free mass (kg)	53.9±12.1	53.1±11.5	54.1±12.2	0.4165
Body fat (%)	28.1±11.0	35.0±9.4	24.8±10.2	<0.0001

Table 4.2. Body composition of participants by fitness level.

	All (N=423) (mean±SD)	**Unfit** (n=138) (mean±SD)	**Fit** (n=285) (mean±SD)	**P value** between group differences
Fat mass (kg)	21.3±10.0	28.8±9.7	17.7±7.9	<0.0001
Fat mass (%)	28.1±11.0	35.0±9.4	24.8±10.2	<0.0001
Fat free mass (kg)	53.9±12.1	53.1±11.5	54.1±12.2	0.4165
Skeletal mass (kg)	27.0±7.5	26.6±7.1	27.2±7.7	0.4115
Skeletal mass (%)	36.0±7.3	32.5±6.5	37.7±7.1	<0.0001
Residual mass (kg)	50.9±11.7	23.4±4.5	23.9±4.4	0.3176
Residual mass (%)	23.8±4.2	28.8±3.6	33.4±3.8	<0.0001
Bone mass (kg)	2.99±0.5	3.05±0.5	2.96±0.5	0.1164
Bone mass (%)	4.0±0.5	3.8±0.6	4.1±0.4	<0.0001

Table 4.3. Oxygen consumption at rest and peak exercise.

	All (N=423) (mean±SD)	**Unfit** (n=138) (mean±SD)	**Fit** (n=285) (mean±SD)	**P value** between group differences
Resting energy expenditure (kcals/day)	1524.8±262.1	1533.2±266.2	1520.8±260.5	0.6486
Resting metabolic rate (mL/kg/min)	2.96±0.4	2.73±2.7	3.08±3.0	<0.0001
REE/FFM ratio (kcals/kg/day)	28.8±3.5	29.4±3.8	28.6±3.3	0.0357
Respiratory quotient	0.79±0.05	0.80±0.05	0.79±0.04	0.0019
Cardiorespiratory fitness (mL/kg/min)	38.5±9.8	29.7±6.5	42.7±8.2	<0.0001
Cardiorespiratory fitness (L/min)	2.88±0.88	2.46±0.77	3.09±0.86	<0.0001

Table 4.4. Time spent in physical activity and energy intake by macronutrients.

	All (N=423) (mean±SD)	Unfit (n=138) (mean±SD)	Fit (n=285) (mean±SD)	P value between group differences
Total daily energy expenditure (kcal/day)	2740.1±511.0	2676.2±474.5	2771.0±525.7	0.0737
Total daily energy expenditure (kcal/kg/day)	37.0±5.6	33.1±4.3	38.9±5.2	<0.0001
Sedentary (min/day)	1086.9±88.0	1135.5±76.3	1063.5±83.8	<0.0001
Light (min/day)	216.1±58.5	213.8±61.2	217.1±57.2	0.5854
Moderate (min/day)	127.6±71.4	86.7±50.5	147.4±71.6	<0.0001
Vigorous (min/day)	6.0±7.4	2.4±3.0	7.7±8.2	<0.0001
Very Vigorous (min/day)	2.4±6.9	0.4±1.7	3.3±8.2	<0.0001
Physical Activity (min/day)	136.0±77.4	89.4±52.1	158.5±77.6	<0.0001
Energy intake (kcal/day)	2078.4±670.7	1912.3±608.1	2158.9±685.7	0.0004
Carbohydrates (% of total kcals)	47.2±9.9	49.1±9.3	46.3±10.1	0.0059
Fat (% of total kcals)	32.9±7.5	32.0±7.0	33.3±7.7	0.0838
Protein (% of total kcals)	17.2±5.0	17.3±4.9	17.2±5.0	0.8631
Alcohol (% of total kcals)	2.8±4.5	1.7±3.6	3.3±4.8	0.0002

Sedentary, 1.0 to ≤1.5 METs; Light, >1.5 to ≤3.0 METs; Moderate, >3.0 to ≤6.0 METs;
Vigorous, >6.0 to ≤9.0 METs; Very Vigorous, >9.0 METs.
Physical Activity, cumulative minutes spent at >3.0 METS.
Mean armband wear time= 23.24±0.8 hours/day; Mean dietary recalls completed= 2.76±0.5

Table 4.5. Pearson correlation coefficients between RMR (kcals/day) and selected variables.

	Resting metabolic rate (kcal/day)		
	All (N=423)	Unfit (N=138)	Fit (N=285)
Weight (kg)	0.76**	0.78**	0.82**
Fat mass (kg)	0.03	0.22*	-0.11
Fat free mass (kg)	0.85**	0.81**	0.87**
Bone mass (kg)	0.69**	0.54**	0.77**
Skeletal mass (kg)	0.82**	0.78**	0.84**
Residual mass (kg)	0.84**	0.78**	0.87**
Physical activity (min/day)	0.08	0.12	0.09
Cardiorespiratory fitness (mL/kg/min)	0.38**	0.50**	0.51**
Cardiorespiratory fitness (L/min)	0.76**	0.79**	0.83**

*Significantly correlated with RMR, P<0.05
**Significantly correlated with RMR, P<0.01

Table 4.6. Analysis of covariance assessing resting metabolic rate between sexes controlling for body compartments (kg), cardiorespiratory fitness (CRF, L/min) and time spent in physical activity (minutes/day) (mean±standard error).

	Unfit	Fit	R^2	P value
Unadjusted	1533.2±22.7	1520.8±15.4	NA	0.6486
Race* + age + gender*	1545.6±16.9	1514.8±11.7	0.45	0.1392
Race* + age + gender + skeletal muscle*	1551.8±12.8	1511.7±8.8	0.69	0.0110
Race* + age + gender + skeletal muscle* + residual mass*	1554.1±11.7	1510.6±8.1	0.74	0.0027
Race* + age + gender + skeletal muscle* + residual mass* + fat mass*	1494.7±12.3	1539.4±7.9	0.79	0.0054
Race + age + gender + skeletal muscle* + residual mass* + fat mass* + bone mass	1494.6±12.3	1539.5±7.9	0.79	0.0053
Race + age + gender + skeletal muscle* + residual mass* + fat mass* + bone mass + CRF*	1513.2±14.9	1530.4±8.9	0.79	0.3949
Race + age + gender + skeletal muscle* + residual mass* + fat mass* + bone mass + physical activity minutes*	1498.0±12.4	1537.8±7.9	0.79	0.0137

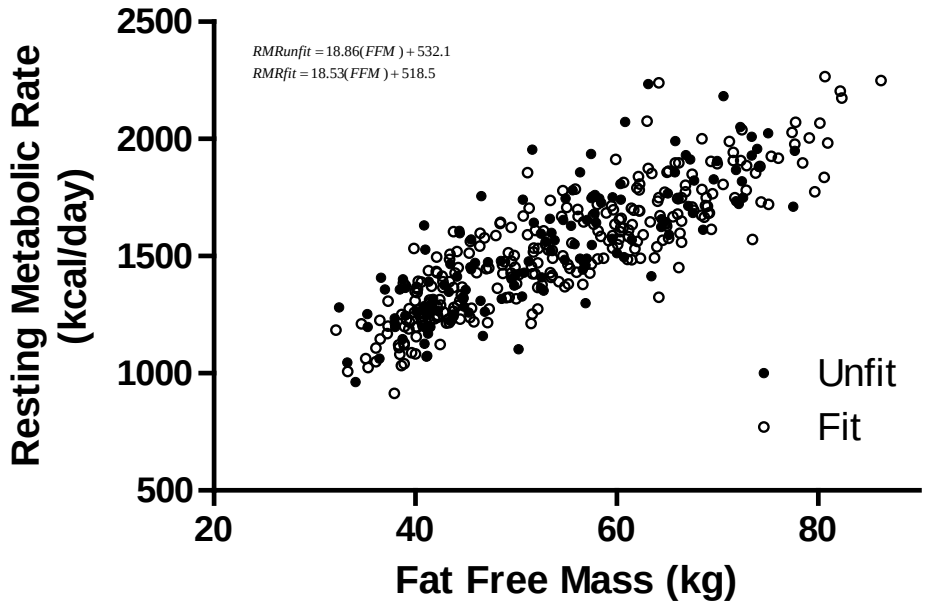

Figure 4.1. RMR was highly correlated with FFM (R^2= 0.85), with unfit individuals having a higher unadjusted RMR at each level of FFM compared to fit individuals (Difference for slopes between groups:P=0.7882, difference for y-intercepts between groups: P=0.0294).

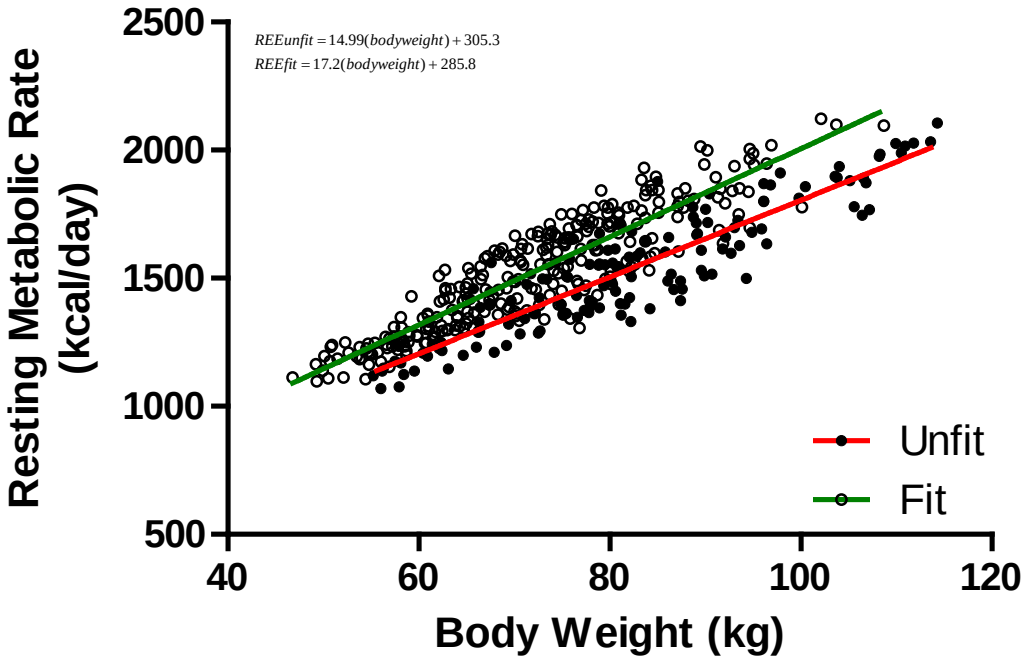

Figure 4.2. After adjusting for differences in body composition, fit individuals had a higher RMR compared to unfit individuals (Difference for slopes between groups: P= 0.0034).

CHAPTER 5

MANUSCRIPT 2: RACIAL DIFFERENCES IN RESTING METABOLIC RATE, CARDIORESPIRATORY FITNESS, AND PHYSICAL ACTIVITY AMONG YOUNG WOMEN

Abstract

Objective: It has been hypothesized that high levels of obesity among African American women may be in part due to a lower resting metabolic rate (RMR) compared to white women. Using state of the art measurement techniques and a large sample, the aim of the current study is to evaluate associations between race, body composition, aerobic fitness, and physical activity with RMR in a group of free living young adult African American (AA) and white (W) women.

Methods: A total of 179 individuals participated in the present study, including 141 W women and 38 AA women with a mean age of 27.7 years. We measured RQ and RMR using indirect calorimetry, along with body weight and body composition using dual energy X-ray absorptiometery, energy expenditure and time spent in physical activity using an arm-based activity monitor, and energy intake using interviewer-administered dietary recalls.

Results: AA women had higher BMI, body weight, body fat percentage, and levels of fat mass and fat free mass compared to white women. There was no difference between AA and W women in RMR when expressed as kcal/day (1378.9±178.4 kcal/day), but AA had a lower RMR when expressed relative to body weight (2.56±0.30 vs. 2.95±0.33 mL/kg/min, $P<0.0001$). After statistical adjustment for differences in body composition between groups using linear regression models, AA women had a lower RMR compared to W women (1400.3±9.1 kcal/day vs. 1299.8±18.9 kcal/day, $P<0.0001$). The addition of peak fitness explained additional variance in the model, though the improvement was modest. The addition of time spent in physical activity did not improve the model.

Conclusion: The present study confirms young adult African-American women have a lower RMR compared to their white peers after statistical adjustments for differences in body composition. Higher levels of fat mass in AA compared to W resulted in elevated RMR beyond the differences in fat free mass. Additionally, CRF was significantly associated with RMR in the present study, but time spent in moderate to very vigorous physical activity was not.

Introduction

The average adult in the United States consumes approximately 1 million calories each year (Food and Agriculture Organization). Despite this large intake of energy, most healthy adults are able to achieve a balance between energy intake and expenditure primarily through mechanisms required to sustain life (resting metabolic rate [RMR]), digest food (thermic effect of food), and perform activities (thermic effect of physical activity). It is a chronic mismatch over time in this energy balance which results in changes of body energy stores. Because the largest contributor to energy expenditure is RMR (60-80%) (Goran, 2000), small changes in it could result in a large number of calories over time (Ravussin & Bogardus, 1989). Given the high volume of energy expended and the large variability between individuals (±25%) (Murgatroyd et al., 1987), it is important to better understand the determinants of RMR and the subsequent effect on body weight and composition.

African American (AA) females have the highest prevalence of overweight (82.1%) and obesity (58.6%) of any racial group in the United States (Flegal, Carroll, Kit, & Ogden, 2012). It is hypothesized that AA females have a harder time losing and

maintaining weight loss (Darga, Holden, Olson, & Lucas, 1994; Kumanyika, Obarzanek, Stevens, Hebert, & Whelton, 1991), and an existing hypothesis suggest a lower RMR may explain part of this problem. A recent review of the literature found 10 out of 15 studies reported lower levels of RMR in AA compared to Whites (W) ranging from 81-274 kcal/day (Gannon et al., 2000). The primary cause of this discrepancy appears to be due to different levels of fat free mass (FFM) in AA populations compared to whites, including greater skeletal muscle mass and bone mineral density and lower residual mass which includes internal organs (Carpenter et al., 1998; Forman et al., 1998; Foster et al., 1997; Jakicic & Wing, 1998; A. Jones, Jr. et al., 2004; Ortiz et al., 1992; Wagner & Heyward, 2000). Since the metabolic activity of bone and skeletal muscle is lower compared to internal organs (Elia, 1992; Gallagher et al., 1998; Holliday et al., 1967), analyses that do not independently account for these variables in FFM may incorrectly find a lower RMR in AA, when in fact it is an appropriate value given the size of metabolically active tissues (Gannon et al., 2000). In practice, few studies have accounted for differences in bone mineral content or skeletal muscle mass when comparing differences between groups.

Previous studies suggest that regular physical activity or high levels of cardiorespiratory fitness (CRF) may also have an effect on RMR via two distinct pathways: 1) the growth of FFM (i.e. skeletal muscle), and 2) the effect on physiological processes that influence RMR (Speakman & Selman, 2003). The physiological effects of physical activity on RMR may have both short (<48 hours post exercise) (Sedlock et al., 1989) and long term (>48 hours post exercise) (Margaria et al., 1933) (Dolezal et al., 2000; Edwards et al., 1935) effects while changes in FFM occur over much longer

periods of time (> 4 weeks). However, the existing research is unclear with some studies showing an independent positive relationship between CRF and RMR and others not.

Using state of the art measurement techniques and a large sample, the aim of the current study is to evaluate associations between race, body composition, aerobic fitness, and physical activity with RMR in a group of free living young adult African American and white women.

Methods

Participants and enrollment process The design and rationale for this study have been described in detail previously (Hand et al., 2013). All study protocols were approved by the University of South Carolina Institutional Review Board, and informed consent was obtained from each participant prior to data collection. Participants were required to have a body mass index (BMI) $\geq$20 and $\leq$35 kg/m^2 and age $\geq$21 and $\leq$35 years. Individuals were excluded if they had or were taking medication for a major medical condition, or for reasons that might influence body weight (taking medications to lose weight, started or stopped smoking in the previous 6 months, planned weight loss surgery, etc.). Individuals with a history of depression, anxiety, or panic were also excluded, as were those taking selective serotonin inhibitors for any reason. All women were eumenorrheic, and those who were planning to begin or stop birth control during the duration of the study also were excluded.

Anthropometrics Dual-energy X-ray absorptiometry (DXA) provided measurements on bone mineral density, fat mass (FM), and FFM, both whole body and for various body regions (arms, legs, etc.). The scan was completed with a Lunar DPX

system (version 3.6; Lunar Radiation Corp, Madison, WI). All anthropomorphic measurements were performed with the participant dressed in surgical scrubs and in bare feet.

Skeletal muscle mass was calculated from appendicular lean soft tissue (ALST) mass using the following linear regression equation:

$$\text{Skeletal mass} = (1.13 \times \text{ALST}) - (0.02 \times \text{age}) + (0.61 \times \text{sex}) + 0.97$$

where sex= 0 for females (Kim et al., 2002). This equation was developed and validated with groups of 321 and 93, respectively, ethnically diverse men and women using magnetic resonance imaging (MRI) and DXA. Correlation between skeletal mass derived from the equation and MRI were high ($R^2 = 0.96$, $P < 0.0001$) during the validation study (Kim et al., 2002). Residual mass was then calculated using the following equation:

Residual mass= body weight – fat mass – skeletal mass – bone mass (A. Jones, Jr. et al., 2004).

Cardiorespiratory fitness All participants completed a maximal fitness test using a Modified Bruce protocol, with respiratory gases sampled using a TrueOne 2400 Metabolic Measurement Cart (ParvoMedics, Salt Lake City, Utah) throughout the duration of the test.

Resting Metabolic Rate RMR was measured via indirect calorimetry using a ventilated hood and an open-circuit system, TrueOne 2400 Metabolic Measurement Cart (ParvoMedics, Salt Lake City, Utah). An initial stabilization period of 15 minutes was followed by a 30 minute data collection period. Participants arrived for a morning visit

(<9:00am) following a 12 hour dietary fast and at least 24 hours after the last bout of structured exercise. RMR was calculated from O_2 consumption and CO_2 production as measured continuously during the testing period with a constant airflow rate into the hood (Branson & Johannigman, 2004; Weir, 1949, 1990). Participants remained quiet and still through the entire RMR procedure and were kept awake with continuous monitoring. The room was maintained in low light, noise was kept at a minimum, and the temperature remained between 26 to 30 degrees centigrade (Branson & Johannigman, 2004; Henry, 2005).

Energy Expenditure Total daily energy expenditure (TDEE) was measured using a validated arm-based activity monitor (SenseWear Mini Armband, BodyMedia Inc. Pittsburgh, PA) (Johannsen et al., 2010; M. St-Onge et al., 2007; Welk et al., 2007). The monitor incorporates tri-axial accelerometry, heat flux, galvanic skin response, skin temperature, and near-body ambient temperature and demographic information to estimate TDEE and time spent in physical activity. The individuals were asked to use the monitor at all times except during periods when the monitor could get wet, and for most individuals this only included periods of showering or bathing. The participants wore the armband for 10 days and compliance criteria for adequate wear time was set at 7 days (including two weekend days) with at least 23 hours of wear time on each of the days.

Energy Intake Energy intake was measured using interviewer-administered 24 hour dietary recalls using the Nutrient Data System for Research software (NDSR, Version 2012). Dietary recalls were collected by a team of experienced (> 6 years using NDSR) registered dietitians employing a multi-pass approach which utilizes prompting to reduce omissions, and standardizes the interview methodology across interviewers

(Dwyer et al., 2001). Prior to data collection, study participants undergo a brief training (10-15 minutes) to estimate portion sizes of commonly eaten foods. Interviews were conducted on randomly selected, non-consecutive days over a 14 day sampling window.

Results

Participant characteristics are presented in Table 5.1, both overall and by race. A total of 179 individuals participated in the present study, including 141 W women and 38 AA women with a mean age of 27.7 years. AA women had higher BMI, body weight, body fat percentage, and levels of fat mass and fat free mass compared to white women. Fat free mass was then compartmentalized into bone mass, skeletal mass, and residual mass, with the later representing internal organs, which have higher rates of metabolic activity compared to fat, bone, and skeletal muscle. AA women had higher bone mass, but there was no statistically significant difference when expressed relative to body weight (Table5. 2). For residual mass, there was no statistical difference when expressed absolutely but AA women had a lower percent when expressed relative to body weight. Skeletal mass was significantly higher in AA compared to W in kg, but lower relative to body weight. Table 5.3 describes oxygen consumption at rest (RMR) and at peak exercise (CRF). There was no difference in RMR between groups of women when expressed as kcal/day, but when expressed relative to body weight white women were significantly higher. There was no difference between groups for respiratory quotients at rest. Oxygen consumption at peak exercise was also higher among white women, both absolutely and relative to body weight.

Detailed information was collected from the arm-based activity monitor regarding energy expenditure and time spent at various intensities of activity (Table 5.4). Compliance with armband was excellent, with 23.2±0.75 hours of daily wear time and no significant difference between groups. There was no difference in total daily energy expenditure between the groups. AA women spent significantly more time in sedentary and light activity, and white women spent significantly more time in moderate, vigorous, and very vigorous activities. Energy intake and diet composition also did not differ between groups, except for percent of kcals from alcohol (Table 5.4).

Univariate correlations between relevant variables and RMR by group are displayed in Table 5.5. As expected, RMR was highly correlated with FFM ($r=0.71$, $P<0.0001$) and this relationship is displayed in Figure 5.1. There was no difference in the slope of the lines relating RMR and FFM between the two groups ($P=0.8789$) indicating no difference between AA and W in the relationship between the two variables. However, there was a nearly significant difference for higher RMR relative to FFM among W women compared to AA women ($P=0.0607$ for Y intercepts).

To explore the relationship between oxygen consumption at rest (RMR) and peak exercise (CRF), the correlation between RMR and CRF by group is displayed in Figure 5.2. The top panel which displays oxygen consumption relative to body weight indicates an attenuated response to peak exercise among AA ($R^2=0.37$, $P=0.0238$) compared to whites ($R^2=0.60$, $P<0.0001$) as indicated by different slopes among the groups ($P=0.01495$). However, these findings can be misleading due to the differences in body composition between the groups, and when RMR was normalized to FFM (Figure 5.2,

bottom panel) there was no relationship between RMR and CRF among AA (r=-0.13, P=0.4544) and significant but small negative relationship for W (r=-0.23, P=0.0064).

Due to statistically significant differences in body composition parameters, linear models were created to adjust RMR (Table 5.6). All models included the covariates race and age, which have previously been shown to influence RMR, and the four body compartments (fat mass, skeletal muscle mass, residual mass, and bone mass) were added in subsequent models. Adjusted RMR values were calculated for each group from each model. The initial model which included race, age, and skeletal muscle mass explained 51% of the variance in RMR, and adding residual mass explained 52%. The addition of FM explained 66% of RMR, and the adjusted mean RMR was now significantly higher for W women compared to AA women (1400.3±9.1 kcal/day vs. 1299.8±18.9 kcal/day, P<0.0001). Adding bone mass to the model did not significantly improve the model.

To determine the role of aerobic capacity on RMR, CRF (L/min) was added to the model described previously. After statistical adjustment for all of the other covariates in the model, CRF was significantly related to RMR (P=0.0115) though the improvement was modest (R^2=0.67). This approach was repeated to determine the influence of moderate to vigorous physical activity on RMR, with time spent in physical activity replacing CRF in the model. Despite the negative univariate correlation described in Table 5.5, time spent in moderate to vigorous physical activity was not statistically significantly related to RMR after adjustment for the body composition covariates in the model (P=0.3917). The individual components of energy expenditure related to physical intensity (time spent in sedentary, light, moderate, vigorous, and very vigorous activity) were also entered into the model both separately and together, but none were statistically

related to RMR (results not shown). Energy intake nor any of the diet composition variables were statistically related to RMR (results not shown).

Conclusions

The primary finding of the present study confirms young adult African-American women have a lower RMR compared to their white peers after statistical adjustments to account for differences in body composition. While often incorrectly considered metabolically inert, the higher levels of fat mass in AA compared to W resulted in elevated RMR beyond the differences in fat free mass. Additionally, CRF was significantly associated with RMR in the present study, but time spent in moderate to very vigorous physical activity was not. As a whole these findings support previous research, but are novel given the characteristics of the population and the assessment techniques utilized.

Unadjusted RMR values presented in Table 5.1 show no statistically significant differences between AA and W women. However, since RMR is primarily determined by the quantity of metabolically active tissues, these values are misleading given the large differences in body size and composition between the two groups. By using DXA, we were able to compartmentalize the body by metabolically active tissues: lean mass, fat mass, bone mass. We further segmented lean mass into skeletal muscle mass and residual mass, the latter representing highly metabolically internal organs, using a validated equation originally derived from magnetic resonance imaging (Kim et al., 2002). After statistically adjusting for the differences in the various body compartments between the two groups, AA women were found to have a lower RMR compared to W women by

approximately 7.2% (100 kcals/day, Table 5.6). This finding is similar to those found elsewhere, though there is wide variability ranging from 87-274 kcals/day due to differences in participant characteristics and rigor of the assessment techniques (Carpenter et al., 1998; Forman et al., 1998; Foster et al., 1997; Gannon et al., 2000; Jakicic & Wing, 1998; A. Jones, Jr. et al., 2004; Ortiz et al., 1992; Wagner & Heyward, 2000).

The hypothesized reason for lower RMR in AA women at a given body weight is differing levels of body composition compared to whites, particularly FFM. Despite often being treated scientifically as metabolically homogenous tissue, fat free mass is composed of multiple components varying in size and metabolic rates. For example, the brain, heart, liver, and kidneys make up just over 5% of total body mass but account for nearly 60% of RMR with a metabolic rate of 330 kcal/kg/day (Elia, 1992). Meanwhile, skeletal muscle is estimated to have a metabolic rate of 13 kcal/kg/day, and bone 12 kcal/kg/day (Elia, 1992). Given this wide range of metabolic properties of fat free mass, it is important to compartmentalize this tissue in order to explain differences in RMR between various groups (Heymsfield et al., 2007; Heymsfield et al., 2012). AA are thought to have higher levels of low-metabolically active bone and skeletal muscle mass, and lower levels of highly metabolically active internal organs. The AA group in the present study had higher levels of bone mass, but when expressed relative to body weight was not different than the W group. FM was higher in AA, both in total mass and relative to body weight, whereas skeletal muscle mass was higher in total mass, but lower relative to body weight. Total residual mass was not higher in AA compared to W, but was

significantly lower relative to body weight. Thus, any differences in RMR would be attributed to differences in skeletal muscle and residual mass and not bone mass.

FM is often overlooked when examining RMR, which was once considered metabolically inert tissue. In the present study FM independently predicted RMR and explained approximately 14% of the variance. The contribution of FM to RMR is estimated to be between 5 kcal/kg/day (Elia, 1992) to 10-13 kcal/kg/day (Goran et al., 1994; M. P. St-Onge, 2005), explaining between 1-10% of the variability in young and middle aged adults (Nelson et al., 1992; Sparti et al., 1997; Tataranni & Ravussin, 1995). The contribution of FM on RMR is slightly higher here than previous studies, likely due to the large differences in FM between the two groups. Of the four body compartments studied here, fat mass differed the most between AA and W women, with W women lower in both absolute (23.7kg vs 31.7 kg) and relative to total body weight (33.8% vs. 39.8%).

It has been suggested 'the factor that causes by far the most dramatic effect on metabolic rate is strenuous exercise' (Guyton, 1997). Physical activity may have an effect on RMR via two distinct pathways: 1) the growth of FFM (i.e. skeletal muscle), and 2) the effect on physiological processes that influence RMR (Speakman & Selman, 2003). It has been known for nearly a century that physical activity will produce acute elevations in RMR (Edwards et al., 1935; Margaria et al., 1933) referred to as excess post-exercise oxygen consumption (EPOC). Much less clear is if regular physical activity results in sustained elevations of RMR. The majority of cross-sectional studies indicate a 5-20% elevated RMR among individuals who participate in regular activity compared to sedentary controls, and CRF(L/min) is highly correlated with RMR in the present study

(r=0.52, P<0.001) (Arciero et al., 1993; Burke et al., 1993; Hill et al., 1984; Ravussin & Bogardus, 1989; Schulz et al., 1991; Sjodin et al., 1996; Tremblay et al., 1992; Tremblay et al., 1985; Tremblay et al., 1986; Tremblay et al., 1990; van Pelt et al., 2001). However, others have failed to find a positive relationship between CRF and RMR, likely due to differences in sample size and statistical power (Broeder et al., 1992a; Hill et al., 1984; LeBlanc, Diamond, Cote, & Labrie, 1984; Ravussin & Bogardus, 1989).

In the present study, CRF was a significant predictor of RMR independent of FFM, though the effect was small (model R^2 increased from 0.66 to 0.67). Figure 5.2 displays the relationship between CRF and RMR, and particularly the role of body composition in this relationship. In the top panel, a positive linear relationship exists between CRF and RMR when RMR is referenced to body weight similar to others (Miller et al., 2011), with a statistically significant blunted response among AA compared to W. However, expressing RMR relative to body weight when comparing to CRF has previously been shown to falsely suggest a positive relationship due to differences in body composition and in particular FM (Broeder et al., 1992a) and this is reflected by lack of association with RMR and CRF when indexed to FFM (Figure5. 2, bottom panel). The results of the linear modeling in Table 5.6 shows CRF predicts RMR independent of FFM, suggesting this relationship is mediated by physiological processes. The mechanisms have not been well-explored, but likely include regulation of the sympathetic nervous system (SNS) (Bell et al., 2004; Bell et al., 2001; Bullough et al., 1995; Ravussin, 1995), changes in muscle cell structure (Hather et al., 1991), immune systems responses (Cannon et al., 1991; Haahr et al., 1991), neuroendocrine function (Herring et al., 1992; Luger et al., 1987), and substrate cycling (Bahr, 1992; Wolfe et al., 1990).

Time spent in moderate to very vigorous physical activity was not related RMR. The current study was the first to explore the role of regular physical activity on RMR using an objective measure rather than self-report. The SensorWear armband has been shown to be highly valid and reliable at assessing both total energy expenditure and the intensity of physical activity and the participants here had excellent compliance, with a mean daily wear time of 23 hours and 15 minutes. Univariate correlations showed low a low association between time spent in physical activity (minutes/day) and CRF (L/min) ($r=0.2035$, $P=0.0063$), so any expected relationship with RMR would have been independent of aerobic fitness effects. However, although we instructed the participants to maintain their normal lifestyle during EE and EI assessment their behavior during this time may not be reflective of their normal lifestyle.

The present study is one of the largest and most thorough examinations of racial differences in RMR to be completed in women. We used state of the art measurement techniques and sound methodology including RMR via indirect calorimetry following a 12 hour dietary fast and >24 hour cessation of strenuous activity. We also had direct measures of maximal CRF and objective measures of physical activity and energy expenditure. Given the cross-sectional study design we cannot identify causality. By compartmentalizing tissue by metabolic rates, we have properly controlled for differences in body composition between the groups. However, despite the use of valid and reliable regression equations, the values for skeletal and residual mass are estimations and not direct measurements.

In summary, after adjustment for differences in body composition, particularly a higher residual mass and lower FM among W, AA had a lower RMR. Additionally, CRF

was independently associated with RMR after adjustment for body composition, but time spent in moderate to very vigorous activity was not. Future research is needed to longitudinally assess the role of low levels on RMR on body composition and weight gain.

Acknowledgements The authors thank the participants of the present study for generously volunteering their time and effort. In addition, we are grateful to the project staff for their tireless efforts with data collection and study management. This study was supported by an unrestricted grant from the Coca-Cola Company.

Table 5.1. Participant anthropometric characteristics overall and by group.

	All (N=179) (mean±SD)	**White** (N=141) (mean±SD)	**AA** (N=38) (mean±SD)	**P value** between group differences
Age (years)	27.7±3.8	27.3±3.4	29.0±4.8	0.0571
Body Mass Index (kg/m^2)	25.5±4.3	24.6±3.9	28.8±4.1	<0.0001
Height (cm)	165.8±6.2	166.2±5.8	164.6±7.3	0.1669
Weight (kg)	70.2±12.4	68.0±11.3	78.3±13.0	<0.0001
Fat free mass (kg)	45.0±5.6	44.5±5.1	47.0±6.9	0.0439
Fat mass (kg)	25.4±9.6	23.7±9.1	31.7±8.7	<0.0001
Body fat (%)	35.0±8.1	33.8±8.2	39.8±6.0	<0.0001

Table 5.2. Participant body compartment characteristics overall and by group.

	All (N=179) (mean±SD)	**White** (N=141) (mean±SD)	**AA** (N=38) (mean±SD)	**P value** between group differences
Bone mass (kg)	2.7±0.4	2.7±0.4	3.0±0.5	<0.0001
Bone (%)	3.9±0.5	4.0±0.5	3.9±0.6	0.7006
Fat mass (kg)	25.4±9.6	23.7±9.1	31.7±8.7	<0.0001
Fat mass (%)	35.0±8.1	33.8±8.2	39.8±6.0	<0.0001
Skeletal mass (kg)	21.3±3.1	20.9±2.7	22.6±3.8	0.0133
Skeletal mass (%)	30.7±4.3	31.1±4.4	28.9±3.4	0.0042
Residual mass (kg)	21.0±2.7	20.9±2.5	21.3±3.3	0.4773
Residual mass (%)	30.4±4.2	31.2±4.0	27.4±3.2	<0.0001

Table 5.3. Oxygen consumption at rest and peak exercise overall and by group.

	All (N=179) (mean±SD)	**White** (N=141) (mean±SD)	**AA** (N=38) (mean±SD)	**P value** between group differences
Resting energy expenditure (kcals/day)	1378.9±178.4	1375.7±173.6	1390.8±197.5	0.6443
Resting metabolic rate (mL/kg/min)	2.87±0.36	2.95±0.33	2.59±0.30	<0.0001
Resting metabolic rate (mL/kg of FFM/min)	4.43±0.41	4.47±0.42	4.28±0.36	0.0128
Respiratory quotient	0.79±0.05	0.79±0.4	0.79±0.06	0.7887
Cardiorespiratory fitness (mL/kg/min)	33.4±7.8	35.3±7.4	26.2±4.2	<0.0001
Cardiorespiratory fitness (L/min)	2.30±0.48	2.37±0.48	2.05±0.41	0.0002

Table 5.4. Energy expended by physical activity intensity.

	All (N=179) (mean±SD)	White (N=141) (mean±SD)	AA (N=38) (mean±SD)	P value between group differences
Total daily energy expenditure (kcal/day)	2419.7±291.1	2413.6±296.0	2442.5±274.7	0.5887
Sedentary (min/day)	1086.0±80.7	1080.4±85.0	1106.7±58.7	0.0302
Light (min/day)	242.0±58.3	237.1±57.9	260.0±56.8	0.0313
Moderate (min/day)	104.0±57.3	113.4±59.5	67.2±25.6	<0.0001
Vigorous (min/day)	4.7±5.5	5.2±5.7	2.7±4.5	0.0112
Very Vigorous (min/day)	2.4±8.1	2.9±9.0	0.7±3.0	0.0142
Physical Activity (min/day)	110.7±62.8	121.5±110.6	70.6±25.7	<0.0001
Energy intake (kcal/day)	1801.9±457.3	1827.6±450.7	1706.6±475.0	0.1483
Carbohydrates (% of total kcals)	48.1±8.4	47.9±8.6	48.6±8.0	0.6567
Fat (% of total kcals)	32.7±6.7	32.3±6.8	33.9±6.3	0.2014
Protein (% of total kcals)	16.7±3.5	16.6±3.5	17.0±3.5	0.4988
Alcohol (% of total kcals)	2.7±4.3	3.3±4.6	0.5±1.1	<0.0001

Sedentary, 1.0 to ≤1.5 METs; Light, >1.5 to ≤3.0 METs; Moderate, >3.0 to ≤6.0 METs;
Vigorous, >6.0 to ≤9.0 METs; Very Vigorous, >9.0 METs.
Physical Activity= cumulative minutes spent at >3.0 METS.
P value represents differences between races

Table 5.5. Pearson correlations coefficients between RMR (kcals/day) and selected variables.

	Resting metabolic rate (kcal/day)		
	All (N=179)	W (N=141)	AA (N=38)
Weight (kg)	0.70**	0.73**	0.78**
Fat mass (kg)	0.49**	0.52**	0.49*
Fat free mass (kg)	0.71**	0.69**	0.83**
Bone mass (kg)	0.51**	0.55**	0.46*
Skeletal mass (kg)	0.70**	0.65**	0.86**
Residual mass (kg)	0.63**	0.61**	0.69**
Physical activity (min/day)	-0.26*	-0.30*	-0.06
Cardiorespiratory fitness (mL/kg/min)	-0.04	-0.03	-0.01
Cardiorespiratory fitness (L/min)	0.52**	0.53**	0.64**

Physical Activity = cumulative minutes spent at >3.0 METS.
*Significantly correlated with RMR, P<0.05
**Significantly correlated with RMR, P<0.0001

Table 5.6. Analysis of covariance assessing resting metabolic rate between sexes controlling for body compartments (kg), cardiorespiratory fitness (CRF, L/min) and time spent in physical activity (minutes/day) (mean±standard error).

	White	AA	R^2	P value
Unadjusted	1375.7±14.6	1390.8±32.0	NA	0.6443
Race + age + skeletal muscle	1391.5±10.8	1332.4±21.3	0.51	0.156
Race + age + skeletal muscle + residual mass	1388.5±10.6	1343.5±21.2	0.52	0.0649
Race + age + skeletal muscle + residual mass + fat mass	1400.3±9.1	1299.8±18.9	0.66	<0.0001
Race + age + skeletal muscle + residual mass + fat mass + bone mass	1400.4±9.2	1299.4±19.2	0.66	<0.0001
Race + age + skeletal muscle + residual mass + fat mass + bone mass + CRF	1395.1±9.3	1318.9±20.3	0.67	0.0017
Race + age + skeletal muscle + residual mass + fat mass + bone mass + physical activity minutes	1399.9±9.2	1301.3±19.3	0.66	<0.0001

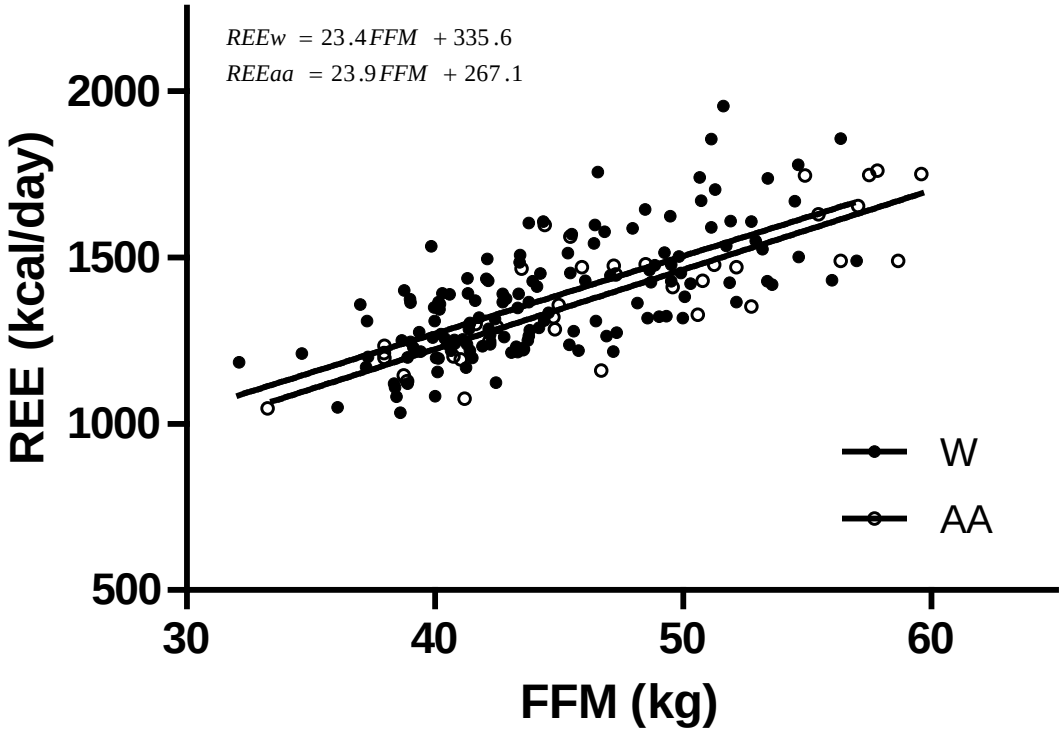

Figure 5.1. The relationship between RMR (kcals/day) and FFM (kg) in 141 white (W) and 38 African-American (AA) women. There is no statistical differences in either the slopes (P=0.8789) or Y-intercepts (P=0.0607) between the groups.

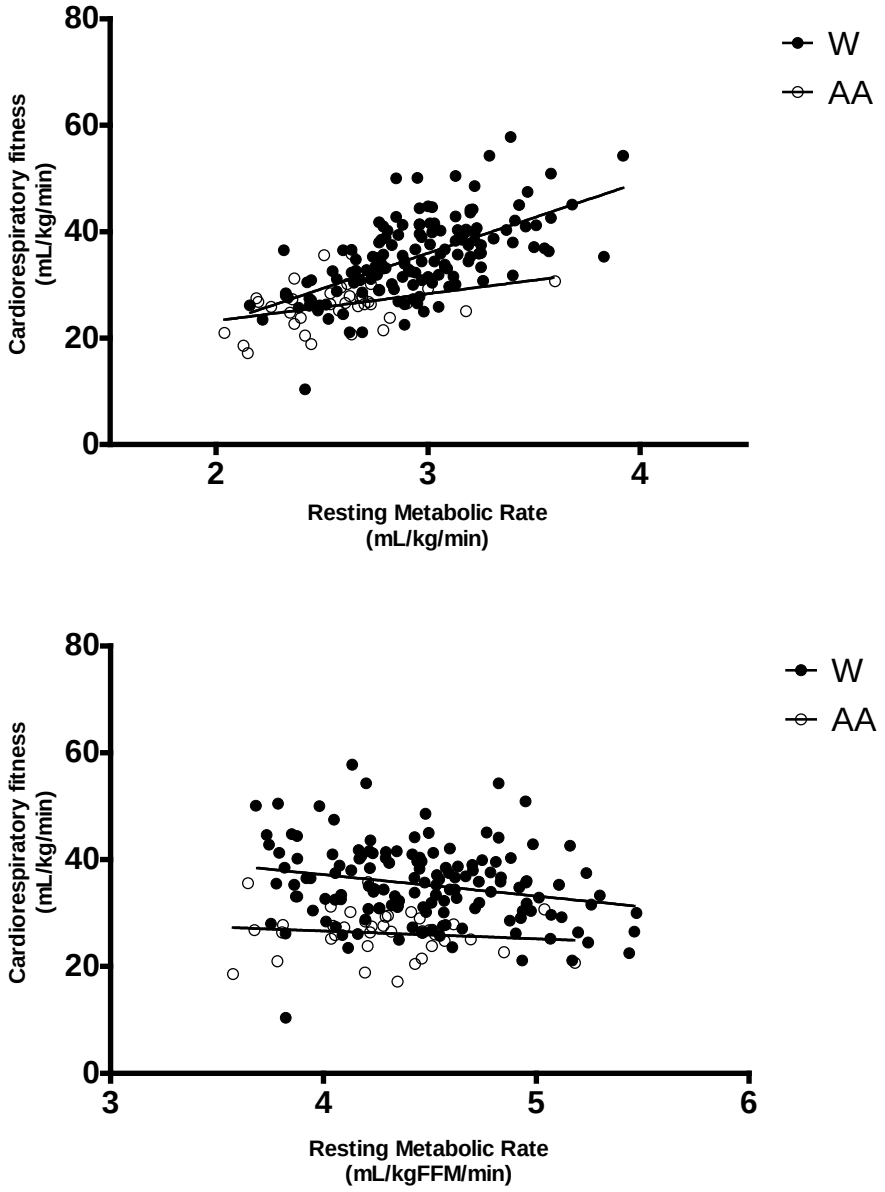

Figure 5.2. Resting oxygen consumption was directly associated with cardiorespiratory fitness (top panel) when referenced to total body weight, though the relationship was different for white women (R2=0.60, P<0.0001) compared to African American women (R2=0.37, P=0.0238). However, when resting oxygen consumption was referenced to fat free mass (bottom panel) there was no relationship for African American women r=-0.13, P=0.45) and a small negative relationship for white women (r=-0.23, P=0.0064).

CHAPTER 6

MANUSCRIPT 3: HIGH RESPIRATORY QUOTIENT IS ASSOCIATED WITH INCREASES IN BODY WEIGHT AND FAT MASS IN YOUNG ADULTS

Abstract

Objective: It has been hypothesized metabolic disturbances such as reduced rates of fat oxidation as demonstrated by a high respiratory quotient (RQ) or low levels of energy expenditure resulting from a low resting metabolic rate (RMR) may contribute to increasing levels of obesity. The purpose of the present study was to determine the role of a high RQ or RMR on subsequent changes in body weight and body composition over a 9 month period.

Methods: We measured RQ and RMR in 317 young adults using indirect calorimetry, along with body weight and body composition using dual energy X-ray absorptiometery, energy expenditure using an arm-based activity monitor, and energy intake using interviewer-administered dietary recalls. Follow up measurements were completed every three months for the duration of the study on all measures except RQ and RMR. Linear mixed modeling was performed to determine the role of RQ and RMR on changes in body weight and fat mass after adjustment for age, race, and changes in energy expenditure and energy intake.

Results: There were no differences in unadjusted changes in body weight or fat mass between individuals with a high RQ or low RMR. Following statistical adjustment for covariates (age, race, and changes in energy expenditure and energy intake), individuals in the highest tertile of RQ had larger gains in body weight and fat mass compared to individuals in the bottom two tertiles after 3 months, which continued through the remainder of the 9 month follow up period. Individuals in the lowest tertile for RMR did

not gain more body weight compared to individuals in the bottom two tertiles, and actually gained less fat mass during the follow up period.

Conclusion: The primary finding of the current study is a high RQ is predictive of gains in body weight and fat mass over a 9 month period among young adults. Additionally, a low RMR was not associated with gains in body weight or fat mass over the same period. These findings suggest reduced levels of fat oxidation may result in obesity over time

Introduction

At the most basic level, obesity is the result of a chronic imbalance between energy intake and energy expenditure. However, the exact etiology is considerably more complex and may involve a variety of physiological and behavioral factors. Metabolic disturbances, including reduced fat oxidation and reduced resting metabolic rate (RMR), have been identified as possible predictors of changes in body weight and body composition.

Respiratory quotient (RQ) reflects the ratio of carbohydrate to fat oxidation, and when measured in a fasting state stored fat is the primary fuel source. If an individual has a low RQ they oxidize more stored fat compared to an individual with a high RQ, and theoretically are protected against future fat accumulation. A high RQ among Pima Indians has previously been shown to be correlated with increased body weight after more than two years of follow up (Zurlo et al., 1990). Additionally, high RQ was found to be a weak but significant predictor of weight gain among 775 adults in the Baltimore Longitudinal of Aging Study (Seidell et al., 1992). However, there was no association

between RQ and weight gain over 5 ½ years in the Quebec Family Study (Katzmarzyk et al., 2000).

RMR, the amount of calories burned from normal physiological functions (E.g. respiration, brain activity), represents the largest contributor (60-80%) of total energy expenditure in humans. Given the intricate balance of energy intake and expenditure in the regulation of body weight, it is hypothesized that small changes in RMR could result in a large reduction in the number of calories burned over time (Ravussin & Bogardus, 1989), but this relationship is uncertain. For example, some prospective studies suggest low RMR is predictive of subsequent weight gain (Astrup, Gotzsche, et al., 1999; Leibel et al., 1995; Ravussin et al., 1986; Ravussin et al., 1988; Zurlo et al., 1990) while others do not (Katzmarzyk et al., 2000; Marra et al., 1998; Seidell et al., 1992).

Thus, the purpose of the present study is to explore the longitudinal associations of RQ and RMR on changes in body weight and body composition over 9 months in a group of healthy young adults.

Methods

Participants and enrollment process A full description of the study design has been described in detail previously (Hand et al., 2013). Briefly, participants were young adults age ≥21 and ≤35 and had a BMI ≥20 and ≤35 kg/m^2. Individuals were excluded for any of the following reasons: use of medications to lose weight, started or stopped smoking in the previous 6 months, or planned weight loss surgery. Individuals were also excluded for resting blood pressure >150 mmHg systolic and/or >90 mmHg diastolic, an ambulatory blood glucose level of greater than 145 mg/dl, current diagnosis or taking

medications for a major chronic health condition. Additionally, individuals with a history of depression, anxiety, or panic were excluded, as were those taking selective serotonin inhibitors for any reason. All women were eumenorrheic, and those who were planning to begin or stop birth control during the study also were excluded. All study protocols were approved by the University of South Carolina Institutional Review Board, and informed consent was obtained from each participant prior to data collection.

Anthropometrics A dual energy X-ray absorptiometer (DXA) was used to measure bone mineral density, fat mass, and fat free mass. The scan was completed with a Lunar DPX system (version 3.6; Lunar Radiation Corp, Madison, WI). All anthropomorphic measurements were performed with the participant dressed in surgical scrubs and in bare feet. Body mass index (BMI; kg/m^2) was calculated from the average of three height and weight measurements using a traditional standiometer and electronic scale and recorded to the nearest 0.1 centimeter and 0.1kg, respectively. All anthropometric measurements were completed once every three months for the duration of the study.

Metabolic measurements RQ and RMR were measured at baseline via indirect calorimetry using a ventilated hood and an open-circuit system, TrueOne 2400 Metabolic Measurement Cart (ParvoMedics, Salt Lake City, Utah). A 15 minute resting period preceded a 30 minute data collection period, and the metabolic cart was calibrated prior to each test using known gas concentrations and volumes as recommended by the manufacturer. All measurements occurred in the morning (<9:00am) following a 12 hour dietary fasting state and at least 24 hours after the last bout of structured exercise. Participants remained quiet and still through the entire RMR procedure and were kept

awake with continuous monitoring. The room was maintained in low light, noise was kept at a minimum, and the temperature remained between 26 to 30 degrees centigrade (Branson & Johannigman, 2004; Henry, 2005). RMR was calculated from O_2 consumption and CO_2 production as measured continuously during the testing period with a constant airflow rate into the hood, and RQ was calculated as VCO_2/VO_2 (Branson & Johannigman, 2004; Weir, 1949, 1990). Given no widely accepted criteria exist to categorize RQ levels, participants were classified with a 'High' if they were in the upper tertile for RQ among the entire cohort for each gender or 'Low/Moderate' if they were in the bottom two tertiles.

Cardiorespiratory fitness Fitness testing was conducted at baseline on a treadmill (Trackmaster 425, Carefusion, Newton, Kansas) with respiratory gases sampled using a TrueOne 2400 Metabolic Measurement Cart (ParvoMedics, Salt Lake City, Utah) using a Modified Bruce protocol, and all participants exercised to volitional fatigue.

Energy Expenditure Energy expenditure was measured using an arm-based activity monitor (SenseWear Mini Armband, BodyMedia Inc. Pittsburgh, PA). The monitor is a portable, multi-sensor device worn on the upper arm, incorporating tri-axial accelerometry and measures of heat flux, galvanic skin response, skin temperature, and near-body ambient temperature. These measures are entered in combination with demographic information into an algorithm to estimate total energy expenditure and time spent in physical activity (> 3 metabolic equivalents or METs). The armband has been shown to be a valid device to measure energy expenditure and activity (Johannsen et al., 2010; M. St-Onge et al., 2007; Welk et al., 2007). The participants wore the armband for 10 days and were deemed compliant if they completed 7 days of wear (including two

weekend days) with at least 23 hours of wear time on each of the days. Measurement of energy expenditure and time spent in physical activity were completed once every three months for the duration of the study.

Energy Intake Energy intake was measured using interviewer-administered 24 hour dietary recalls. The Nutrient Data System for Research software (NDSR, Version 2012), a state-of-the-art research software for conducting dietary recalls (F. E. Thompson & Subar, 2013), was utilized to conduct the dietary interviews. The dietary recalls were collected by a team of experienced (> 6 years using NDSR) registered dietitians employing a multi-pass approach which utilizes prompting to reduce food omissions, and standardizes the interview methodology across interviewers (Dwyer et al., 2001). Interviews occurred on randomly selected non-consecutive days over 14 days to minimize preparation that could bias recall by the participants (Hebert et al., 2002). Measurement of energy intake was completed once every three months for the duration of the study.

Statistical Analyses Participant characteristics were based on demographic and physiological measurements using means and standard deviations for continuous variables and percentages for categorical variables. Statistical significance for comparison between groups were tested using t-tests for continuous variables and chi-square for categorical variables. A linear mixed models (LMM) regression random intercept growth model was used to analyze the longitudinal data (West et al., 2007). An advantage of the LMM approach is it allows for unbalanced observations over time making it ideal to analyze longitudinal data. The dependent variable was change in body weight and fat mass over 9 months expressed in kg. The LLM was repeated multiple

times using various covariance structures, unstructured, first-order autoregressive, and compound symmetry. The unstructured covariance structure did not converge, and the results presented here utilized the first-order autoregressive structure due to lower Akaike Information Criteria (AIC) value compared to compound symmetry covariance structure. All analyses were initially completed using groups based on tertiles of RQ and repeated using groups based on tertiles of RMR. Data presented here display the RQ grouping findings and the results from RMR grouping are presented only when appropriate. All computations were performed using SAS 9.2 (Cary, N.C.).

Results

Selected demographic and anthropometric variables are reported overall and for each group in Table 1. Overall, our participants (n=317) were young adults (27.7±3.8 years) with nearly equal numbers of males (50.8%) and females (49.2%). The percentage of female in low/moderate and high RQ groups was similar. There were no differences at baseline between the low/moderate and high RQ groups for body weight, fat free mass, fat mass, BMI or body fat percentage. In terms of race, the low/moderate RQ group primarily consisted of 68.5% whites, 11.7% African Americans, and 7% Asians, and the high RQ group primarily consisted of 58.7% whites, 12.5% African Americans, and 19.2% Asians.

The RQ was significantly elevated for the high RQ group (0.838±0.030 vs. 0.766±0.024, $P<0.0001$) and is displayed in Table 2. Resting energy expenditure (kcals/day) was not different between the groups, but resting metabolic rate was higher in the low/moderate RQ compared to the high RQ group (3.01±0.36 vs. 2.90±0.36

mL/kg/min, P=0.0096). There was no difference in total energy intake or in diet composition between the groups. Likewise, there was no difference total energy expenditure or time spent in physical activity between the groups.

At the end of the 9 month follow-up period, the average weight gain was 1.0±3.4 kg with no significant differences between the groups (Table 3). The average gain in fat mass was 0.7±3.2 kg with no significant differences between the groups. Likewise, there were no significant differences between baseline and 9 months in the change of variables potentially predictive of weight change between the groups (energy intake, diet composition, energy expenditure, time spent in physical activity). However, there were significant differences in change of body weight and fat mass at three and six months, with the high RQ group gaining more at each time point compared to the low/moderate RQ group (Figure 1). In terms of RMR, the only change when comparing the low group to moderate/high group was a higher change in fat mass at 9 months (1.08±2.77 vs. 0.09±3.74 kg, P=0.0156).

Despite no difference at baseline and no change from baseline at 9 months for energy expenditure, physical activity, and percent of kilocalories from carbohydrates, there were significant differences at 9 months and various time points in between (Figure 2). Individuals in the high RQ group expended fewer total kcals (2638±419 vs. 2755±516 kcals/day, P=0.0324), spent less time in moderate to vigorous activity (113±72 vs. 133±76 min/day, P=0.0270), and consumed a higher percent of their diet in carbohydrates (47±9 vs. 45±9 percent, P=0.0490) at 9 months. There were no significant differences between groups during any of the follow up periods for energy intake or percent of kcals from fat, protein, or alcohol (results not shown).

Given the differences between groups among covariates that could potentially influence body weight and composition changes, linear mixed modeling was completed to describe the role of RQ in the process. After adjustment for differences in gender, age, race, and change in physical activity level, energy intake, and energy expenditure from baseline between the low/moderate and high RQ groups, the high RQ group had gained more weight at each time point (Figure 3, 9 month weight gain difference 1.545±0.232 vs. 0.829±0.1819 kg, P=0.0040). The high RQ also gained more fat mass at each time point compared to the low/moderate RQ group (Figure 3, 9 month weight gain difference 1.193±0.226 vs. 0.603±0.178 kg, P=0.0150). In terms of RMR, there were no differences in changes in body weight between the low and moderate/high groups after adjustment for the previously mentioned covariates. Interestingly, the moderate/high RMR group gained more fat mass compared to the low group (1.04±0.1871 vs. 0.44±0.2182 kg, P=0.0141) following the same statistical adjustments.

C**onclusions**

The primary finding of the current study is that a high baseline RQ was predictive of gains in body weight and fat mass over a 9 month period among young adults. Additionally, a low RMR was not associated with gains in body weight over the same period, and gains in fat mass were smaller when compared to individuals with a moderate or high RMR. These findings suggest that lower levels of fat oxidation, independent of changes in energy intake, energy expenditure, macronutrient composition of the diet, and physical activity, contribute to changes in body weight and fat mass, while lower energy expenditure from RMR does not.

Previous research has been ambiguous regarding the role of fasting substrate oxidation on subsequent weight gain. In the current study, we found that individuals in the top tertile for RQ (mean RQ=0.838±0.030) had larger gains in body weight and fat mass after 9 months compared to individuals in the bottom two tertiles (Figure 3). Studies involving Pima Indians found RQ to be an independent predictor of gains in both body weight ($P<0.001$) and fat mass ($P=0.004$) at 25 months, suggesting weight gain is a result of reduced rates of fat oxidation (Zurlo et al., 1990). The Baltimore Longitudinal Study of Aging is the largest study to examine the role of RMR and RQ on weight gain in men (N=775) over 10.3 years of follow-up (Seidell et al., 1992). After multivariate adjustments for age, BMI, and FFM, RQ was significantly associated with weight change at $P<0.05$, and RMR was significantly associated at $P<0.10$. Additionally, those with an RQ of >0.85 (individuals with low rates of fat oxidation) were 2.42 times more likely to gain at least 5 kg compared to those with an RQ of <0.76 (individuals with high rates of fat oxidation). A study of Italian women (N=58) found similar results, with those who gained >3kg over a three year follow-up period having an RQ of 0.91 vs. 0.84 of those who did not (Marra et al., 1998). Based on these studies, it is oxidation of energy stores, not RMR, which is predictive of weight gain. These results are in direct contrast to the Quebec Family Study, which found no association between RMR or RQ on changes in body weight or fat during a 5.5 year follow-up (Katzmarzyk et al., 2000). In that study, the correlations were low between measures of body weight/fatness (E.g. weight, BMI, or sum of skinfolds) and RMR (r= -0.03 to 0.16, not significant) or RQ (r= -0.05 to 0.12, not significant). Neither RMR nor RQ were significant predictors of increases in body weight or fatness from Cox regressions.

The variations in findings are likely due to many factors. The studies that have shown significant relationships between RMR and weight gain have consisted of young adult populations, and likely because this age group is more likely to gain weight compared to older adults (Sheehan et al., 2003). For example, the mean age of the Pima Indians in a separate study was 26 years with 11.9% of participants gaining at least 10 kg over four years of follow-up (Ravussin et al., 1988), while the mean age of the Baltimore Longitudinal Study of Aging was 49 years and weight gain was 0.07 kg over a mean follow-up of 10.3 years and the mean age in the Quebec Family Study was 39 years and mean weight gain was 2.8 kg for men and 3.5 kg for women over 5.5 years. In the current study of young adults (mean age=27.7±3.8 years), the mean weight gain for all participants was 1.0±3.4 kg after 9 months. There are also methodological differences across studies. For example, RMR was assessed in the Pima Indians over a 24-hour period in a respiratory chamber, while RMR in the Baltimore Longitudinal Study of Aging was measured using multiple techniques over a 19 year period. Also, baseline values in the Baltimore Longitudinal Study of Aging were not reported; instead mean RMR values from all visits over the 19 year follow-up were used for data analysis which may dilute the findings. Additionally, body composition assessment techniques varied in quality from hydrostatic weighing in the Pima Indians, skinfolds in Baltimore Longitudinal Study of Aging and Quebec Family Study, and not reported in the study from Italy, which makes direct comparisons of adjusted RMR values difficult.

The determinants of elevated RQ are not well understood. The studies involving Pima Indians found approximately 28% of the variability in RQ values was due to family membership and 18% was due to prior change in weight, energy balance, body fat, and

sex (Zurlo et al., 1990). More recent studies support the genetic influence on RQ levels (Loos et al., 2007) and perhaps fitness and fat mass (Astrup et al., 1994; Colberg, Simoneau, Thaete, & Kelley, 1995; Kelley, 2005; Kunz, Schorr, Rommling, Klaus, & Sharma, 2002; Ukropcova et al., 2005). Individuals with high levels of CRF and low levels of fat mass have been shown to be 'metabolically flexible', meaning their skeletal muscle possesses the ability to switch between glucose and fat oxidation in response to homeostatic signals, such as after a meal or during a dietary fast (Kelley, 2005). When glucose is not present (i.e. following an overnight fast), a transition to reliance on fat oxidation occurs in metabolically flexible individuals as seen in low RQ values (Henriksson, 1995). Among obese and unfit individuals with low metabolic flexibility, this transition in fuel source is less robust and there is a blunted preference on fat oxidation as seen in high RQ values (Kelley, 2005). In the current study it is possible individuals in the low/moderate RQ group were more metabolically flexible due to higher levels of CRF and physical activity at baseline (significant at P=0.0578 and P=0.0667, respectively, Table 2).

It is worth noting the significant differences between groups in changes body weight and fat mass observed as soon as the third month of follow up (Figure 3). Additionally, differences in body weight and fat mass observed at 3 months became smaller at 6 and 9 months. Zurlo et al. noted that of all variables studied, RQ was most highly correlated with acute (<3 days) changes in body weight (r=0.32, P<0.00.1) (1990). Additionally, among a subsample of their population who returned for follow up metabolic assessments, RQ was negatively associated with changes in body weight (r=-0.42, P=0.07). This suggests that an elevated RQ may be associated with relatively short

term gains in weight, and after this weight gain reductions in RQ values occur. While all studies to date have focused on long term (>24 months) changes in weight and body composition, further research should explore the role of RQ on short term changes, in addition to changes in RQ following changes in weight.

A limitation of the current study is the relatively short follow up period (9 months) for assessing changes in body weight and fat mass. Other studies that have examined this topic have ranged from 25 months (Zurlo et al., 1990) to an average of 10 years (Seidell et al., 1992). We did find statistically significant differences in weight change, but further follow up is needed to assess longer term changes.

In summary, the current study has found a high RQ is predictive of gains in body weight and fat mass over a 9 month period among young adults when compared to individuals with a low/moderate or low RQ value. A low RMR was not associated with gains in body weight or fat mass over the same period. These findings support previous research which suggests that lower levels of fat oxidation, independent of changes in energy intake, energy expenditure, macronutrient composition of the diet, and physical activity, contribute to changes in body weight and fat mass.

Acknowledgements The authors thank the participants of the present study for volunteering their time and effort. In addition, we are appreciative of the staff for their efforts collecting and managing the data and all other aspects of the study. This study was supported by an unrestricted research grant from The Coca-Cola Company.

Table 6.1. Participant anthropometric characteristics overall and by group.

	All (N=317) (mean±SD)	**Low/Mod RQ** (N=213) (mean±SD)	**High RQ** (N=104) (mean±SD)	**P value** between group differences
Percent female (%)	49.2	49.3	49.0	0.9657
Percent white (%)	65.3	68.5	58.7	0.0374
Age (years)	27.7±3.8	27.4±4.0	28.1±3.6	0.1321
Body Mass Index (kg/m^2)	25.6±3.9	25.5±3.8	25.8±4.1	0.6187
Height (cm)	171.3±9.4	171.6±9.8	170.7±8.3	0.4155
Weight (kg)	75.3±13.9	75.4±13.9	75.2±13.7	0.8990
Fat free mass (kg)	54.0±12.1	54.7±12.5	52.8±11.3	0.2116
Fat mass (kg)	21.3±10.3	20.7±10.2	22.3±10.4	0.1908
Body fat (%)	27.9±11.3	27.3±11.2	29.3±11.4	0.1375

Table 6.2. Descriptive statistics overall and by group**.**

	All (N=317) (mean±SD)	**Low/Mod RQ** (N=213) (mean±SD)	**High RQ** (N=104) (mean±SD)	**P value** between group differences
Respiratory quotient	0.790±0.045	0.766±0.024	0.838±0.030	<0.0001
Resting energy expenditure (kcals/day)	1533.2±260.5	1544.1±271.0	1510.9±237.1	0.2868
Resting metabolic rate (mL/kg/min)	2.97±0.36	3.01±0.36	2.90±0.36	0.0096
Cardiorespiratory fitness (mL/kg/min)	38.4±9.7	39.1±9.6	36.9±9.8	0.0578
Energy intake (kcals/day)	2114±678	2141±735	2058±542	0.2611
Carbohydrates (% of total kcals)	47.1±9.7	46.6±9.9	48.3±9.3	0.1380
Fat (% of total kcals)	33.3±7.3	33.5±7.5	32.7±7.2	0.3386
Protein (% of total kcals)	17.1±4.7	17.4±5.0	16.5±4.0	0.0861
Alcohol (% of total kcals)	2.6±4.0	2.6±4.0	2.6±4.0	0.9851
Energy expenditure (kcals/day)	2754±495	2785±512	2689±456	0.1050
Physical activity (minutes/day)	138±79	143±79	126±79	0.0667

Physical activity= cumulative minutes spent at >3.0 METS/day.
Mean armband wear time= 23.24±0.8 hours/day; Mean dietary recalls completed= 2.76±0.5

Table 6.3. Changes in key variables between baseline and 9 months both overall and by group.

	All (N=317) (mean±SD)	**Low/Mod RQ** (N=213) (mean±SD)	**High RQ** (N=104) (mean±SD)	**P value** between group differences
Weight (kg)	1.0±3.4	0.9±3.4	1.4±3.2	0.1651
Fat mass (kg)	0.7±3.2	0.6±3.4	1.1±2.7	0.1796
Body fat (%)	0.6±3.1	0.4±3.3	0.8±2.6	0.3343
Energy intake (kcals/day)	-5±19	-27±648	40±707	0.4066
Carbohydrates (% of total kcals)	-1.4±8.8	-1.5±8.4	-1.1±9.7	0.6787
Fat (% of total kcals)	-0.3±7.7	-0.5±7.6	0.1±8.1	0.4633
Protein (% of total kcals)	0.8±5.0	0.9±5.3	0.7±4.6	0.8004
Alcohol (% of total kcals)	0.9±4.5	1.2±4.4	0.2±4.7	0.0803
Energy expenditure (kcals/day)	-37±232	-30±243	-51±207	0.4586
Physical activity (minutes/day)	-11.0±52.2	-10.1±56.4	-12.7±42.6	0.6839

Physical activity= cumulative minutes spent at >3.0 METS/day.

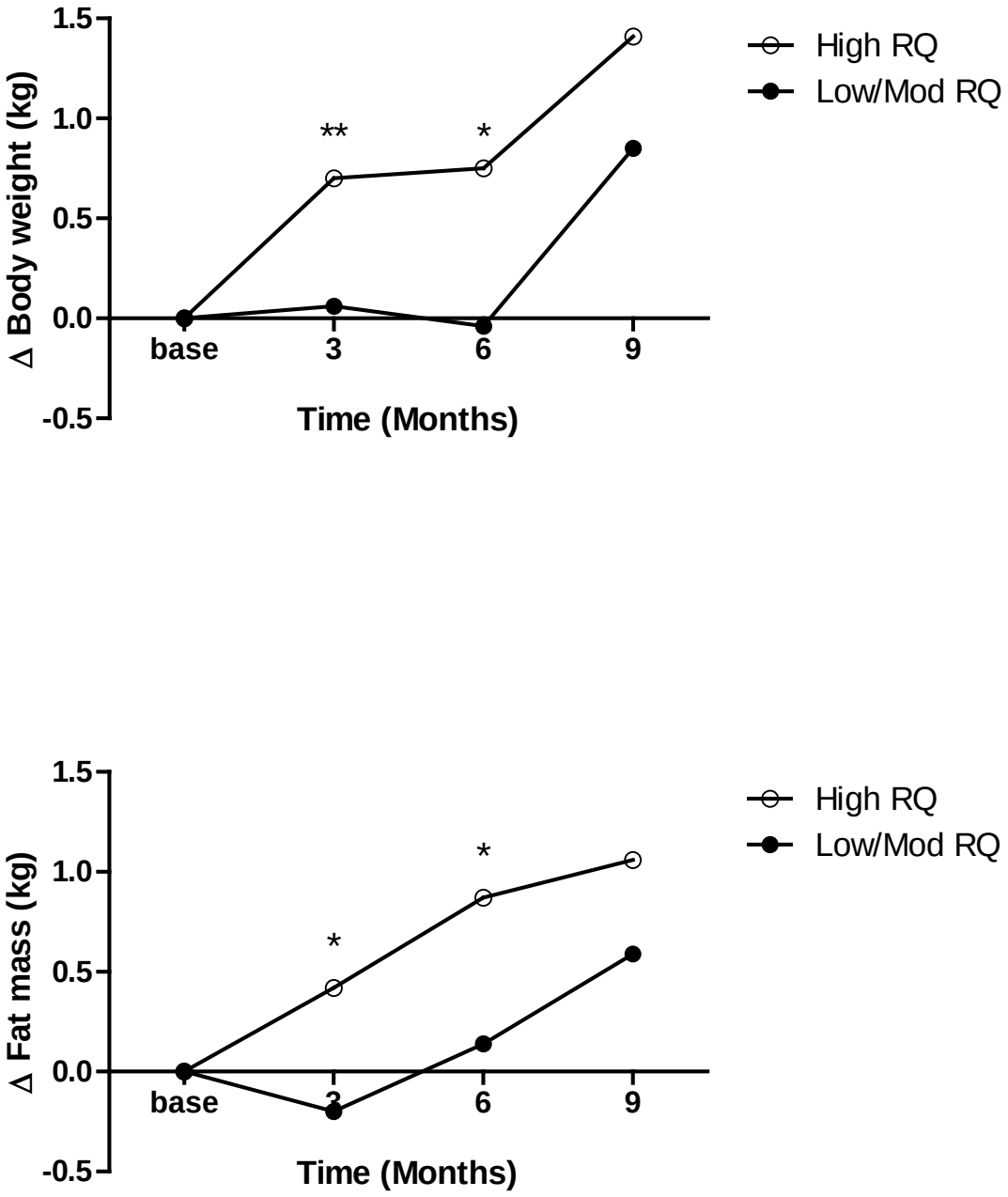

Figure 6.1. Unadjusted changes in body weight and fat mass at each time point for each group. Between group differences for each time point: * P<0.05, ** P<0.001.

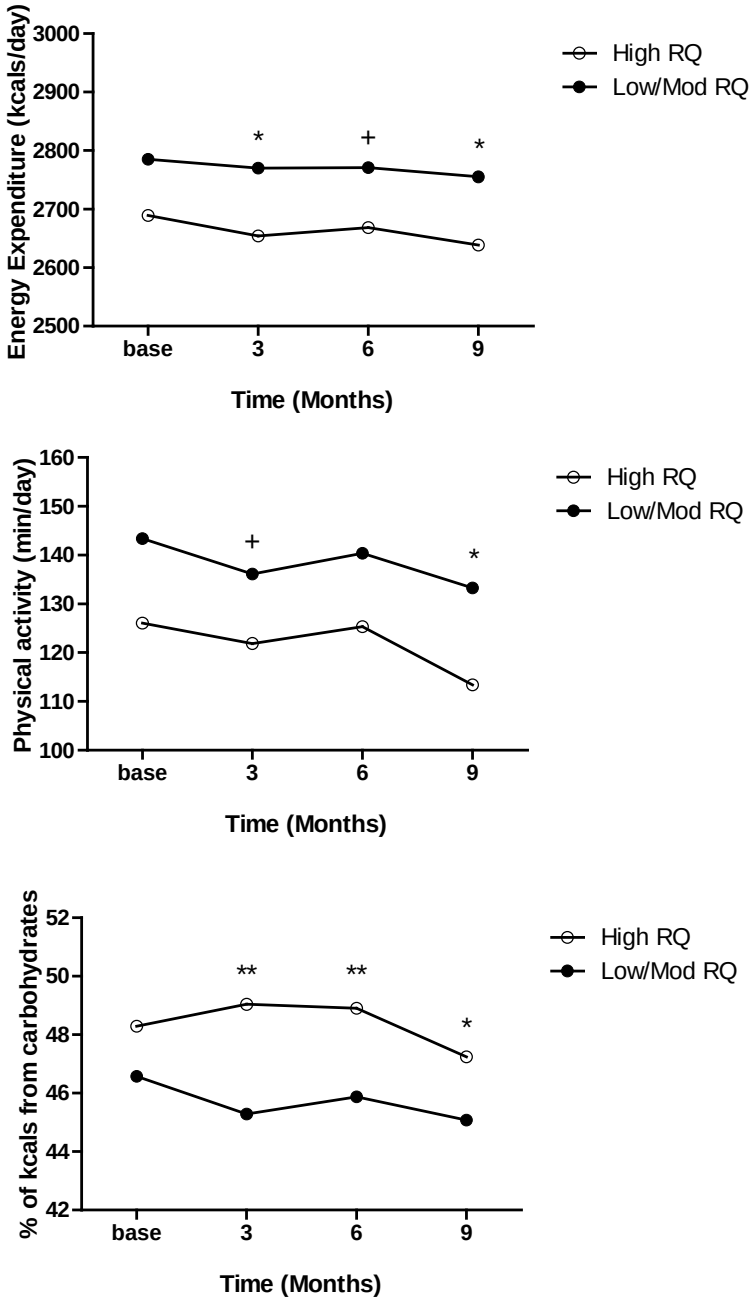

Figure 6.2. Changes in energy expenditure, physical activity, and carbohydrate composition of the diet at each time point for each group. Between group differences for each time point: + P<0.10, * P<0.05, ** P<0.001.

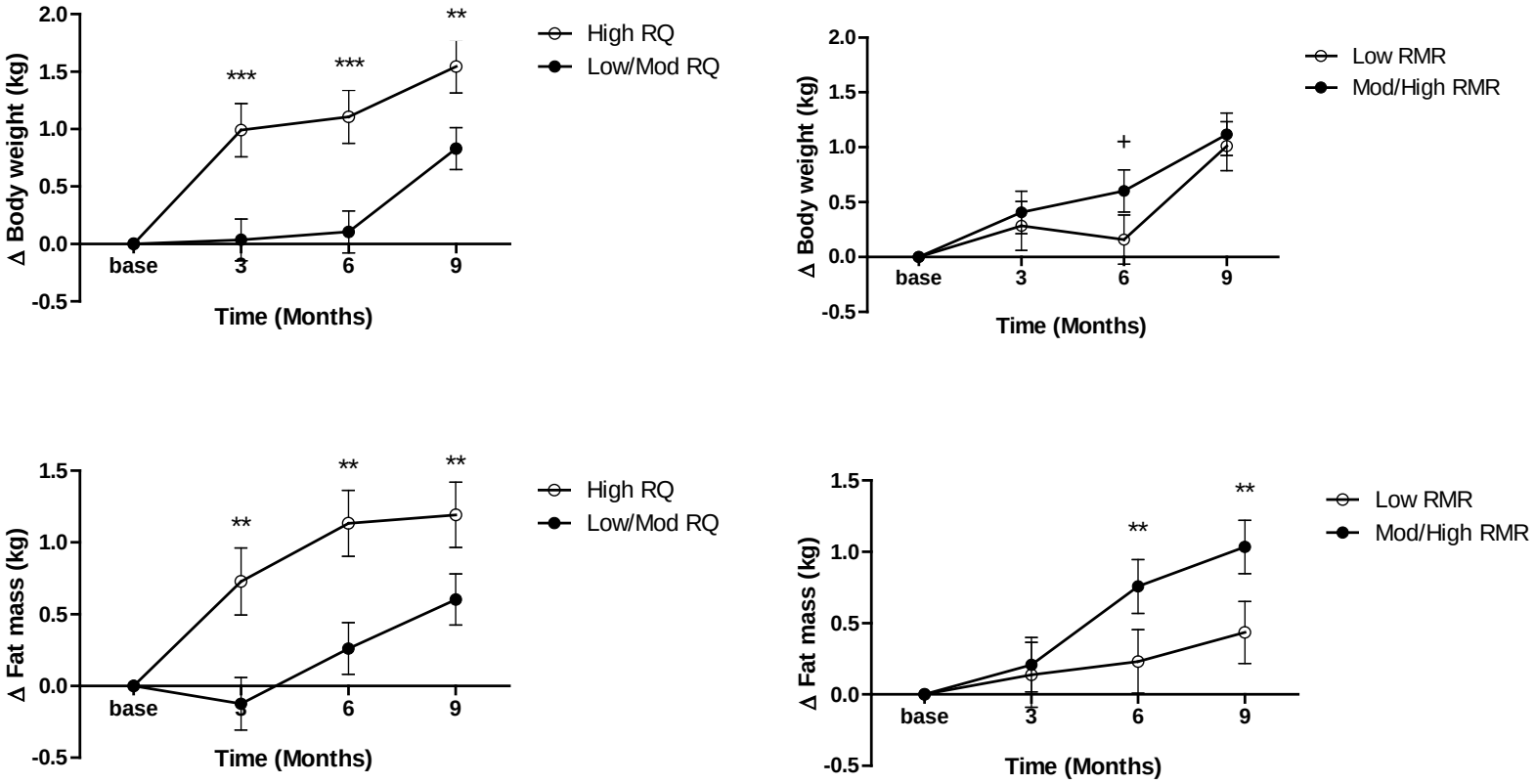

Figure 6.3. Adjusted changes in body weight and fat mass at each time point for each group. Between group differences for each time point: + P<0.10, * P<0.05, ** P<0.001.

CHAPTER 7

OVERALL DISCUSSION

While the current high levels of obesity and their associated health consequences are well documented, specific causes of the epidemic are subject to debate with most research focusing on increasing levels of energy intake or decreasing levels of energy expenditure through sedentary behavior. However, the causes of obesity are likely multifactorial and there is some evidence metabolic disturbances, such as low resting metabolic rate or reduced rates of fat oxidation, also are involved. The cause of these metabolic disturbances is not well established and the extent of their role in weight gain is unclear.

The purpose of this dissertation was to 1) Identify correlates of RMR among behavioral and physiological variables in a cohort of young adult men and women; 2) Examine racial differences in RMR, body weight, and body composition among young adult women; and 3) Explore the longitudinal effects of RMR, RQ, physical activity, and dietary intake on subsequent changes in body weight and body composition in young adults followed for nine months.

The three studies that form this dissertation were completed using data collected in The Energy Balance Study, a prospective observational study following young adults (N=430) for 24 months. The purpose of The Energy Balance Study is to examine the extent to which variation in total energy expenditure and variation in total energy intake

contribute to changes in body weight and fat among young adults. Extensive assessments of anthropometric and metabolic variables, in addition to estimations of energy intake and expenditure, were completed at baseline and every three months for the duration of the study.

Studies 1 and 2 consisted of cross-sectional analyses of baseline data. Univariate correlations were used to identify relationships between dependent variables and candidate independent variables. Generalized linear modeling was then conducted in which differences in body composition were statistically accounted for and adjusted mean values were calculated. Study 3 included baseline assessments of RMR and RQ followed by longitudinal monitoring of changes in body weight and body composition. A linear mixed models (LLM) regression random intercept growth model which allowed for unbalanced observations overtime was used to analyze the changes in body weight and fat from baseline through 9 months of follow up.

The primary results from this dissertation are the following:

1. Fit individuals have a higher RMR compared to those who are unfit. Differences in body composition, specifically skeletal muscle mass, residual mass, and fat mass, explained a large portion of the variability in RMR between fit and unfit individuals. Additionally, while time spent in moderate to vigorous physical activity was also significantly related to RMR and varied considerably between the groups, the influence was small and had little predictive value over adjustments for body composition.
2. Young adult African-American women have a lower RMR compared to their white peers after statistical adjustments for differences in body composition.

Higher levels of fat mass in AA compared to W resulted in elevated RMR beyond the differences in fat free mass. Additionally, CRF was significantly associated with RMR in the present study, but time spent in moderate to very vigorous physical activity was not.

3. High RQ is predictive of gains in body weight and fat mass over a 9 month period among young adults. Additionally, a low RMR was not associated with gains in body weight or fat mass over the same period. These findings support previous research which suggests that lower levels of fat oxidation, independent of changes in energy intake, energy expenditure, macronutrient composition of the diet, and physical activity, contribute to changes in body weight and fat mass.

These findings of this dissertation are important because they provide important information about the correlates of RMR, levels of RMR in a population at a high risk of obesity, and the role of RMR and RQ on longitudinal changes in body weight and body composition. Additionally, the role of CRF, physical activity, energy intake, and diet composition were explored in each study. The results from studies 1 and 2 suggest high CRF and physical activity play a statistically significant but modest role in elevating RMR. Findings from study 3 also low CRF and physical activity may be associated with reductions fat oxidation, ultimately resulting in gains in body weight and fat mass.

Several potential research questions have arisen based on the findings presented here. During studies 1 and 2 attempts were made to explain the inter-individual variance in RMR values, specifically by exploring CRF and physical activity levels. The influence of each of these variables on RMR was modest at best, but future research should explore

this relationship with more specificity, examining different levels of CRF and doses of physical activity rather simply high or low fitness or high or low physical activity. Of specific interest is the duration and intensity of physical activity on RMR. All studies included in this dissertation analyzed cumulative minutes of activity at moderate intensity or higher regardless of the duration of the bout. Future research should impose bout criteria (E.g. ten consecutive minutes of activity at >3.0 METs and ≤6.0 METs) to better answer questions regarding physical activity dose on RMR values.

Second, studies 1 and 2 were cross sectional in design so no causal effects can be determined from the findings, and study 3 was limited by a relatively short follow up period. Future research should include not only longitudinal assessment of body weight and composition but also measure trajectories of change in RMR, RQ, CRF, physical activity, and diet. For example, while it is thought that RMR and RQ is relatively stable within individuals with ±5% day to day variability, there is limited evidence suggesting wider variability long term, particularly in response to changes in diet, energy expenditure, and weight change. By assessing the trajectory of RMR and RQ in relation to changes in weight and other covariates we can better understand the dynamic and multifactorial etiology of obesity.

In conclusion, low levels of CRF are associated with lower levels of RMR in a population of young adults independent of levels of FFM. However, the decrease in RMR from low levels fitness compared to moderate or high levels of fitness is modest and represents approximately 3%. Young adult African-American women have a lower RMR compared to their white peers after statistical adjustments for differences in body composition. High levels of fat mass, often considered to be metabolically inactive,

resulted in elevated RMR beyond the differences in fat free mass. Finally, high RQ at baseline is predictive of gains in body weight and fat mass over a 9 month period among young adults, while low RMR was not.

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APPENDIX A

Participant Screening Chart

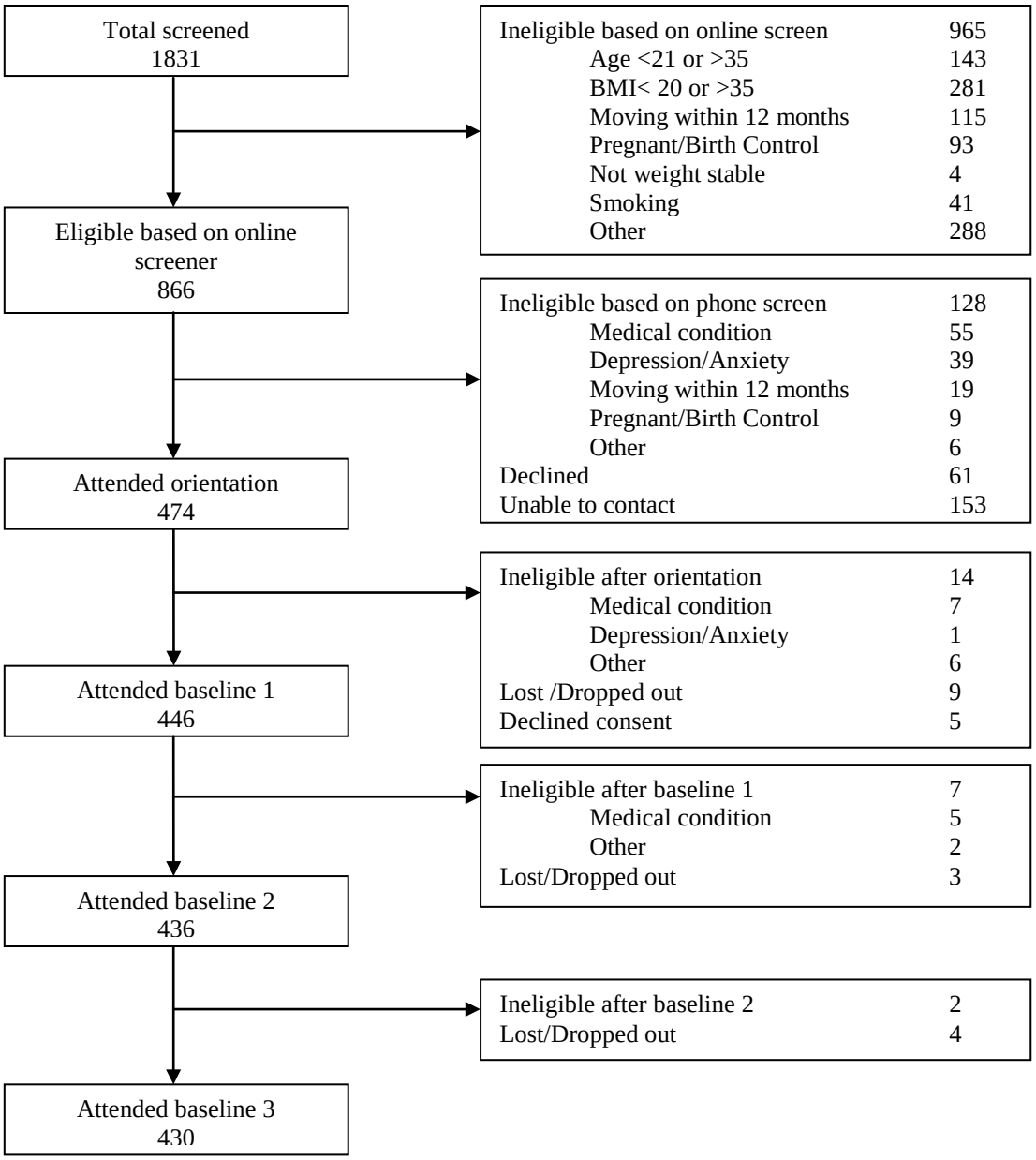

Figure A.1. Participant screening chart.