THE RELATIONSHIP BETWEEN DIET, BODY COMPOSITION, CARDIORESPIRATORY FITNESS AND RESPIRATORY QUOTIENT

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Abstract

Background: Respiratory Quotient (RQ) indicates the oxidation of fat or carbohydrate as a fuel source for the body during energy expenditure. Resting Metabolic Rate (RMR) and RQ are often determined during the same metabolic test, yet 73% is known of what explains RMR and none is known of what explains RQ.

Objective: This thesis aimed to partition the variance in RQ and explore the relationship between determinants thought to explain RQ, such as diet, body composition (fat mass (FM), skeletal muscle, and residual mass), and cardiorespiratory fitness (CRF).

Design: This is a retrospective cohort design using data from the Energy Balance Study including 430 participants, healthy men and women between the ages of 21-35. Partitioning of RQ was be measured by conducting a linear regression model.

Results: Only 4.8% of the variation in RQ was explained from the linear regression model including models for cardiorespiratory fitness, diet, and body composition. Of this explained variance, majority was explained by percent of total calories from carbohydrate (2.4%, $P<0.01$). 95.2% variance is still unknown about RQ.

Conclusion: This data confirms that percent of total calories from carbohydrate is a significant contributor to RQ. Although the variables of interest in this thesis are not all encompassing, groundwork has been set for future research endeavors to determine RQ's role in contributing information to personalized healthcare.

KEY WORDS: Respiratory Quotient, Respiratory Exchange Ratio, Diet, Body Composition, Cardiorespiratory Fitness, Fitness

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Chapter I: Introduction

Resting metabolic rate (RMR) was thought to be a strong determinant of weight gain over time but respiratory quotient (RQ) is found to be better (1). Beyond determining weight gain over time, not much else is known about RQ. RQ measures the utilization of macronutrients (carbohydrate and fat) for energy and typically ranges from 0.7 to 1.0 (2). RMR is similar to RQ in that it is measured when an individual is at rest in order to understand their metabolism more in depth (3, 4). RMR measures the number of calories burned at rest and has been studied to determine the variables that influence RMR results (4). RMR is 63% determined by fat free mass (FFM), 6% determined by fat mass (FM) and 2% determined by age (5). Variables such as height, sex, and bone mineral content were not significant. Another study expanded these results by discovering that fitness also plays a role, determining approximately 4% of RMR (6). Similar to RMR, an investigation on which variables significantly determine RQ must be done in order to fully understand RQ.

Most of the research has been done on the relationship between diet and RQ (2), specifically high fat diets (7-9), and found that RQ is strongly influenced by the fuel substrate primarily consumed (3). Studies are consistent in identifying that a high fat diet will yield a lower RQ compared to a high carbohydrate diet (3, 9, 10).

Less is known about the relationship between body composition and RQ. Previous research has focused on how RQ is associated with weight status over time (11). Body composition represents total lean mass and total fat mass. Higher fat mass may indicate better oxidation of fat as fuel, thus impacting RQ.

Lastly, cardiorespiratory fitness (CRF) has not been explored. It is well known that different exercise intensities will cause the body to utilize different fuel sources (12). For

example, higher intensity exercise greater than or equal to 75% VO_{2max}, will result in using more carbohydrate for energy than fat (12). However, it is less understood if CRF influences RQ at rest. The study mentioned above analyzed the impact of CRF on RMR and determined that there was a significant ($P<0.05$), positive relationship between CRF and RMR (6). Given the relationship between the two, it is important to analyze the relationship between CRF and RQ to determine if the same relationship exists. CRF and RQ is an area of research that must be further explored to fully understand the significance of RQ.

The body of literature to examine the relationships between diet, body composition, CRF and RQ is insufficient. A clear pattern is lacking; however, we can see several areas emphasized, such as diet. Several of the studies agree with one another; while, several are completely independent.

Therefore, the purpose is to investigate how significantly diet, body composition, and CRF determine RQ. Given that the majority of literature is focused on diet and RQ, it is logical to assume that diet composition will be the greatest determinant of RQ. Body composition is likely to be a strong determinant of RQ; FM and FFM were both strong predictors for RMR (5), therefore they may also strongly influence RQ. Lastly, CRF's influence on RQ at rest has very little evidence to support that it will be a strong determinant of RQ; however, the relationship remains unknown. Thus, leading us to the current research question: What is the relationship between diet, body composition, CRF and RQ? This question will support not only future research questions surrounding RQ but will establish a foundation to further understand the relationship between all factors and RQ and how we may use RQ to individualize healthcare recommendations.

Chapter II: Literature Review

Personalized Healthcare and Metabolic Tests

The prevalence of overweight and obesity has increased over the past 25 years (13, 14). With obesity there is an increase in weight gain which is mostly seen as adipose tissue (15). Obesity is linked to an increased risk of developing chronic diseases such as type 2 diabetes and hypertension (16). An increase in adipose tissue impacts metabolic flexibility, otherwise known as the ability to efficiently use fat and carbohydrate as a fuel source (17-19). Respiratory Quotient (RQ) measures the utilization of macronutrients and typically ranges from 0.7 to 1.0 with 0.7 representing 100% fat use and 1.0 representing 100% carbohydrate use (2, 3). RQ is greatly associated to metabolic flexibility as this represents which substrate, carbohydrate or fat, will most readily be oxidized for energy (17). Healthcare interventions targeting the outcomes of specific metabolic tests, such as RQ, may lead researchers to new treatments for preventing increased adiposity. Interventions developed to reduce increases in adiposity focus mostly on diet and lifestyle changes (i.e. increased physical activity) (20, 21). Understanding which variables, and to what extent they influence RQ, will help health professionals and researchers better understand this RQ's influence on adiposity and determine interventions to improve overall health of an individual.

Resting Metabolic Rate and Respiratory Quotient

To determine the potential variables influencing RQ, we must first look to what has already been investigated. Resting Metabolic Rate (RMR) is a similar metabolic test to RQ and has been investigated for the variables that influence its results (5). RMR represents the number of calories burned while performing normal physiological functions such as breathing or brain activity (3). RMR is the largest contributor of total energy expenditure in humans, comprising

approximately 60-80% of total energy expenditure (3). When measuring RMR, a 4 hr. fast is required and the person is in a rested position (4). Basal metabolic rate (BMR) and RMR are typically used interchangeably, although, BMR is the amount of energy expended to sustain life, i.e. respiration, heartbeat, renal function and blood circulation (4). BMR is measured after a 12 hr. fast in a supine, motionless position (4). Variables known to affect RMR include FFM, FM, age, and cardiorespiratory fitness (CRF) (5, 6). The extent to which these variables explain RMR is still being studied. However, current literature shows that FFM explains 63% variance, FM explains 6%, age explains 2%, and CRF explains 4% of the variation of RMR between persons (5, 6).

Assessment of whole body energy metabolism by indirect calorimetry is a tool used to understand energy homeostasis in humans (22). Understanding the results of metabolic tests is important to individualize health care and understand whole-body energy metabolism. RMR is a metabolic test that allows healthcare providers to give personalized health recommendations to patients. RQ is a metabolic test whose results are not well understood and therefore healthcare providers are unable to use its results in providing personalized care to patients. RMR and RQ are often determined during the same metabolic test, however it is better understood the determinants of RMR rather than RQ (5, 6, 23, 24). It is logical to assume that some of the variables that affect RMR may also affect RQ. Variables of interest include diet, body composition (FFM, FM), and fitness. The purpose of this literature review is to determine to the relationship between diet, body composition, CRF, and RQ. This question conveys a particular importance when rising obesity and chronic conditions are considered.

Differences Between RQ and RER

Similar to BMR and RMR, RQ is commonly interchanged with respiratory exchange ratio (RER). RQ and RER are used to determine the volume of CO_2 produced over the volume of O_2 consumed by the body ($\frac{VCO_2}{VO_2}$). They are equivalent at rest, however, there are two distinct characteristics that cause RQ and RER to differ. RQ is the ratio of metabolic gas exchange at the cellular level, and RER includes measurement of CO_2 produced as a result of buffering (25). In other words, RQ is measured at the cellular level, and RER measures the air expelled.

Since RQ is determined at the cellular level, its measurement requires invasive procedures, such as inserting a catheter through the venous or arterial veins (26). RER is measured at the mouth; therefore, it does not require invasive procedures, but does require equipment such as a metabolic cart (25). RER at rest is a strong predictor of RQ but does not accurately reflect RQ when the body is past the ventilatory threshold, either during strenuous exercise, or during hyperventilation (25). RER reflects the same ratio of substrate being used for energy as RQ (0.7 – 1.0); however, RER can exceed 1.0 unlike RQ due to the extra CO_2 being measured during exhalation. For the purpose of this literature review, RQ is the variable in question, therefore only studies measuring RER at a rested state will be included.

The Relationship Between Diet and Respiratory Quotient

The macronutrient composition of food intake affects how the body utilizes energy. RQ is reported to be strongly influenced by the intake of fat and carbohydrates (2). A diet primarily composed of carbohydrates will cause RQ be closer to 1.0 (3). This occurs because of the ratio of hydrogen to oxygen atoms in a glucose molecule. The complete oxidation of glucose yields 6 CO_2 and uses 6 O_2, thus, $\frac{6CO_2}{6O_2}$ yields a RQ of 1.0 (27). A diet that includes an equal ratio of fat and carbohydrates will have a RQ near 0.8-0.85 (26). Lipids on the other hand is generally found

with an even number of carbons (27). For example, Palmitic Acid which has a carbon chain of 16 carbons. With the oxidation of Palmitic acid, 16 CO_2 is produced and 23 O2 is used. Thus, $\frac{16CO_2}{23O_2}$ yields a RQ of 0.7. Fat oxidation relates to the amount of fat being used to provide energy (7). A high fat diet is negatively correlated with RQ (7-9), whereas a high carbohydrate diet is positively correlated with RQ (10, 28, 29).

One unbalanced meal caused a shift in RQ. Twenty-nine healthy men and women aged 55 to 75 years old were randomly assigned to either a high fat (HF) or a high carbohydrate (HC) breakfast (7). The objective of this study was to determine the effect of consumption of either a HF or a HC breakfast for 4 weeks on daily substrate utilization determined by RQ. The HF breakfast consisted of 45%, 35%, and 20% fat, carbohydrate, and protein, respectively; the HC breakfast consisted of 20%, 60%, and 20% fat, carbohydrate, and protein, respectively; lunch and dinner were neutral with 30% fat, 50% carbohydrate, and 20% protein. A HF breakfast increased fat oxidation throughout the 24 hr. day; however, those who consumed the HC breakfast showed a decrease fat oxidation, resulting in a higher RQ. Therefore, we see the effect that one unbalanced meal has on RQ. Although this study shows a relationship between high fat meals and fat oxidation, these results are only in acute settings. Longer term studies are needed to determine the role of macronutrient composition of meals on RQ.

Six healthy men were studied three times, each time after 7 days of a different diet with 8-10 weeks between the beginning of a new experiment where they consumed their habitual diet (8). The high fat diet consisted of 83% of energy from fat and 2% from carbohydrates, the high carbohydrate diet consisted of 85% of energy from carbohydrate and the control diet consisted of 44% of energy from carbohydrate and 41% from fat. RQs were 0.82 after 7 days following the high carbohydrate diet, 0.78 after the control diet, and 0.72 after the high fat diet ($P<0.05$).

Whole body fat oxidation was increased by 47% on the high fat diet as well. The composition of the high fat diet is considered ketogenic; therefore, the macronutrient composition may have led to overestimation of the amount of fat needed to influence RQ. To fully understand where this shift in macronutrient composition's influence on RQ takes place, diets less extreme in composition and longer in duration are needed.

Longer term high fat diets (>2 weeks) reduce and maintain a low RQ according to one study (30). Thirty-two healthy females were divided into two groups. The first group followed a ketogenic diet for 20 days with a macronutrient distribution of 43% protein, 14% carbohydrate, and 42% fat. The control group followed a Mediterranean-style diet with a balanced macronutrient distribution of 15% protein, 60% carbohydrate, and 25% fat. After 20 days of the ketogenic diet, a significant reduction in RQ from baseline was evident ($P<0.01$). This group of women then followed a modified ketogenic diet for an additional 20 days comprised of 27.5% protein, 34% carbohydrate, and 38% fat. After 20 days of this modified diet, the women's RQ remained significantly lower from baseline. Therefore, a high fat diet followed for 20 days may significantly reduce RQ and maintain that RQ for an additional 20 days. A ketogenic diet is defined as very high-fat, low-carbohydrate diet that restricts carbohydrate to ≤10% of total calories (31) Despite not being a true ketogenic, or a true modified ketogenic diet, we still see that high fat has an impact. Therefore, the effects of high-fat diets are seen to be inversely correlated with RQ.

The Relationship Between Body Composition and Respiratory Quotient

Higher FFM contributes greater energy expenditure than FM (5); therefore, it is likely that body composition may also affect RQ. To the author's knowledge only two studies have investigated the effects of body composition (FM and FFM) on RQ (32, 33). A cross-sectional

study compared Asian men and women's body composition, total energy expenditure, and RQ to American men and women (32). After comparing Asians to white Americans, Asian males and females had on average 5% more FM than white Americans. Total energy expenditure and RQ were calculated over 24 hr. in a respiratory chamber. Despite a difference in body fat percentage in participants, fat oxidation did not differentiate RQ at rest (RQ Americans = 0.92, Asians = 0.91). Another study comparing body compositions among participants also noted similar findings that FM may not influence RQ.

When comparing lean and obese individuals, but including a dietary component, FFM appeared to effect fat oxidation (33). Low fat and high fat groups were determined by self-report of the participants' habitual diet. Diets were considered low fat if <30% of calories came from fat, or high fat if >40% of calories came from fat. Of the thirty participants, 10 were in the lean (BMI <25kg/m^2) low fat (LLF) group, 10 in the lean high fat (LHF) group, and 10 in the obese (BMI >30kg/m^2) high fat group (OHF). There were no significant differences in RQ between groups, but the LHF and OHF groups were more efficient at fat oxidation than the LLF group. The individuals in the LHF and OHF group had an RQ of 0.91 and 0.94 respectively, while the LLF group had an RQ of 0.96. The LHF and OHF groups are more efficient at oxidizing fat as a fuel substrate potentially causing their RQ to be lower compared to the LLF group. The authors of this study speculated that the LHF participants had a greater capacity for fat oxidation, which may be protective against weight gain; whereas the OHF participants had a blunted appetite hormonal response to the high-fat test meal which may have increased their energy intake. Although both the LHF and OHF groups were more efficient at oxidizing fat, their weight is attributed to other causes unrelated to their FFM or FM. This creates speculation in the role of body composition and its influence on RQ.

Other studies focused specifically on FFM and found it may have a significant role in fat oxidation (33, 34). Whole-body peak fat oxidation was investigated in trained compared to untrained individuals (34). Sixteen young, healthy male participants were divided into trained and untrained groups based on their VO_{2max} kg^{-1}, body mass, and training habits. The trained group had significantly more FFM (kg) and a significantly higher VO_{2max} compared to the untrained group. The trained group also had higher whole-body peak fat oxidation and at higher relative exercise loads than the untrained participants. Since fat oxidation is positively correlated to FFM, the difference in FFM between the trained and untrained groups may play a significant role in the participants' whole-body peak fat oxidation and therefore RQ. Although these findings were measured by means of determining RER while the individuals were exercising, these findings present a relationship between FFM and RQ that is important to understand. In this study, FFM and CRF are related in terms of trained individuals (higher fitness) who have more FFM than the untrained group. FFM's relationship to RQ is seen during exercise when muscles are at work; therefore, RER is determined during exercise. Here it is seen that fat oxidation is in fact impacted by individuals of different fitness levels and FFM.

Previous literature on the predictors of RQ is limited because much of the research has focused on explaining the relationship between RQ and body composition prediction. A 10-year observational study shows a higher RQ (i.e., greater carbohydrate oxidation) was related to greater weight gain (11). 775 men aged 18-98 participated in this study. RER was measured by indirect calorimetry at rest on their first visit and related to subsequent weight change. This study found a higher RQ is linked to more prospective weight gain compared to those with a lower RQ. Those with a resting RER $\geq$0.85 had 2.4 times higher risk of gaining at least 5 kg compared to those with an RER <0.76. Due to the public and current medical interest in preventing or

reducing obesity, the topic of RQ's effect on body composition is of much more interest than the effect of body composition on RQ; however, in the light of the current obesity epidemic, it may be beneficial to examine how body composition predicts RQ due to the relationship in weight gain.

The Relationship Between Cardiorespiratory Fitness and Respiratory Quotient

As previously discussed, comparing trained and untrained individuals (34), the quantity of FFM may also be dependent upon fitness level. Cardiorespiratory fitness (CRF), is a measure of the maximum amount of oxygen that an individual can use during vigorous physical activity (2). Less strenuous activity (<85% VO_{2max}) yields a lower RQ indicating fat is primarily oxidized, while more strenuous activities have a higher RQ indicating carbohydrates are primarily oxidized (12). CRF is measured by a fitness test to determine the maximal oxygen uptake during exercise (VO_{2max}) (2). Measuring VO_{2max} requires activation of large muscle groups typically required for running, cycling, or climbing, therefore this test is typically conducted using a treadmill or cycle ergometer (2). CRF is an important metabolic test as it is been positively associated with insulin sensitivity and, when adjusted for FFM, it is negatively associated with metabolic syndrome (35, 36).

Different levels of activity affect RQ during exercise (12), but it is unknown if CRF plays a role in RQ at rest. CRF in 423 young adults was determined in a cross-sectional study (37). Participants with lower CRF had a higher RQ (RQ=0.80) compared to participants with higher CRF (RQ=0.79). Although the difference is small, its significance ($P<0.01$) increases speculation that baseline CRF, in addition to the level of exercise intensity, may drive which fuel substrate is being used.

Substrate utilization during exercise was examined in eight male cyclists while exercising at different VO_{2max} intensities. Increasing the exercise intensity to 75% VO_{2max} resulted in a decrease in total fat oxidation and an increase in total carbohydrate oxidation compared to other intensities (12). Therefore, varying levels of intensity allow for different substrates (carbohydrate or fat) to be oxidized for energy. Metabolic flexibility is one proposed concept to explain the mechanism between CRF and RQ.

It is important to understand how metabolic flexibility and RQ are related. Metabolic flexibility is the ability to respond or adapt to acute changes in nutrient availability and energy demand and may be represented by RQ (22). Insulin plays an important role in metabolic flexibility as it directs which fuel is oxidized for energy (17). When insulin levels are high, fat oxidation is down-regulated, and carbohydrates are the primary source of energy (17). During exercise, muscle becomes more sensitive to insulin, therefore we see that exercise impacts insulin levels and perhaps fuel selection as well (17). An example of reduced metabolic flexibility or, metabolic inflexibility, is presented in those with type 2 diabetes and obesity (17). RQ defines the preferred fuel substrate of an individual, while metabolic flexibility determines how well multiple fuel substrates can be used (18). Metabolic flexibility is not being studied in this thesis, but provides context of how diet, body composition, and CRF may impact each other's relationship to RQ.

The Significance of Understanding Respiratory Quotient

Current literature on RQ has taken a one variable at-a-time approach, such as "the effect of diet on RQ" or "the effect of FM on RQ" where only one variable is being investigated for its relationship to RQ. Research investigating multiple factors and their impact on RQ has yet to be done. This thesis project will lay the foundation for further research exploring multiple variables

and RQ. It is important to understand RQ as it is an important metabolic indicator that may help better understand whole-body metabolism and individualize healthcare practices such as exercise regimens and diet.

The relationship between diet and RQ show the most consistent results, while the results from body composition and CRF vary more frequently; however, it is evident that each variable discussed can be manipulated through lifestyle efforts and does, in some capacity, influence RQ. Lifestyle changes and their influence on RQ could potentially negate certain risk factors for developing preventable chronic diseases.

It is evident that diet may affect substrate energy utilization (7-10, 28, 29). Findings between diet composition and RQ suggest that an individual meal has the effect of influencing substrate utilization throughout the remainder of the day (7). What is still unknown is at what percent of total calories the shift in RQ begins. Through examining other articles on diet and RQ, it is evident that diet composition does not have to be as extreme as 83% of total calories from fat to see a significant change in RQ (7, 30).

The relationship between body composition RQ are not clearly understood. Parameters of body composition beyond FM and FFM must be studied in order to learn more about its role in determining RQ. FFM is an area to further explore as it is shown to be positively correlated with fat oxidation (34). Additionally, the quantity of muscle (34, 38) as well as how the body performs during exercise can influence RQ (12).

RQ not only determines which fuel substrate is best used by the body but also impacts metabolic flexibility which may contribute to high or low fat and carbohydrate oxidation during activity and at rest (17). Exercising at different intensities determines the metabolic energy

selection between glucose and fatty acids (17); although, the composition of FFM may be the pivoting factor between metabolic flexibility and determining of RQ.

Discussion and Conclusion

The presented studies show variety in study design and number of participants. A strength of many studies is that RQ and RER were estimated by using a metabolic chamber and/or method of indirect calorimetry, providing consistency between methodology. For the studies of fewer participants, their designs were either crossover studies, or had multiple arms allowing for results to be compared to a strong control group.

Although strong in design, the number of participants is also a limitation in translatability of findings. With the exception of a few longitudinal studies, most had less than 50 participants. This low number reduces the opportunity to generalize and make strong assumptions. Other limitations resided within the methods used to determine diet composition, exercise intensity, and analyzing multiple measurements of body composition. Diet composition varied in most of these studies with some high fat diets consisting of 45% total calories from fat, and others being as high as 83%. With this variation in composition, it becomes challenging to pinpoint when the shift in RQ occurs. Body composition was determined using different methods, i.e. DXA scan, infrared reactance, or a 3-compartment calculation. Without standardizing body composition measurements, conclusions may vary significantly, and little will be learned about the significance of body composition on RQ. Current literature that has analyzed the relationship between CRF and RQ are characterized by limitations in methodology such as they measure CRF and RER during exercise rather than at rest; however, these studies were not referenced in this literature review. Due to the significant limitations in these studies, more research needs to

be done in the area of CRF in order to make more specific assumptions about fitness and RQ. With more studies, the range of factors involved in RQ can become more apparent.

Findings over the last 30 years have improved upon the understanding of RQ as well as remained in congruence. Studies focusing on diet and RQ have similar findings that there is an inverse relationship between RQ and percent of total calories from fat. There are not enough studies focused on the relationship between body composition, CRF and RQ; therefore, it is not yet known if a pattern exists.

RQ may be influenced by factors beyond diet, body composition, and CRF. In addition, RQ is being studied more in depth for its role in metabolic flexibility. RQ is a metabolic test that may have a stronger influence in determining preventative health strategies than is recognized. Conclusions being made about RQ and the variables discussed, bring clinical relevance to dietitians, physicians, athletic trainers, and other health professionals. To determine the amount of influence each of these variables has on RQ, variables such as diet, body composition and CRF, there will need to be more studies with standardized methods and a higher number of participants to make generalized claims.

Chapter III: Methods

Overview

The purpose of this thesis was to determine to the relationship between diet composition, body composition, CRF, and RQ. Data was provided by the Energy Balance Study (EBS) (1). The EBS was a 12-month study with 430 participants to determine the association between caloric intake and energy expenditure on body weight and composition.

Inclusion and Exclusion Criteria

Participants in the EBS were healthy women and men aged 21 to 35 years old with a BMI between 20-35 kg/m^2. Stratification by age group took place to recruit an equal amount of men and women within the age range of 21 to 35 years old. Men and women were stratified by the ages 21 to 27 and 28 to 35. Participants must have had access to a telephone for receiving diet recall interviews and be available to participate in the study for 15 months.

Exclusion criteria consisted of no acute or chronic conditions in order to select only healthy individuals. No large changes in health behaviors were allowed to take place in the previous months leading up to the start of the study. Exclusion criteria also included use of weight-loss medications, a change in smoking status in the past 6 months, or planned weight loss surgery. Individuals with a resting blood pressure exceeding 150 mmHg systolic and/or 90 mmHg diastolic were also excluded, as well as those having an ambulatory blood glucose level of 145 mg/dl or greater. Those were excluded if currently diagnosed with, or taking medications for chronic health conditions, if diagnosed with, or a history of major depression, anxiety disorder, or panic disorder, if taking selective serotonin inhibitors, or pregnancy or planned births in the previous 12 months. Lastly, given the cyclical changes in body water and potential changes in appetite associated with contraceptive medications, only women planning to begin or

to stop birth control during the 12month observational period were excluded. Temporary exclusion applied for those who had begun or changed their birth control regimen in the last 3 months.

Design

An extensive baseline assessment took place at the beginning of the study and was repeated quarterly. An orientation session was first held for all eligible participants. This visit included a 25 min. presentation describing the study protocols and all associated expectations for the participant. Height and weight were also taken at this visit to verify BMI. All anthropometric measurements were taken while the participant was barefoot and dressed in scrubs. The average of three heights and weights were taken using a traditional stadiometer and electronic scale and recorded to the nearest 0.1 centimeter and 0.1 kg respectively. After the orientation, 3 additional visits were performed over the following 3 weeks to gather baseline measurements.

The purpose of visit 1, following the orientation, was to complete demographics, psychometrics, and activity recall questionnaires for each participant. Visit 2 included the following measurements: resting blood pressure, height, weight, waist and hip circumference, body composition via dual x-ray absorptiometry, and a maximal fitness test using a modified Bruce protocol with 12-lead electrocardiogram (ECG). Visit 3 consisted of height, weight, a blood draw, and a resting metabolic rate test while the participant was 12 hrs. fasted and had abstained from physical activity in the past 24 hrs.

Dual energy X-ray absorptiometry (DXA) was used to measure total fat and lean tissue mass. The scans were completed with a Lunar fan-beam DXA scanner (GE Healthcare model 8743, Waukesha, WI). Measurement of body composition using a DXA scanner has been validated and is an accurate technique (39, 40). Diet was analyzed for estimates of energy and

nutrient intakes and was calculated using the Nutrient Data System Research software (NDSR Version 2012) from self-report. This information was collected using multiple telephone-administered 24 hr. recall interviews. This method is not devoid of error but is considered an imperfect "gold standard" for estimating dietary intake (41). Participants received three 24 hr. dietary recalls on random days, 2 weekdays and 1 weekend day, conducted by licensed dietitians. CRF was determined using the validated Modified Bruce graded exercise test (GXT) (42-44) and a treadmill (Trackmaster 435; CareFusion Corp), with respiratory gases sampled using a TrueOne 2400 metabolic measurement cart (ParvoMedics). This test begins at a speed of 1.7 mph at 0% grade for 2 min. then progresses to 1.7 mph. at 5% grade for 3 min. After this stage the protocol is identical to that of the Bruce Protocol. The Modified Bruce Protocol was used for its lower initial intensity to fit a generally deconditioned population. Research has shown a high correlation between the Modified Bruce and the Bruce protocols for peak VO_{2max} measurements (45). This GXT was conducted by a trained exercise physiologist who prepared eligible participants for the test. All participants exercised to volitional fatigue followed by continued walking at a slow pace until heart rate and blood pressure returned near baseline levels. Criteria for a maximal test included two of the following variables: a heart rate plateau or a VO_{2max} plateau with increasing workload, a RER $\geq$ 1.15, and a rate of perceived exertion (RPE) $\geq$ 17 using the Borg scale of perceived exertion.

RMR and RQ

RMR and RQ were determined for each participant using standard indirect calorimetry with a ventilated hood and an open-circuit system, TrueOne 2400 Metabolic Measurement Cart (ParvoMedics, Salt Lake City, UT, USA), following a standardized protocol (46, 47). The metabolic cart was calibrated before each test using known gas concentrations and volumes as

recommended by the manufacturer. A 15 min. resting period preceded a 30 min. data-collection period in the supine position; All measurements occurred in the morning (before 9:00am) following a 12-hr. dietary fasting state and at least 24 hrs. after the last bout of any exercise. Participants remained quiet throughout the entire procedure and were awake with continuous monitoring; The room as maintained in low light and noise was kept at a minimum. RMR was determined as the average value of 10 consecutive minutes with the lowest coefficient of variation. RMR was calculated from O_2 consumption and CO_2 production measured continuously during the procedure with a constant airflow rate into the ventilated hood. Airflow rate was based on body weight with a goal of maintaining the fraction of end tidal CO_2 between 1.0 and 1.2% (L/min). The flow rate was set to not exceed 33 L/min for a person weighing 68 kg or 40 L/min for a person weighing 91 kg. RQ was calculated as $\frac{VCO_2}{VO_2}$ (46, 48, 49).

Ethics

All study protocols were approved by the University of South Carolina Institutional Review Board. All measurements were obtained by trained research staff members who demonstrated competency and were certified in each specific measurement technique required. Participants received $500 for completing the study, as well as a report with their collected information. Participants were able to schedule a one-on-one counseling session with a member of the research staff to review the information collected on them.

Data Analysis

Participant characteristics were based on demographic and physiologic measurements. Means and standard deviations were used for continuous and categorical variables and percentages. Linear regression models were used to determine the variance between variables comprising RMR. Initially the model was adjusted by race, age, and sex. Subsequently skeletal

muscle mass, residual mass, FM and bone mass were included. Additional models were created to include all previously mentioned covariates as well as (1) time spent in sedentary, light, moderate, vigorous, and very vigorous activity, (2) time spent in moderate to vigorous physical activity (MVPA), (3) energy intake, and (4) the macronutrient content of the diet (i.e. kilocalories and the percent of daily kilocalories from carbohydrates). Linear modeling results were presented as least squares means with standard errors. Analysis of covariance was used to compare RMR among low-, moderate-, and high-fitness groups. Participants were classified as low if they were in the bottom tertiles for CRF among the entire cohort for each sex. If in the middle tertile they were considered moderate CRF, or if in the upper tertile they were considered high CRF. Statistical significance was defined as $P \leq 0.05$ (2-tailed). SAS software, version 9.3 was used for all computations.

Secondary data analysis performed for this thesis consisted of participant characteristics based on demographic and physiological measurements and conducted with means and standard deviations as mentioned above. One linear regression model analyzing covariance, similar to the model described in the study above, was used to explain the variances between variables influencing RQ. Initially the model was adjusted by race, age, and sex. Subsequently, models included percentage of calories from carbohydrates, protein, and fat, skeletal muscle mass (kg), residual mass (kg) (residual mass= body weight – FM – skeletal mass – bone mass) which includes brain, liver, kidneys, heart, gastrointestinal tract, and other organs and tissues, and FM (kg). 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 women and 1 for men. This equation was validated with ethnically diverse men and women using magnetic resonance imaging and DXA (50). Skeletal muscle mass and residual mass are both highly metabolic parts of the body that may explain the variation in different metabolic processes relationship to RQ. Additionally, analysis was used to compare RQ among the low-, moderate-, and high-fitness groups, given by tertiles explained above, after adjustment for covariates as was done in the EBS. Statistics were conducted using SAS software, version 9.4 with statistical significance defined aa $P \leq 0.05$. It is hypothesized that diet will have the greatest influence on RQ, followed by body composition (FM, skeletal muscle mass, and residual mass) then CRF.

Chapter IV: Results

The purpose of this thesis was to determine the relationship between diet, body composition, cardiorespiratory fitness (CRF), and RQ. All tables and figures are given in the section labeled Tables and Figures. Participants were separated into tertiles by sex based on their fitness level.

Group Characteristics

Table 1 demonstrates participant's characteristics by group. Weight and BMI were statistically significant between groups ($P<0.001$). However, the groups did not differ based on age, height, bone mass, or residual mass. Groups were statistically significantly different from each other in terms of percent of fat mass ($P<0.001$), with group 1 having the highest percent fat mass and group 3 having the lowest. Percent of skeletal mass was also statistically significant between groups with group 3 having the highest percent skeletal mass followed by group 2 then group 1 ($P<0.001$).

Metabolism, Expenditure, and Intake

Table 2 demonstrates expenditure and metabolism by group. Group 1 had the highest RQ (0.801 ± 0.050), followed by group 3 (0.785 ± 0.045), then group 2 (0.788 ± 0.043). Differences in VO_{2max} among the three groups were statistically significant ($P<.001$). Group 1 is low CRF (29.8 mL/kg/min), Group 2 is moderate CRF (38.1 ml/kg/min), and Group 3 is high CRF (47.4 ml/kg/min). Group 3 had the highest total daily energy expenditure, which was expected as group 3 is the highest fit group; however, this was not statistically significant ($P=0.13$). In addition, figure 1 and table 3 show group 1's diet was highest in percent of total calories from carbohydrates (49.1%) with group 2 consuming 46.5% total calories from carbohydrates and

group 3 consuming 45.9% total calories from carbohydrates (P=0.02). Percent of total calories from fat were 31.9%, 33.4%, and 33.2% for group 1, group 2, and group 3 respectively (P=0.19).

Variables Determining RQ

Table 4 shows the linear regression model, separated by fitness groups. Unadjusted, the RQs were statistically significant between groups 1 and 2 (P=0.04) and 1 and 3 (P=0.01). However, RQ was not statistically significantly between groups 2 and 3 (P=0.90). CRF explained 2.1% variation in RQ. Age, race and sex had no impact on RQ and the significance between CRF groups remained the same as the unadjusted RQ model. Once percent of total calories from carbohydrates was added to the model, an additional 2.4% variation was explained. At this point, RQ was no longer statistically significant between any of the CRF groups for the remainder of the model. Percent of calories from protein did not impact RQ; however, percent of calories from fat explained 0.1% variation in RQ. Skeletal muscle (kg) and residual mass (kg) explained 0.2% variation in RQ. Overall, as seen in figure 2, the model explained 4.8% variation in RQ, compared to the 77.4% variation that is known about RMR.

Tables and Figures

Table 1. Participant Characteristics Overall and by Fitness Level[a]

		Fitness Group			
Characteristic	All (N=426)	Low (n=140)	Moderate (n=145)	High (n=141)	Between-group differences (*P* value)
Female sex (%)	50.94	50.71	51.03	51.06	>.99
Age (y)	27.6 ± 3.8	28.4 ± 3.7	27.4 ± 3.8	27.2 ± 3.7	.02
Height (cm)	171.6 ± 9.5	170.9 ± 9.2	172.1 ± 10.2	171.9 ± 9.1	.58
Weight (kg)	74.9 ± 13.8	81.7 ± 14.7	73.8 ± 12.7	69.4 ± 10.8	<.001
Body mass index	25.4 ± 3.8	27.9 ± 4.0	24.9 ± 3.4	23.4 ± 2.5	<.001
Fat mass (kg)	21.3 ± 8.6	27.9 ± 8.6	20.6 ± 6.8	15.6 ± 5.2	<.001
Fat mas (%)	28.2 ± 9.0	33.9 ± 7.6	28.0 ± 8.1	22.8 ± 7.6	<.001
Fat-free mass (kg)	53.6 ± 11.1	53.6 ± 10.4	53.2 ± 11.5	53.9 ± 11.4	.87
Skeletal mass (kg)	29.3 ± 7.6	29.3 ± 6.8	29.1 ± 7.5	29.7 ± 8.5	.77
Skeletal mass (%)	39.2±6.8	35.9 ± 5.5	39.2 ± 6.1	42.3 ± 7.3	<.001
Residual mass (kg)	21.4 ± 4.0	21.5 ± 3.6	21.4 ± 3.9	21.4± 4.4	.96
Residual mass (%)	28.9 ± 3.7	26.6 ± 2.8	29.0 ± 2.8	30.9 ± 4.2	<.001
Bone Mass (kg)	2.8 ± 0.5	2.8 ± 0.5	2.8 ± 0.5	2.8 ± 0.5	.98
Bone Mass (%)	3.8 ± 0.5	3.5 ± 0.5	3.8 ± 0.4	4.0 ± 0.4	<.001

[a]Data are given as mean ± SD except for female sex

Table 2. Time spent in Physical Activity and Oxygen Consumption at Rest and at Peak Exercise[a]

Characteristic	Fitness Group				
	All (N=426)	Low (n=140)	Moderate (n=145)	High (n=141)	Between-group differences (*P* value)
Cardiorespiratory fitness (L/min)	2.9 ± 0.9	2.5 ± 0.8	2.8 ± 0.8	3.3 ± 0.9	<.001
Cardiorespiratory fitness (mL/kg per minute)	38.4 ± 9.8	29.8 ± 6.4	38.1 ± 6.1	47.4 ± 7.2	<.001
Respiratory quotient	0.790 ± 0.05	0.801 ± 0.050	0.788 ± 0.043	0.785 ± 0.045	.008
Resting energy expenditure	1526.7 ± 262.9	1536.9 ± 268.6	1519.7 ± 267.6	1523.5 ± 253.8	.85
RMR[2] (mL/kg per minute)	2.96 ± 0.4	2.7 ± 0.3	2.98 ± 0.3	3.2 ± 0.3	<.001
Total daily energy expenditure (kcal/d)	2742.9 ± 512.2	2681.7 ± 479.5	2740.9 ± 541.5	2805.7 ± 508.9	.13

[a]Data are given as mean ± SD

Table 3. Energy Intake by Macronutrients[a]

Characteristic	All (N=426)	Fitness Group: Low (n=140)	Moderate (n=145)	High (n=141)	Between-group differences (*P* value)
Energy Intake (kcal/d)	2086.8 ± 677.2	1916.9 ± 607.9	2127.5 ± 719.7	2213.6 ± 667.8	<.001
Carbohydrates (% of total kcal)	47.2 ± 9.9	49.1 ± 9.2	46.5 ± 9.7	45.9 ± 10.6	.02
Fat (% of total kcal)	32.9 ± 7.5	31.9 ± 6.9	33.4 ± 7.1	33.2 ± 8.5	.19
Protein (% of total kcal)	17.2 ± 4.9	17.3 ± 4.9	17.1 ± 5.0	17.2 ± 4.8	.96
Alcohol (% of total kcal)	2.8 ± 4.5	1.8 ± 3.7	3.0 ± 4.7	3.6 ± 5.0	.002

[a]Data are given as mean ± SD

Figure 1.

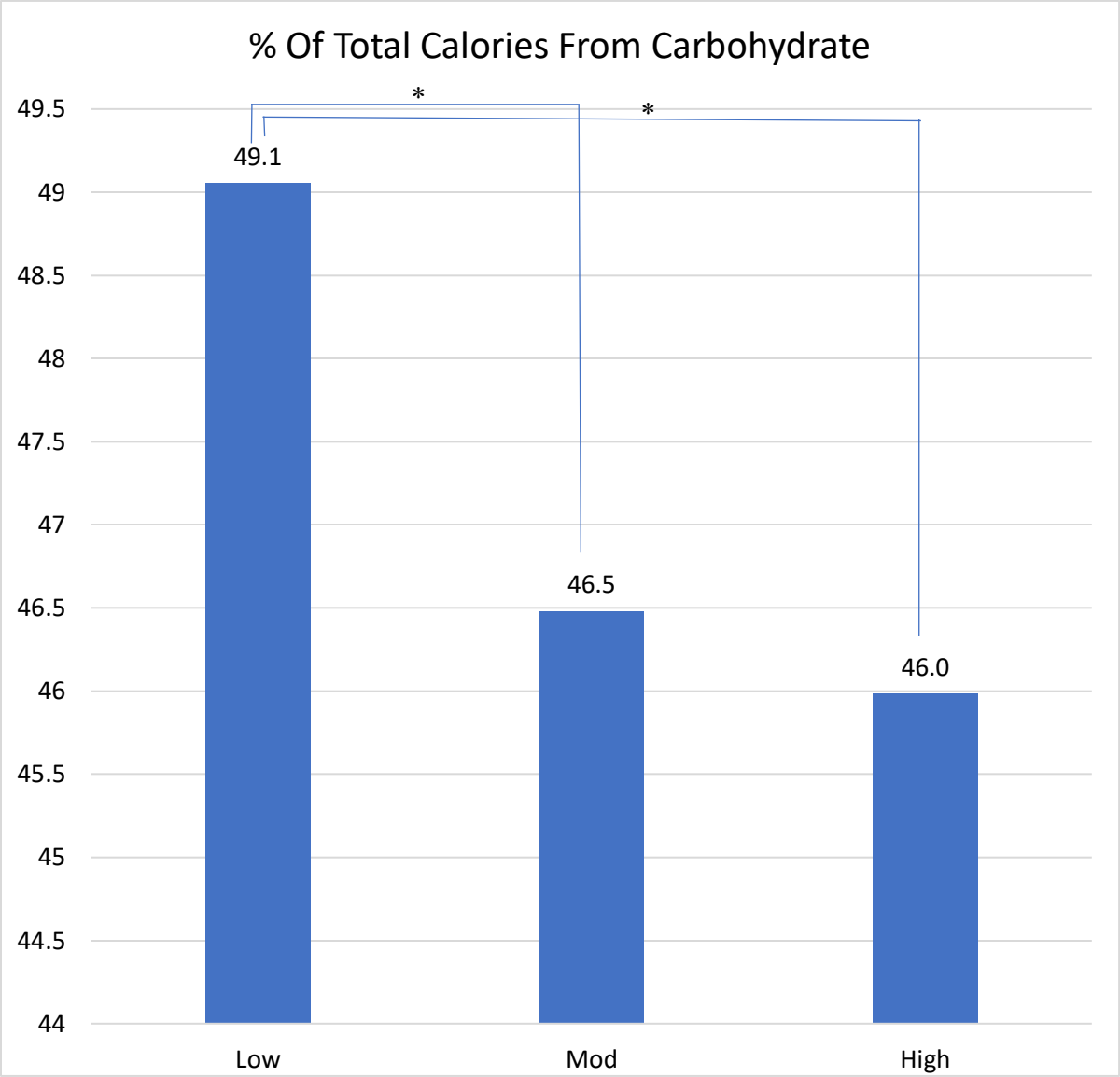

FIGURE 1. % of Total Calories from Carbohydrates Per Group
The association between fitness groups and % total calories from carbohydrates. Group 1v2, and Group 1v3 are significantly different (*P<0.05).

Table 4. Analysis of Covariance Assessing Respiratory Quotient Among Fitness Groups Controlling for Diet and Body Compartments

Characteristic	Fitness Group			R^2	Between-group differences (*P* value)		
	Low (n=140)	Moderate (n=145)	High (n=141)		Low vs moderate	Moderate vs high	Low vs high
Unadjusted (mean ± SD)	0.801	0.787	0.786	0.021	0.04	0.90	0.01
Age + sex + race	0.801	0.787	0.786	0.021	0.05	0.92	0.02
Age + sex + race + carbohydrates	0.799	0.788	0.786	0.045	0.10	0.93	0.05
Age + sex + race + carbohydrates + protein	0.799	0.788	0.786	0.045	0.12	0.93	0.05
Age + sex + race + carbohydrates + protein + fat	0.799	0.788	0.786	0.046	0.12	0.94	0.06
Age + sex + race + carbohydrates + protein + fat + fat mass + skeletal muscle + residual mass	0.799	0.788	0.788	0.048	0.23	0.99	0.30

[a]Data are given as mean ± SD

Figure 2

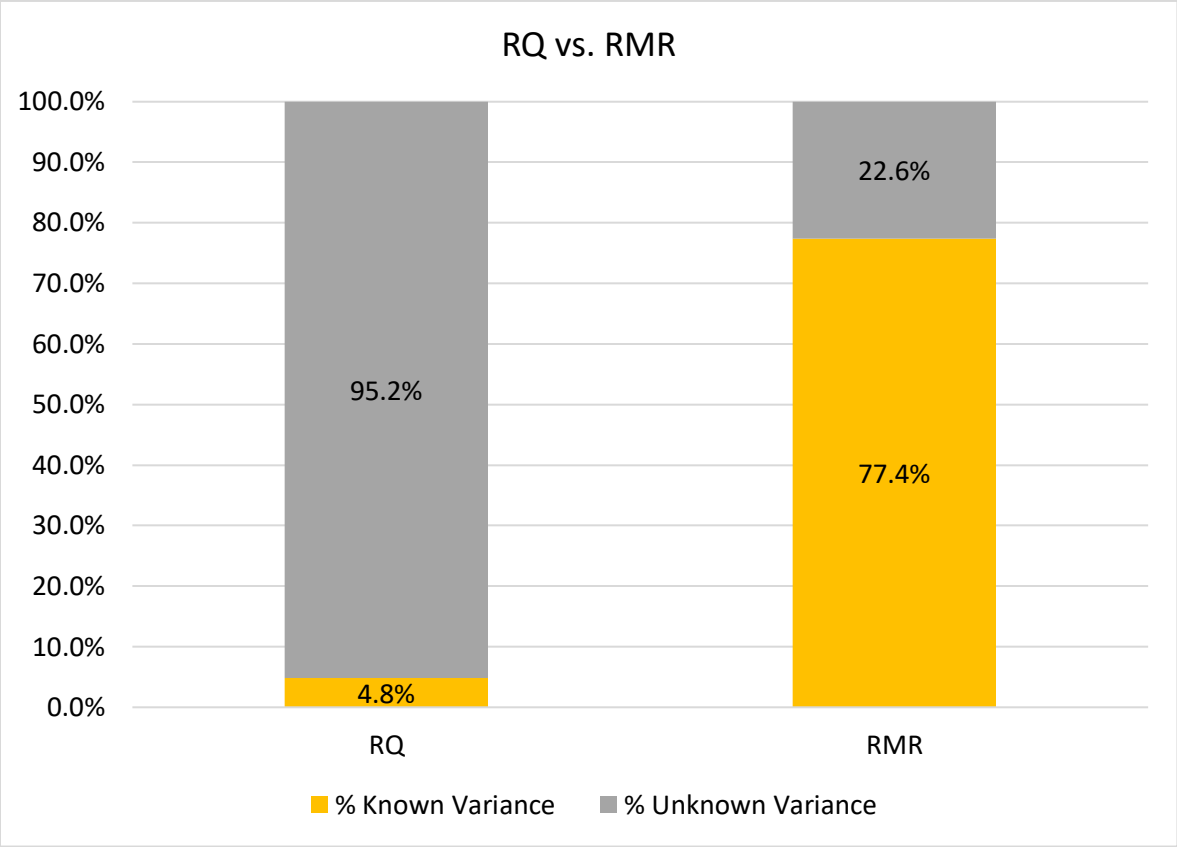

FIGURE 2. RQ vs. RMR Variance
Partitioning of the variance in respiratory quotient (left) was measured in 430 adults. Most of the variation was explained by percent of total calories from carbohydrates (2.4%, $P < 0.01$). There were smaller effects of CRF (2.1%) and body composition (0.02%). A large portion of the variance is unexplained. Partitioning of the variance in resting metabolic rate (RMR) (right) was measured in 150 adults in one study by Johnstone, 2005, and 430 in another study by Shook, 2014. 77.4% of variance is known about RMR, 22.6% variance is unexplained.

Chapter V: Discussion

The hypothesis stated that diet will have the greatest influence on RQ, followed by body composition (FM, skeletal muscle mass, and residual mass) then CRF. This set of data did show that diet had the greatest influence on RQ. Similar to other studies focused on diet and RQ (7, 8, 30), the group who consumed the highest percent of calories from carbohydrates also had the highest RQ. Group 1(low fitness) consumed significantly more calories from carbohydrates than any other group and their RQ was significantly higher than all groups ($P<0.05$). The percent of total calories from carbohydrates explained the most variation in RQ, 2.4%, protein did not explain any variation, and fat explained 0.1%. Carbohydrates consisted of the largest portion of all three groups' diets which may explain why percent of total calories from carbohydrates had a more significant influence on RQ than percent of calories from protein or fat.

Body composition was added to the model as percent of fat, skeletal muscle, and residual mass. Fat explained 0.1% variation of RQ, and skeletal and residual mass explained 0.2%. Compared to the studies analyzing the variables that influence RMR (5, 6), fat mass explained 6.7%, fat-free mass explained 63.0%, and CRF explained 4.0% of the variance of RMR.

CRF was significantly different between all groups ($P<0.01$) and explained 2.1% variation in RQ. However, after percent of total calories from carbohydrates were added to the model, CRF was no longer significant in predicting RQ between groups. This was to be expected as there have been few studies focused on the relationship between RQ and CRF, therefore their relationship was predicted to be minimal.

Implication of Findings

Given the difference in variances between RMR and RQ, it seems the two metabolic indicators are not comparable. RMR is a metabolic indicator in which one of its uses in

healthcare is to determine caloric needs for individuals by registered dietitians. RQ is not well understood, therefore it is not utilized as frequently by healthcare professionals. This thesis only explained 4.8% of the variance, leaving 95.2% of variance unknown. Without complete understanding of what influences RQ, health professionals are unable to give evidenced-based recommendations on how to utilize this metabolic indicator in recommendations.

Given percent of total calories from carbohydrates explains most of the variance, more research needs to be done examining how various types of carbohydrates and amounts can increase the amount of determined variance.

Limitations and Strengths

This thesis project examined the effects of various variables, including diet composition, body composition, and CRF on RQ. Currently, there is no research to date examining the roles of each of these variables simultaneously on RQ. Literature focuses on exploring the effects of one variable at-a-time rather than seeking to understand the entirety of this specific metabolic indicator.

Secondary data analysis was performed using the EBS study consisting of young, healthy adults. Majority of the research on RQ focusing on body composition, diet composition, and fitness have also used young, healthy individuals. Limitations to this thesis project are that secondary data analyses are unable to control for its own methods and procedures but are limited to the original study design. The age of participants is limiting to males and females younger than 40 years old thus restricting generalizability. Data analysis was conducted using baseline measurements rather than being able to control for confounding factors by examining two sets of data at different time points.

The EBS was strong in study design and methodology, therefore secondary data analyses were conducted using strong data. The large number of participants in the EBS is another strength as most of the literature on this topic has fewer than 50 participants and the EBS provides 430 participants.

Conclusion

This thesis project sets the foundation for the determinants of RQ, similar to studies examining the determinants of RMR (5, 6). Although the variables of interest in this thesis are not all encompassing, groundwork has been set for future research endeavors.

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