ABSTRACT

Title of Document:	ROOM CALORIMETRY AS A METHOD OF MEASURING METABOLIC FLEXIBILITY
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Metabolic flexibility is commonly measured as the change in respiratory quotient (RQ) from the fasted state to the insulin-stimulated state. This measurement is performed using a hyperinsulinemic-euglycemic clamp and hood calorimetry. A study was performed to evaluate if room calorimetry alone could provide the same results and to examine how diet composition and exercise affect metabolic flexibility. Sixteen healthy males and females stayed overnight in a room calorimeter on four separate occasions. While in the calorimeter, they participated in meal and exercise challenges. The subjects took part in one of two exercise sessions on the treadmill (high intensity – short duration or low intensity – long duration). They were also provided one of two treatment beverages for lunch (high carbohydrate or high fat shake). Blood samples were collected before and 30, 60, 90, and 120 minutes after breakfast and lunch to measure glucose, insulin, and non-esterified fatty acids (NEFAs). Metabolic flexibility was calculated in a manner similar to the literature, and new metabolic flexibility variables were also considered including the rate of

change of RQ (slope) following exercise or a meal and the lag in RQ following exercise. Delta RQ - calculated as the difference between a two hour average night RQ and a two hour average morning RQ - was negatively correlated to % body fat, positively correlated to VO_2peak, and trending negatively with age. The post-lunch slope of RQ was significantly larger following the high carbohydrate shake compared to the high fat shake. The slope of the increase in RQ following the start of exercise was negatively correlated with age and % body fat and positively correlated with VO_2peak. This study shows that the non-invasive room calorimetry method can be used to measure many metabolic flexibility variables.

.ROOM CALORIMETRY AS A METHOD OF MEASURING METABOLIC FLEXIBILITY

By

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Dedication

To my husband, Greg, for all his love, support, and patience during this long process of changing careers.

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Abbreviations

AC	Acylcarnitines
AMPK	Adenosine monophosphate-activated protein kinase
AUC	Area under the curve
BG	Blood glucose
BHNRC	Beltsville Human Nutrition Research Center
BMI	Body mass index
CBC	Complete blood count
CDC	Centers for Disease Control and Prevention
CGMS	Continuous glucose monitoring system
CK	Creatine kinase
CPT 1b	Carnitine palmitoyltransferase 1b
CS	Citrate synthase
CT	Computed tomography
DXA	Dual-energy X-ray absorptiometry
EPA	Eicosapentaenoic acid
FAT/CD36	Fatty acid translocase/cluster of differentiation 36
FCHL	Food Components and Health Lab
FFA	Free fatty acid
FFQ	Food frequency questionnaire
FQ	Food quotient
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GDR	Glucose disposal rate
^{1}H NMR	Proton nuclear magnetic resonance
HC	High carbohydrate
HF	High fat
HI	High intensity – short duration
HOMA-IR	Homeostatic model assessment – insulin resistance
HR	Heart rate
HSF	Human Studies Facility
HU	Hounsfield units
IFG	Impaired fasting glycemia
IGT	Impaired glucose tolerance
IHL	Intrahepatic lipid
IMCL	Intramyocellular lipid
IR	Insulin resistance
IRβ	Insulin receptor β
IRB	Institutional Review Board
IS	Insulin sensitivity
LA	Linoleic acid
LI	Low intensity – long duration
LXR	Liver X receptor
MCD1	Malonyl-coenzyme A decarboxylase 1
MF	Metabolic flexibility

MS	Multiple sclerosis
mtDNA	Mitochondrial deoxyribonucleic acid
NAFLD	Non-alcoholic fatty liver disease
NEFA	Non-esterified fatty acid
NGT	Normal glucose tolerance
NHANES	National Health and Nutrition Examination Survey
OGTT	Oral glucose tolerance test
PA	Palmitic acid
PAL	Physical activity level
PCK1	Phosphoenolpyruvate carboxykinase 1
PDK2 (or 4)	Pyruvate dehydrogenase kinase isozyme 2 (or 4)
PCOS	Polycystic ovary syndrome
PFK	Phosphofructokinase
PGC1α	Peroxisome proliferator-activated receptor gamma coactivator-1α
PPARα	Peroxisome proliferator-activated receptor-α
PPARγ2	Peroxisome proliferator-activated receptor-γ2
RER	Respiratory exchange ratio
RPE	Rate of perceived exertion
RQ	Respiratory quotient
SCD1	Stearoyl-Coenzyme A desaturase 1
SEM	Sensor electronic module
sTNFR1(or 2)	Soluble tumor necrosis factor-α receptors
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
TSH	Thyroid-stimulating hormone
VCO_2	Volume of carbon dioxide
VO_2	Volume of oxygen
UN	Urinary nitrogen
USDA	United States Department of Agriculture

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Introduction

Metabolic flexibility is the ability to switch substrate use based on substrate availability. For example, while sleeping at night, a person is in a fasted state and therefore is using more fat. When they wake up and eat breakfast, they are now in an insulin-stimulated state and will switch to using glucose as their fuel source. Much of the literature measures metabolic flexibility as the change in respiratory quotient (RQ) between an insulin-stimulated state and a fasting state. This measurement is done using a hyperinsulinemic-euglycemic clamp and a hood calorimeter. By utilizing a smoothing method that reduces equipment noise, it is possible to use a room calorimeter to study metabolic flexibility. Using a room calorimeter is beneficial for several reasons. First, it is less invasive that the clamp method, which involves infusion of insulin and glucose. Second, the room calorimeter allows the body to have a more physiological response to diet or exercise challenges than the clamp which involves controlling glucose levels. Finally, the clamp would not allow for eating meals or exercising during the procedure; therefore using the room calorimeter and smoothing the data allows the subjects to consume actual meals and be ambulatory to exercise so that RQ response can be monitored continuously.

The first chapter will provide background on metabolic flexibility and the second chapter will focus on using room calorimeter to measure metabolic flexibility and evaluating how subjects adapt their RQ to diet and exercise challenges. The primary objectives were (1) to verify that measuring metabolic flexibility using a room calorimeter would provide similar results to other methodologies; (2) to examine the effects of diet composition and exercise interventions on metabolic flexibility variables (ΔRQ, slope of RQ, area under the curve (AUC)

of RQ); and (3) to see if there are any correlations that can be established between the metabolic flexibility variables and the physical characteristics of the subjects (age, BMI, % body fat, VO_2peak, and HOMA-IR).

Chapter 1: Literature Review

1.1.1 Introduction

Obesity is a major health concern that has reached epidemic proportions in the United States. According to the National Health and Nutrition Examination Survey (NHANES) from 2009 – 2010, 35.7% of adults (35.8% women; 35.5% men) in the U.S. were obese [1, 2] and 16.9% of children and adolescents in the U.S. were obese [2, 3]. Healthy People 2010 set a goal to reduce the incidence of obesity to 15%, and in 2009, using self-reported heights and weights, all states' obesity rates exceeded this percentage [4]. The Healthy People 2020 objective is to reduce the incidence of obesity to 30.6% [5], and nine states from the previous study had an obesity prevalence of $\geq$ 30% [4]. In 2010, 36 states had an obesity prevalence of 25% or more with 12 states $\geq$ 30%. Much of this has to do with energy balance: we are taking in more calories than we are using. With the increased incidence of obesity comes the higher risk of developing other health problems including Type 2 diabetes (T2D). According to the CDC National Diabetes Fact Sheet, 2011, 25.8 million people in the US (8.3% of the population) had diabetes in 2010 [6]. The role of skeletal muscle in the development of insulin resistance and T2D has been studied extensively. Skeletal muscle plays a large role in glucose and fatty acid oxidation. Skeletal muscle dysfunction may cause improper fuel usage (oxidation), which may contribute to T2D. This review will focus on one form of skeletal muscle dysfunction, referred to as "metabolic inflexibility"; how it develops and how it relates to obesity and T2D. Factors that may contribute to the development of metabolic inflexibility, such as diet, physical activity, family history, will be included and discussed.

"Metabolic flexibility" (MF) is a term that has only been recently developed and is most commonly examined in relation to T2D. Most studies involve evaluating how MF differs among various populations (lean, obese nondiabetics, and obese diabetics) with the intention of determining if changes in MF develop prior to or along with the development of T2D. Many researchers have defined MF as the change in respiratory quotient (ΔRQ; insulin-stimulated RQ – fasting RQ) [7-11]. In one recent study, MF was defined as the change in non-protein RQ (ΔNPRQ) at the end of a refeeding diet period compared to the end of a caloric restriction diet period [12]. However, some authors don't explicitly calculate ΔRQ, but still use the terminology. For example, Prior et al. looked at the changes in RQ from a fasted state to various low intensity exercise sessions which they considered "metabolic flexibility during exercise" [13]. Others look at how subjects adapt their fat oxidation to a high fat diet and consider this metabolic flexibility [14, 15]. Bergouignan at al. [16] has also pointed out the variety of MF definitions and came up with another way to measure MF – by the variance of NPRQ and the variance of insulin. At this point, there has not been enough research done to develop a quantitative index of metabolic flexibility. There may be ΔRQ values that are significantly different between the previously mentioned populations, but the numbers don't mean anything yet. A "normal" range hasn't been identified, nor has a value or range that could indicate an increased risk for developing T2D, etc. Therefore, this literature review will take the reader through the developing research on MF, which may potentially lead to a quantitative index.

To begin, it is essential to understand what MF means in terms of appropriate fuel usage. This means being able to switch fuel sources so that fat is used when in a fasted state and carbohydrates are used when insulin-stimulated. In the laboratory it is possible to monitor fuel

utilization by measuring RQ, which also increases with exercise. Multiple factors contribute to the way the body handles fuel. These include the secretion of insulin by the β-cells of the pancreas and the functionality of skeletal muscle. Insulin and amylin control glucose levels in the body, while smaller mitochondria or the amount of lipid in the skeletal muscle can affect the ability of the skeletal muscle to use the fuel that it obtains. Another topic that will be addressed is whether this inflexibility is an intrinsic factor in the skeletal muscle cells (myotubes) as opposed to something that develops over time as someone becomes obese or insulin resistant. By understanding these metabolic and tissue factors, the reader can better comprehend the MF research that is presented.

1.1.2 Overview

MF research spans a variety of topics; therefore, this literature review is arranged by these topics to aid the reader in navigating this document. Initially, correlations will be introduced in order to see how MF relates to the characteristics of the populations evaluated, especially body mass index (BMI), body fat, aerobic capacity (VO_2max), and insulin sensitivity. Since metabolic inflexibility seems to be related to T2D, populations with a family history of T2D will be examined to see whether or not this inflexibility is seen prior to the development of T2D. Next, gender and race differences in MF will be briefly discussed, since disease risks vary between men and women and among different ethnicities. Recently, some researchers have been examining MF in subjects with various conditions other than T2D, such as subjects with multiple sclerosis. Finally, environmental factors such as diet and exercise will be considered. Metabolic inflexibility demonstrates inefficient fuel usage, so diets of various macronutrient compositions will be addressed to see if the type of fuel affects MF or how quickly RQ is

adjusted to the type of diet. Exercise could potentially be part of the solution for metabolic inflexibility if it results in weight loss and better insulin sensitivity.

1.2 Metabolic Factors

1.2.1 Substrate Utilization

RQ is the ratio of the volume of CO_2 expired to the volume of O_2 consumed (VCO_2/VO_2), which reflects substrate oxidation. For example, RQs of 1.000, 0.710, and 0.835 would indicate exclusive carbohydrate, fat, and protein oxidation respectively [17]. RQ is commonly used interchangeably with respiratory exchange ratio (RER). However, RER is the ratio of VCO_2/VO_2 measured from expired gases and RQ is the ratio of VCO_2/VO_2 for cells [18]. It is thought that RER reflects ongoing cellular processes [18].

Fuel usage among various populations, including lean, obese, and diabetics, have been evaluated in many studies. Blood samples (arterial and femoral venous) drawn from the leg of obese and lean subjects to measure the O_2 and CO_2 content in the blood were compared [19]. Obese subjects had significantly higher fasting leg respiratory quotients (RQs) than those of lean subjects (0.90±0.01 vs. 0.83±0.02, means ± SE) [19]. This higher fasting RQ suggests that obese subjects use less fat as an energy source. Lean subjects had significantly higher insulin-stimulated leg RQs, indicating that they are using carbohydrates (glucose) for energy and at the same time suppressing fat oxidation [19]. This pattern of fuel usage (oxidation) was confirmed when total substrate oxidation was calculated using indirect calorimetry equations along with VO_2 and VCO_2 as variables [19]. In addition, Type 2 diabetics have been shown to have reduced uptake of free fatty acids (FFA), higher leg RQ, and lower lipid oxidation than nondiabetics during fasting [20]. After insulin stimulation, FFA uptake was not significantly different than in the fasted state for the Type 2 diabetics. They also had lower mean lipid oxidation, higher mean

glucose oxidation, and their leg RQ remained higher than the nondiabetics after insulin stimulation [20]. This demonstrates that diabetics in a fasted state were not using fat, and in a fed state, glucose oxidation was not suppressing FFA uptake as much. This impairment in fat oxidation observed in obese subjects and Type 2 diabetics has suggested a new potential marker in the development of IR and T2D: metabolic flexibility (MF).

MF is the ability to switch fuel sources based on substrate availability. For example, using fat while in a fasted state and carbohydrates during insulin stimulation is an indicator of being metabolically flexible. The phrase "metabolic flexibility" was created by Kelley and Mandarino [21] and is specifically defined as "the capacity to switch from predominantly lipid oxidation and high rates of fatty acid uptake during fasting conditions to the suppression of lipid oxidation and increased glucose uptake, oxidation, and storage under insulin-stimulated conditions." One author compared metabolic inflexibility to gridlock that might be experienced during rush hour traffic [22]. Chronic overeating would cause gridlock in the mitochondria with the excess fuel resulting in substrate competition and unclear signals regarding which substrate to use [22]. **Appendix A** provides a summary of important studies related to MF and is organized by the year published.

1.2.2 Insulin

Insulin has been well established as a pancreatic hormone that contributes to the regulation of intake by acting as an adiposity signal [23]. Type 2 diabetes (insulin resistance) interferes with intake regulation [23]. When food is consumed, insulin is released from the β-cells of the pancreas in order to control blood glucose level by promoting the uptake of glucose by skeletal muscle [24]. In a fed state, gluconeogenesis is inhibited and glycogenesis occurs in the liver and muscle [24]. Insulin also causes glucose uptake in adipose tissue which results in

fatty acid synthesis [24]. Individuals with Type 2 diabetes are insulin resistant and are less efficient in controlling blood glucose. FFA release is not inhibited in adipose tissue, and glucose uptake is not stimulated by the skeletal muscle because of the resistance to the release of insulin [24]. Blood glucose levels remain elevated, which causes the release of more insulin in an attempt to control glucose levels, and FFA levels are elevated as well [24]. Kelley et al. [25] examined healthy subjects with no history of T2D during "physiological hyperinsulinemia and euglycemia." Under those conditions, glucose is primarily oxidized in skeletal muscle, while lipid oxidation is suppressed. The leg RQ also demonstrates the increase in glucose oxidation with a hyperinsulinemic value of close to 1 (0.99±0.02, means ± SE) compared to the basal value of 0.74±0.02 which would be representative of increased lipid oxidation. This response demonstrates appropriate fuel usage by skeletal muscle. It bears repeating that this ability of skeletal muscle to change substrate use is a key characteristic of "metabolic flexibility."

1.2.3 Calorimetry

Indirect room calorimetry is a way to measure MF, since it provides data for the calculation of RQ (see next paragraph). Energy expenditure and substrate use can also be calculated. Unlike a direct calorimeter, an indirect calorimeter does not involve measuring heat production. Typically room calorimeters are approximately 9'x12' and each are equipped with a bed, desk, computer, TV, DVD player, phone, radio, treadmill, and bathroom facilities (**Figure 1.1**). Further descriptions are provided in Rumpler et al. [26] and Seale et al. [27]. Subjects usually remain in the room for approximately 23.5 hours. They receive their standardized meals through an airlock in the door, and an arm port in the window allows them to have their blood drawn while inside the room. There is a schedule they must follow which includes the time they

go to bed, wake up, eat, and perform any activities involved in the study (exercise, blood draws, etc.) (**Figure 1.2**). Subjects have free time when they are not performing study tasks.

Figure 1.1: Room calorimeter
The exterior (a) and interior (b) of the respiratory calorimeters at the USDA Beltsville Human Nutrition Research Center.

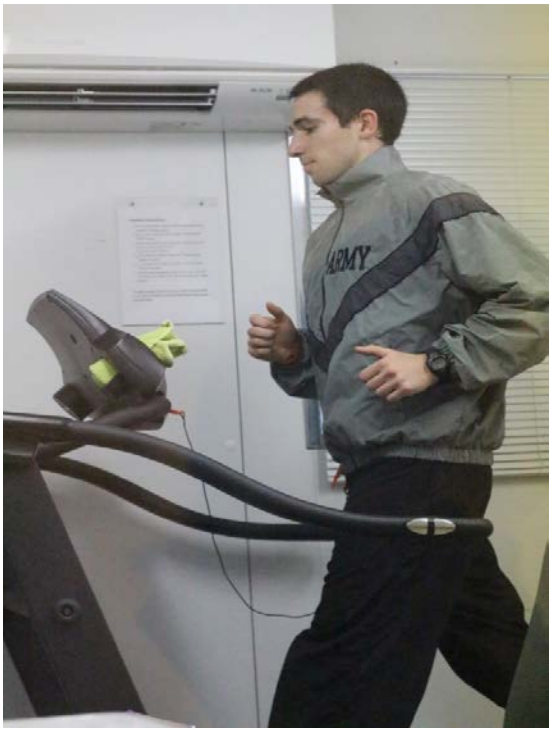

Figure 1.2: Study participant
Subject running on the treadmill in the calorimeter as part of a study protocol.

Calorimetry involves measuring five variables which include: air flow, fractional O_2 inspired, fractional O_2 expired, fractional CO_2 inspired, and fractional CO_2 expired by measuring mass and composition (via mass spectroscopy) and volume (via mass flow) of air into and out of the calorimeter. From these variables, the volume of O_2 (VO_2) in L/min, volume of CO_2 (VCO_2) in L/min, and RQ can be calculated. VO_2 and VCO_2 would then be multiplied by the duration of the calorimeter stay to calculate the total volume of each. Urinary nitrogen (UN) is also measured during the calorimeter session. From these data, the equations in Livesey et al. [28] can be applied to calculate substrate oxidation and total energy expenditure. In addition, substrate balances can be determined using the dietary intake and substrate oxidation data.

Ideally, observations of the changes in substrate oxidation rates would be made nearly instantaneously allowing for the assignment of these changes to subtle differences in the conditions (food intake, physical activity, etc.) impacting the individual in the calorimeter. However, variability in the collected calorimeter data is a function of both the volunteer (signal) and the instrumentation (noise). Assuming that the noise is random, averaging of the data over extended periods of time minimizes the effect of the noise. However, averaging the data obscures the short term changes and rapidly occurring responses of the volunteer in the calorimeter. The USDA Food Components and Health Laboratory recently published a method [29] using data collected while the calorimeters were empty and while a known amount of ethanol was combusted to characterize the "noise" inherent in the system. Estimates of the "noise" are included in the algorithms used to calculate the variability in the O_2 consumption and CO_2 production to improve the estimate of the variability from the volunteer. The method dampens the noise and simultaneously smoothes the room calorimeter data using regularized least squares derivatives so that changes in gas composition are evaluated over periods of

several minutes [29]. This means that variations in RQ, O_2 consumption, and CO_2 production are observed immediately after meals or exercise, and the rate at which a subject switches fuel sources after an event is estimated, thus allowing a new insight into the data. For example, after a high carbohydrate meal, a subject's RQ should increase to reflect use of carbohydrates for energy, and it typically would increase almost simultaneously with VO_2 (energy expenditure). During exercise, some subjects have demonstrated a lag in how quickly their RQ adjusts when compared to VO_2. In some cases, the RQ peaks after the VO_2 is returning to baseline. This lag is illustrated in **Figure 1.3** where after the exercise period (around 1200 min elapsed time), the peak in RQ occurs after the VO_2 peak. Using cross-correlation, RQ and VO_2 can be compared, and the maximum of the cross-correlation function is defined as the lag and is measured in minutes. Lag has been shown to be positively correlated to age (**Figure 1.4**). It has also been verified using alcohol control trials that this new method allows observation of the changes due to physiological responses independent of calorimeter instrumentation noise (unpublished data).

Alcohol has an RQ of 0.667 [17] when combusted. Combusting alcohol in the calorimeter allows for the characterization of the variation in O_2 and CO_2 composition under controlled conditions which can then be compared to the variation observed when subjects are present in the calorimeters. Using this new biomarker, factors that cause the variations in the lag times can be examined. A recent examination of the calorimeter data at the USDA has identified other potential variables that might be worth considering including the slope of RQ. Analyzing variables such as lag time or the slope of RQ could provide valuable information to quantify an index of MF using just room calorimetry [30]. With the calorimeter data collected, MF (ΔRQ) can still be calculated in a manner similar to the literature by taking the average RQ of a time interval during the day and subtracting the average RQ of a night interval.

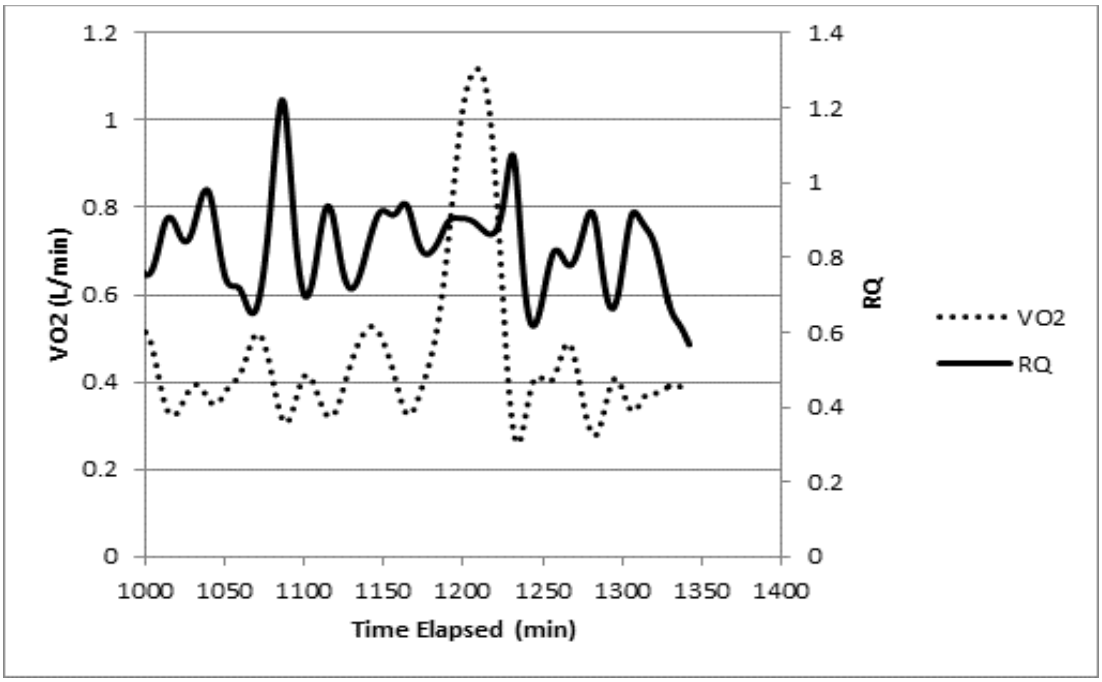

Figure 1.3: Subject lag following exercise
Example of a subject demonstrating a lag in RQ after an exercise period (the largest VO_2 peak). (Presented at NFSC Research Day) [31]

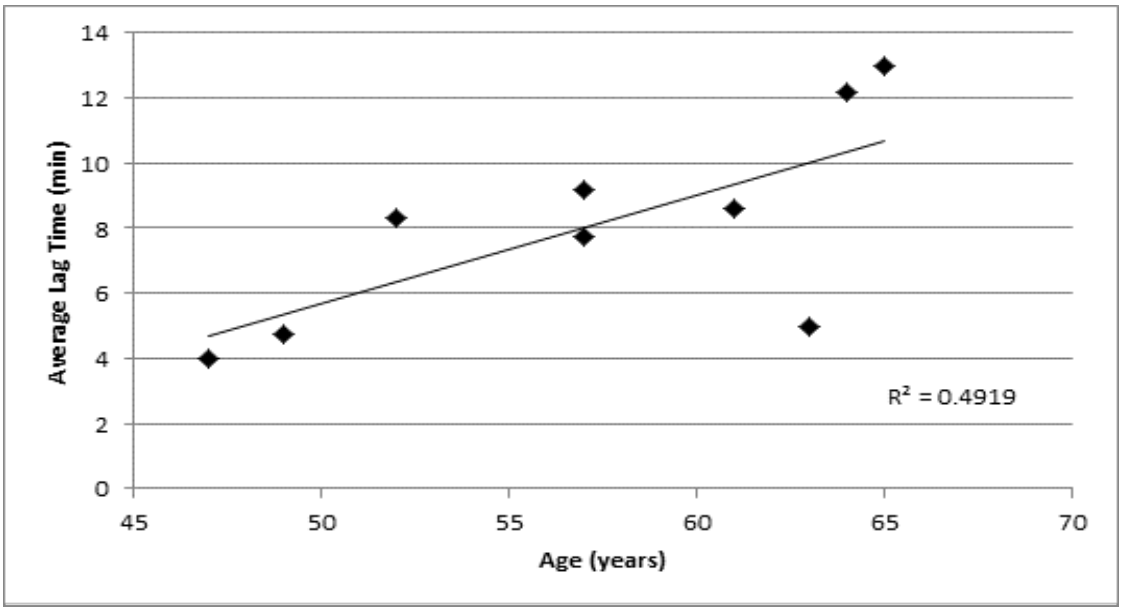

Figure 1.4: Average lag time vs. age
In a study of healthy adult males, ages 47-65 (BMI: 24.6 – 31.2), there was a positive correlation between the subjects' average lag time following exercise and age ($r = 0.70$, $p = 0.0352$) [31].

1.3 Tissue Factors

1.3.1 Skeletal Muscle Mitochondria

In addition to the role of insulin, several investigators have evaluated skeletal muscle, focusing on the question: "Does impaired fat oxidation contribute to the development of IR and T2D?" These studies examine skeletal muscle mitochondria, lipid content of skeletal muscle, and skeletal muscle cells. Skeletal muscle mitochondria are smaller or damaged in obese and Type 2 diabetic subjects compared to lean subjects, and the size correlates positively with insulin sensitivity (IS), as measured by glucose disposal rate (GDR) [32]. Smaller muscle mitochondria may result in the mitochondria not performing to their full capacity in terms of generating energy from available sources. For example, small or damaged mitochondria results in less β-oxidation converting fat to energy. The capacity of the oxidative and glycolytic enzymes in skeletal muscle is also important to consider when examining the energy capability of the muscle. In a group of 17 women, low Hounsfield attenuation muscle was negatively correlated to citrate synthase (CS) activity (aerobic-oxidative capacity) and positively correlated with both creatine kinase (CK) activity (anaerobic capacity) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) activity (glycolytic capacity). The ratio of phosphofructokinase to citrate synthase (PFK/CS) was also positively correlated to low attenuation muscle demonstrating that there is more glycolytic capacity as the area of low attenuation muscle increases. [33]. Attenuation was determined by a computed tomography (CT) scan, where normal attenuation muscle was measured from 35-100 Hounsfield units (HU) and low attenuation muscle was measured from 0-34 HU [33]. Based on the Hounsfield scale where the reference is water (0 HU), muscle has a positive attenuation and fat has a negative attenuation [34]. Obese women also had more low

attenuation muscle, and area of muscle with low attenuation was negatively correlated to rates of leg glucose storage [33]. Using step-wise multiple regression, the authors found that 57% of the variance in leg glucose storage was accounted for by low attenuation muscle and visceral adiposity, with low attenuation muscle having the strongest predictive value for insulin resistance [33].

1.3.2 Skeletal Muscle IMCL

Intramyocellular lipid (IMCL) content in skeletal muscle was negatively correlated to insulin action at the low dose of insulin infusion during the clamp for a group of nondiabetic male Pima Indians, but was not significantly correlated with BMI, percent body fat, or waist-to-hip ratio [35]. IMCL also tends to be higher in obesity [36]. It has been suggested that the cause of this increase is due to the regulation of fat oxidation being "inflexible," and this deregulation is associated to IR [19]. In healthy, normal weight men and women, IMCL is negatively correlated with M-value (whole body glucose uptake) during insulin stimulation [37]. The authors concluded that IMCL, which they measured via ^{1}H NMR spectroscopy, is a good predictor of IS. IMCL was higher in offspring of type 2 diabetic parents compared to controls, but there was no correlation between IMCL in either the tibialis anterior or soleus and fasting lipid oxidation in either group [38]. Lipid content and oxidative enzyme capacity has been examined by fiber type as well [39, 40]. Type 2 diabetics and obese subjects had a significantly higher lipid content and a significantly lower oxidative enzyme activity in each muscle fiber type (type 1, type IIa, and type IIb). He et al. [39] also found that muscle glycogen was lower in Type 2 diabetics and negatively correlated with hyperglycemia, suggesting a negative correlation between muscle glycogen and IR.

Curiously, endurance athletes have more IMCL, but still remain insulin sensitive. Goodpaster et al. [41] found that endurance-trained athletes had more IMCL than did lean subjects, and trained subjects had a higher oxidative capacity in their muscle compared to lean, obese nondiabetics, and obese diabetics. In addition, lean and trained subjects were more insulin sensitive than both obese groups. After 16 weeks of moderate exercise training, older, obese men and women had an increase in IMCL, insulin sensitivity, oxidative capacity, and glycogen content and a decrease in diacylglycerol and ceramide [42]. In a group of healthy older adults, IMCL and oxidative capacity of the muscle both increased after participation in 12 weeks of exercise training [43]. Exercise-induced fat oxidation increased. Trained subjects seem to show a better ability to use the fat that is in their muscle, so that they don't become insulin resistant.

Although obese, sedentary men and women who participated in a weight loss and physical activity intervention did not have a significant change in their IMCL content, they did have a significant decrease in lipid droplet size. The change in size of the droplets was negatively correlated to the change in IS; thus, the more the size decreased, the more IS improved [44]. Using succinate dehydrogenase staining, there was an increase in oxidative enzyme capacity after this intervention. When reexamining biopsy samples from a previous study, the authors found that the lipid droplets were larger in Type 2 diabetic subjects compared to those from lean subjects who were nondiabetics and sedentary. Lipid droplets from obese subjects who were also nondiabetic and sedentary were similar in size to those from Type 2 diabetics. Lipid droplet size seems to also play a role in the development of insulin resistance, but it appears to be how the lipid is dispersed that matters and not necessarily the amount of fat. Loss of fat mass was not significantly correlated with decreased lipid droplet size [44].

1.3.3 Skeletal Muscle Cells

Another question that needs to be addressed is whether or not this inflexibility is an intrinsic factor in the skeletal muscle or is it something that develops over time as someone becomes obese or insulin resistant. When examining myotubes, or muscle cells, from lean, obese, and obese Type 2 diabetics, it was found that glucose oxidation and palmitate oxidation were insulin-sensitive for all groups [45]. Myotubes from all groups remained insulin-sensitive even after supplementation with palmitate reduced basal and insulin-stimulated glucose oxidation. This led to the conclusion that metabolic inflexibility was not an intrinsic defect but an extramuscular mechanism, meaning that some outside issue was contributing to skeletal muscle performance in these groups. By contrast, Ukropcova et al. [46] found that metabolic switching, defined as the changes in fat oxidation in muscle cells, reflects the metabolic characteristics of the donor. It would be expected that someone who is metabolically flexible would be efficient in their metabolic switching both in vivo and in vitro. This would suggest that if the donor was metabolically flexible, their myotubes would be metabolically flexible as well. As anticipated, in the absence of insulin, suppression of fat oxidation by glucose in the myotubes (suppressibility) was negatively correlated with MF and IS and positively correlated with body fat and fasting FFA levels [46]. The ability of the myotubes to increase fat oxidation (adaptability) was positively correlated with MF, IS, and aerobic capacity and negatively correlated with % body fat and fasting insulin levels [46]. Delta RQ was not determined in the myotubes.

The result is that there is a disagreement in whether MF is an intrinsic defect or an extrinsic defect in skeletal muscle. However, MF of the subjects was not actually measured in vivo by Gaster [45] who had measured glucose and palmitate oxidation and was using IS as the

determining factor. It would have been interesting to identify which subjects were metabolically flexible to better compare the subject to the myotubes. In addition, establishing whether or not myotubes were insulin-sensitive does not give the whole picture. These myotubes were judged by whether insulin-stimulated glucose or fat oxidation was significantly different from basal, and all three groups were. However, Type 2 diabetics had significantly higher basal and insulin-stimulated glucose oxidation than leans suggesting that the myotubes may be insulin-sensitive, but their response is altered [45].

Hessvik et al. [47] also evaluated myotubes, but pretreated them with the liver X receptor (LXR) agonist T0901317 and different fatty acids. In addition to looking at suppressibility and adaptability, they looked at a new parameter that they called: substrate-regulated flexibility. This parameter was defined as "the ability of the myotubes to increase the acute fatty acid oxidation while changing from a 'fed' (low fatty acid and high glucose concentration) to a 'fasted' condition (high fatty acid and no glucose added)." Pretreatment with eicosapentaenoic acid (EPA) increased the suppressibility of the cells. Adaptability was significantly increased by pretreatment with EPA, linoleic acid (LA), and palmitic acid (PA). EPA, along with LA and PA, increased substrate-regulated flexibility. It was also found that EPA regulated the most genes of the fatty acids, and that myotubes that were exposed to EPA had stimulation of carbohydrate metabolism pathways. The conclusion was that n-3 fatty acids may improve metabolic switching in skeletal muscle [47]. These samples were taken from eight healthy subjects with no family history of diabetes. More research is needed to examine whether this could provide improvement to the myotubes of obese or Type 2 diabetic subjects.

Myotubes from healthy subjects were examined to see the effect of chronic hyperglycemia on metabolic switching [48]. Suppressibility was significantly less in the cells

that were placed in a state of chronic hyperglycemia when compared to the normoglycemic cells; however, there was no difference between the cells in adaptability. After hyperglycemia, glucose oxidation was reduced, but mitochondrial DNA was not different between groups.

Expression of mitochondrial enzymes involved in substrate switching were also examined from skeletal muscle of subjects with normal glucose tolerance or T2D [49]. The enzymes included in the evaluation were pyruvate dehydrogenase kinase (PDK) 4, PDK2, malonyl-coenzyme A decarboxylase (MCD1), and carnitine palmitoyltransferase 1b (CPT1b). Subjects with T2D had an increased messenger RNA (mRNA) expression of PDK4, PDK2, and MCD1. Some subjects underwent a 4 month intervention which involved increasing weekly physical activity by 5 hours of Nordic walking. Messenger RNA expression of PDK4 increased only in the normal glucose tolerant subjects; however, the exercise was not monitored.

Another study examined mitochondrial respiration in human skeletal muscle cells to see the response to changes in substrate availability in obese versus lean subjects [50]. There was a large increase in mitochondrial respiration (state 3) after 24 hour lipid incubation in the lean cells, and this was not seen in the obese cells. In addition, mtDNA copy number increased in the lean cells after the incubation, while the mtDNA copy number deceased in the obese cells. With all these studies, it would beneficial to evaluate the metabolic flexibility of the actual subjects for comparison to their skeletal muscle cells. These factors discussed in the previous sections have the potential to affect fuel usage in the body. Insulin affects the glucose levels in the body, and the skeletal muscle must be fully functional to be able to use the fuel efficiently. Therefore, these factors may contribute to a person's metabolic flexibility.

1.3.4 MF Correlations

Due to the trend of increasing obesity which can lead to an increased risk for IR and T2D, it is worthwhile to evaluate the relationships between MF, obesity, and diabetes risk. It is also necessary to identify who may develop this inflexibility and what the contributing factors are. Important variables to consider for these health problems include percent body fat, body mass index (BMI), insulin sensitivity (IS), and aerobic capacity (VO_2max). It has been reported that IS, which is commonly measured in terms of glucose disposal rate (GDR), is negatively correlated with BMI, fat mass, % body fat, and visceral fat in young, healthy men with or without a family history of T2D [8, 46]. These correlations are not surprising, since it is well established that obese individuals are more likely to become insulin resistant. Young, healthy men have a positive correlation between IS and both VO_{2max} and mitochondrial DNA [8]. Physical activity is known to increase IS; therefore, a positive correlation is expected. MF is inversely correlated with body fat and FFA levels and positively correlated with IS, VO_{2max}, and mitochondrial DNA in the above population [8, 46]. The correlations between MF and both IS (determined by glucose disposal rate) and FFA levels were also confirmed in a population of obese men and women, with or without T2D [10]. In this study, Type 2 diabetics had a significantly lower metabolic flexibility than obese nondiabetics; however, there was no difference after adjusting for GDR [10]. Another evaluation was done by dividing MF into quartiles for comparison purposes, and it identified the lowest quartile as being associated with larger fat cells, higher % body fat, and lower serum adiponectin levels [51]. Adamska et al. [52] also identified a positive correlation between adiponectin and MF as well as a negative correlation between both sTNFR1 and sTNFR2 (soluble TNF-α receptors) and MF in a group of lean, overweight, and obese men and women. MF was found to be inversely correlated to age,

positively correlated to IS, and higher in nondiabetics than Type 2 diabetics in a population of men and women of various race, age, BMI, and diabetes status [11]. These studies demonstrate that an individual is more likely to have this inflexibility if they have a high level of body fat (obese) and are insulin resistant. It is not surprising that MF decreases as people age, since there is a tendency to lose muscle mass and many people become less active for reasons such as health conditions. In a study of men and women of various insulin sensitivities, MF was significantly lower in the insulin resistant-non diabetic group (BMI: 28 kg/m^2 or higher) and the Type 2 diabetic group when compared to the insulin sensitive-lean control group [53]. The significantly lower MF in T2D subjects compared to controls was also seen in a study of obese men [54]. This study also determined that whole body glucose disposal predicts MF and insulin-stimulated RER, and mitochondrial function predicts basal RER. Obese adolescents with IR have a significantly lower MF than obese adolescents that are insulin sensitive [55]. A positive correlation was found between intermyofibrillar mitochondria content and MF, but there was no correlation between subsarcolemmal mitochondria content and MF [53]. Reduced ability to switch fuel sources has also been reported in obese men with impaired glucose tolerance, a prediabetic state [56]. Perhaps this inflexibility could be an early factor or marker in the development of T2D [56]. However, it remains unknown as to whether or not the inflexibility developed before or after these conditions and if family history plays any role. When examining men and women in two different prediabetic states, isolated impaired fasting glycemia (i-IFG) and isolated impaired glucose tolerance (i-IGT), MF was significantly lower in both groups when comparing them to the normal glucose tolerance (NGT) group [57]. This remained true after adjusting for insulin sensitivity and BMI. The authors think that the inflexibility may precede the development of insulin resistance in pre-diabetics, but again more studies need to be

performed. Soluble E-selectin (sE-selectin), a marker of endothelial dysfunction, was found to be negatively correlated to both MF and insulin sensitivity in a group of lean and obese women with normal glucose function [58]. Metabolic syndrome total Z-score was also negatively correlated to MF and insulin sensitivity and positively correlated to sE-selectin. The authors suggest that maybe the endothelial dysfunction leads to MF (improper fuel usage) and then insulin resistance follows.

1.4 Genetic, Gender, and Racial Factors

1.4.1 Family History

Family history of T2D may be a factor in the development of metabolic inflexibility. This idea has been evaluated in two feeding studies and one that involved passive stretching and a glucose load. There is a trend towards decreased MF in subjects with a family history of T2D and a decrease in mitochondrial DNA [8]. Mitochondrial DNA (mtDNA) content correlated inversely with sleep RQ during a high fat diet and positively with MF and IS. MtDNA content was also negatively correlated with BMI and body fatness and positively correlated with VO_{2max} [8]. This decrease in mitochondrial DNA may not allow the generation of enough energy to enable efficient substrate switching, and it is another factor related to obesity and a more sedentary lifestyle. After a high carbohydrate meal, serum insulin tended to be increased in subjects with a family history of T2D, but they were not significantly different from the controls in regards to their RQs [59]. After a high fat meal, subjects with a family history of T2D had a diminished capacity to reduce RQ compared to controls, suggesting an impaired ability to switch between fuel sources [59]. This was also confirmed over a three day high fat diet where subjects

with a family history of T2D had a significantly higher sleep RQ on the third day, which indicated a reduction in fat oxidation [8]. Sleep RQ measured when subjects were fed a high fat diet was also inversely related to MF, suggesting that subjects with a family history of T2D were already showing early signs of metabolic inflexibility [8]. Russell et al, used hood calorimetry to evaluate RER when subjects were passively stretched and consumed a glucose load [60]. Subjects had a 10 minute break from the hood after the stretching session. This is when the glucose load was consumed, and they returned to the hood. The subjects included controls, subjects with a family history of T2D, and subjects with T2D. Passive stretching increased carbohydrate use for all groups with a significantly greater CHO rate change for the subjects with T2D compared to the controls. There were no differences between groups in ΔRER during the stretching. The controls were more metabolically flexible than the subjects with T2D and the subjects with a family history of T2D. This was observed both in ΔRER AUC and the ΔRER at 15 minute intervals (up to 1 hour). There were no differences in MF between the subjects with a family history of T2D and the subjects with T2D. Taken together, these studies show that a genetic factor in skeletal muscle could predispose individuals to develop this inflexibility. However, two of the above studies do not clearly define how many of the subjects with a family history of T2D were already overweight or obese, a state which would increase their disease risk even without the history. Russell et al. [60] required that the control subjects and the subjects with a family history of T2D had a BMI in the normal range. The subjects with T2D had BMIs in the overweight range and were significantly older than the other groups. In addition, subjects with a family history of T2D had a significantly different response of the genes PGC1α (peroxisome proliferator-activated receptor coactivator-1α) and FAT/CD36 (fatty acid

translocase) to a high fat meal [59]. A high fat meal upregulates PGC1α and FAT/CD36 in lean controls, but suppresses both genes in the subjects with a family history of T2D.

1.4.2 Gender

When examining gender differences in healthy, young subjects, it has been determined that women had higher MF than men, even though they had significantly more body fat than the men [9]. This may be surprising due to the negative correlation found between % body fat and MF as mentioned previously in a population of men. It would seem that women should be less flexible. We speculate that it is the distribution of the fat that may be more important. Visceral adipose tissue mass was negatively correlated with MF for both the women and men, and women had less [9]. In addition, fat cell size was not significantly different between the groups, again suggesting that it is not how much fat, but how it is distributed that influences MF. Fasting and insulin-suppressed non-esterified fatty acids differed between men and women; women had significantly higher fasting non-esterified fatty acids (NEFAs) than men but had significantly lower insulin-suppressed NEFAs than men [9]. MF was negatively correlated with insulin-suppressed NEFAs. Adiponectin levels were higher in women than men, and adiponectin was positively correlated with MF in men; however, the correlation was not significant in women. Women also had a higher expression of the genes involved in adipogenesis (peroxisome proliferator-activated receptor- γ2, PPARγ2), lipid storage (phosphoenolpyruvate carboxykinase 1, PCK1 and stearoyl-Coenzyme A desaturase 1, SCD1), and lipid oxidation (peroxisome proliferator-activated receptor-α, PPARα). Sparks et al. [9] suggests that adipose tissue inflammation could affect MF, since it has a negative impact on IS. Men and women displayed

no differences in macrophage content markers, expression of chemokines, or classic inflammatory pathway (M1). However, women had a significantly higher anti-inflammatory pathway (M2) marker leading to the hypothesis that higher MF in women could be due to their macrophages being predominantly activated along an anti-inflammatory pathway [9]. In addition, each gender only had two adipose tissue factors that correlated with MF. For women, they were SCD1 and PCK1 mRNA, and for men they were serum adiponectin and insulin-suppressed NEFAs. Also, men and women both demonstrate a positive correlation between intermyofibrillar mitochondria content and MF [53].

1.4.3 Race

Due to the prevalence of obesity being higher in African Americans, and African Americans being at high risk for T2D [6], does this also mean that they are metabolically inflexible? Berk et al. [61] found that Caucasian women had significantly higher fat oxidation during a high fat diet and higher carbohydrate oxidation during the low fat diet, whereas African American women had no significant differences. However, Stull et al. [11] reported that MF was greater in African American men and women compared to Caucasian men and women and remained greater after adjusting for IS, diabetes status, triglyceride concentrations, and fasting RQ. Adjusting for diabetes status was important due to the unbalanced number of African Americans (n=19; 4 men, 15 women) and Caucasians (n= 60; 35 men, 25 women) with T2D. Delta carbohydrate oxidation was not different between the races, but Δ fat oxidation was significantly higher in African Americans than Caucasians even after adjusting for IS, diabetes status, fasting triglyceride concentrations, and fasting fat oxidation [11]. These studies gave conflicting reports in regards to how race affects MF. Interestingly one study only examined

women and did not actually measure MF (ΔRQ). Instead, fat and carbohydrate oxidation was examined to determine if there was an impaired ability to switch substrate use. The comparison of the articles would have been more interesting if they both measured (MF), or if Stull et al. [11] considered comparing the role of race in men and women separately as well.

Another study examined South Asians due to their high T2D risk compared to Europeans [62]. Middle-aged men with a family history of T2D consumed a very low calorie diet for 8 days. After the 8 days, the shift from glucose oxidation to lipid oxidation was significantly less for the South Asian men than the European men. Based on MF in terms of fat oxidation rates, the South Asian men were less flexible when adapting to the diet.

1.4.4 Medical Conditions

Metabolic flexibility of subjects with various medical conditions (other than T2D or IR) has been reviewed recently. . In a study of men with (n=11) or without (n=11) Type 1 diabetes (T1D), men with T1D had a significantly lower basal RQ and a significantly lower MF than the controls [63]. During the clamp, the subjects with T1D had a significantly higher lipid oxidation rate throughout the clamp and a significantly lower carbohydrate oxidation rate during periods I and II of the clamp when compared to the controls. When evaluating 16 subjects with Multiple Sclerosis (MS) and 16 control subjects, the MS subjects had a higher MF than the controls [64]. The higher RQ after the glucose load is indicative of a higher carbohydrate oxidation rate. When receiving the oral glucose load immediately prior to exercise, the MS subjects increased their RQ to a higher level than the controls, demonstrating that they were using more carbohydrates. However, the MS subjects had a sharp decline in RQ after 20 minutes of exercise and continued to decline after completion of the exercise. There was no difference in MF between subjects

with anorexia nervosa and controls [65]. There were conflicting results in two studies involving women with polycystic ovary syndrome (PCOS). In one study involving lean and obese women with or without PCOS, it was found that there was no difference in MF between the subjects with PCOS and the controls; however, MF of the obese subjects (with or without PCOS) was significantly lower than the lean subjects [66]. It was further reported that women with PCOS had a significantly lower MF than data previously obtained from healthy women [67]. Normo-androgenemic PCOS women had a significantly higher MF than hyper-androgenemic PCOS women. For this group of women, MF was positively correlated with glucose disposal rate and negatively correlated with BMI, free testosterone, fasting insulin, and baseline RQ. A possible reason for this variation in results is that there were more PCOS subjects in first article that were lean with a BMI in the normal range (21.4 ± 2.0 kg/m^2, mean ± SD; 40 out of 92 total PCOS subjects vs. 24 out of 89 PCOS subjects). PCOS women were also found to use less lipid during an overnight fast compared to women without PCOS [68].

MF has also been examined in obese adolescents with and without non-alcoholic fatty liver disease (NAFLD) [69]. The adolescents with NAFLD had a significantly higher fasting RQ than the ones without the disease. Both groups had a significant increase in RQ from fasting to insulin-stimulated; however, the adolescents without the disease had a significantly higher MF that the adolescents with NAFLD. This significantly higher MF in adolescents without NAFLD was also shown by Perseghin et al.[70] The authors also found that the adolescents with NAFLD had a higher fasting RQ than the adolescents without the disease.

1.5 Environmental Factors

1.5.1 Diet

There have been a variety of metabolic flexibility studies that have related to diet. Diet composition does not have an effect on daily energy expenditure, but daily substrate oxidation shifts so that it is closer to matching substrate intake [71]. This means that a person will use the same amount of energy (kcals) no matter what the diet composition is, but the percentage of kcals from each substrate changes with the composition. For example, a high fat intake will result in a shift toward increased fat oxidation, or a higher percentage of daily energy from fat. High fat diets promote positive fat and protein balances and a negative carbohydrate balance is achieved [72]. Fasting insulin was found to be positively correlated to fat balance over the four days on a high-fat diet, and VO_{2max} and carbohydrate balance were negatively correlated to fat balance [72]. When shifted to a high fat diet, physical activity improved fat oxidation in young, healthy men [73]. Fat balance (intake – oxidation) over the four days was lower during high activity. In comparison, carbohydrate and protein balances were higher during high activity.

In addition to analyzing the role of family history, Ukropcova et al.'s [8] diet study identified that during a 3-day high fat diet, sleep RQ was negatively correlated with MF and positively correlated with VO_2max. Fat balance over the three days was positively correlated with sleep RQ and 24-h RQ data from day three. These findings demonstrate that subjects accumulated more fat when they had reduced fat oxidation and therefore a higher 24-h RQ, resulting in impaired switching and an inability to match oxidation to intake [8]. After the high fat diet, mtDNA was positively correlated with plasma adiponectin. Plasma adiponectin levels have been shown to be lower in type 2 diabetics than nondiabetics [74], and mtDNA is positively

correlated with MF [8]; therefore, the positive correlation between mtDNA and adiponectin is not surprising.

When on a high fat diet for 3 weeks, overweight men had a decrease in MF and an increase in intrahepatic lipid (IHL) content when compared to their low fat diet counterparts [75]. Insulin sensitivity and IMCL were not affected by the diet. Fat oxidation was significantly increased in the high fat group during the clamp which demonstrates a decrease in MF; however, the authors indicate that this could just be the result of the body adapting to the high fat diet.

After one high fat meal, a group of healthy, lean men and women had an increase in mRNA content of peroxisome proliferator activated receptor α (PPAR-α) whereas the obese, insulin resistant men and women did not [76]. After continuing on the high fat diet for 5 days, mRNA content for various genes involved in lipid oxidation (PPAR-α, pyruvate dehydrogenase kinase 4, PPAR γ coactivator-1α, and uncoupling protein 3) increase in lean, but decreased in the obese subjects. The authors deemed the ability to up-regulate these lipid oxidation genes in response to a high fat diet as indicative of metabolic flexibility.

A three month intervention involving obese men and women (65 ± 1 years old, mean ± SEM) performing aerobic exercise and consuming either a high or low glycemic diet found that metabolic flexibility was significantly improved post intervention for both diet groups [77]. However, MF was not significantly different between diet groups pre or post intervention.

The use of salsalate after meals has been examined for improving MF. Nine healthy sedentary men (21.8 ± 1.0 years old, mean ± SE) participated in hyperinsulinemic-euglycemic clamp protocols [78]. There were three treatments: control, Intralipid, and Intralipid with salsalate. During the clamps, the control and Intralipid treatments were co-infused with glycerol and Intralipid, respectively. The Intralipid with salsalate treatment involved consumption of

salsalate after meals for 4 days prior to the clamp (4000mg/d), an additional dose of 1500 mg of salsalate on the morning of the clamp, and co-infusion of Intralipid during the clamp. The Intralipid infusion was to induce insulin resistance. It was found that MF was significantly reduced in the Intralipid and Intralipid w/salsalate groups compared to the controls. Intralipid w/salsalate group tended to have a higher MF than the Intralipid group, but the difference failed to be statistically significant. However, the carbohydrate oxidation rate during the clamp was higher for the Intralipid w/salsalate group compared to the Intralipid group. There were no differences in the density or functioning of the mitochondria taken from muscle fiber sample. When overweight men and women were supplemented with epigallocatechin-gallate (E) and resveratrol (R) for three days, MF increased compared to the subjects that consumed the placebo or E+R+soy isoflavones but only in the men [79]. There was no difference in women. MF was measured as the difference between the highest RQ postprandially and fasting RQ. The ratio of respiratory exchange ratio to food quotient (RER:FQ) while fasted was used as a way to evaluate MF with a value greater than 1 indicative of positive fat balance (fat intake>fat oxidation) [80]. The RER:FQ ratio was not significantly different between the insulin sensitive and insulin resistant groups. Adjusting for age and % body fat resulted in RER:FQ being significantly lower in subjects with a family history of T2D compared to no family history, and also significantly lower in insulin resistant subjects (homeostasis model assessment (HOMA) ≥ 1.95). The main limitation with this study was that fat intake was assessed using a food frequency questionnaire (FFQ).

Metabolic flexibility has also been defined as an increase in fat oxidation in response to a high fat diet. Lean men significantly increased fatty acid oxidation (measured by complete palmitate oxidation in skeletal muscle biopsies) after 3 days on a high fat diet compared to obese

men [14]. Seven days of exercise increased both groups' fatty acid oxidation compared to pre-diet; however, adding 3 days of a high fat diet to the exercise did not result in a significant increase in fatty acid oxidation. Also, the lean and obese groups were not significantly different after the exercise. Exercise may have helped the obese group. Another group examined the effects of the infusion of Intralipid during the hyperinsulinemic-euglycemic clamp among lean endurance-trained, lean sedentary, and obese sedentary males and females [15]. They determined that the trained subjects were more metabolically flexible because they were better able to increase their fat oxidation in response to the lipid overload than both the lean and obese sedentary subjects. This highlights how exercise helps with substrate utilization.

Metabolically inflexible subjects also require more days to adjust their 24-h RQ to a high fat or high carbohydrate diet, but eventually they will regulate their 24-h RQ to around the same level as their metabolically flexible counterparts [81]. This reveals how inefficient the subjects are at switching so that substrate oxidation matches intake even over the course of days.

1.5.2 Caloric restriction

Another evaluation method was used to determine how 25% caloric restriction affects MF [82]. MF was determined by examining the concentration differences in various metabolic intermediates between the fasting and postprandial state. Caloric restriction resulted in significantly larger changes in acylcarnitines (AC) over time and trends toward larger changes in FFA over time compared to the controls. The authors determined that this was indicative of an increase in MF (AC and FFA concentrations higher during fasting).

Healthy lean and obese men and women underwent 48 hours of fasting [83]. After 24 and 48 hours of fasting, lipid oxidation increased and glucose oxidation decreased from baseline,

which would be the expected metabolic response to these conditions. However, the obese subjects had a significantly lower increase in fat oxidation and a significantly lower decrease in glucose oxidation than the lean subjects, which demonstrates metabolic inflexibility. This study also found that the skeletal muscle of obese subjects had a lower expression of insulin receptor-β (IRβ) at baseline, and fasting resulted in a reduction in AMP-activated protein kinase (AMPK) activity only in lean subjects.

1.5.3 Exercise

Exercise can improve insulin sensitivity, giving rise to the question: "Can exercise and weight loss improve MF as well especially with the positive correlation between IS and MF?" Obese men and women without diabetes improved their VO_2max and IS after undergoing an exercise and calorie reduction weight loss intervention [84]. In addition, their fasting fat oxidation rates increased as well as their energy derived from fats. This change in fat oxidation rates demonstrates that these obese subjects were already showing some impaired substrate use and possibly impaired substrate switching, but losing weight provided some improvement. Obese subjects also showed improvements in IS and suppression of fat oxidation by insulin after weight loss, but no change in their fasting leg RQs [19]. In Type 2 diabetics, a 3-month exercise protocol increased their whole-body GDR and non-oxidative GDR; however, glucose oxidation only tended to increase [85]. In obese men with impaired glucose tolerance (IGT), weight loss improved their response to a meal by increasing their forearm muscle RQs [56]. This increase in RQ was blunted prior to the weight loss when compared to subjects with normal glucose tolerance. Fasting fat oxidation improved as well in the subjects with IGT, but intramyocellular

triglycerides only tended to decrease. After a one-year lifestyle intervention to lose weight via increased physical activity and decreased caloric intake, type 2 diabetic subjects experienced an increase in both glucose disposal rate and MF to glucose [10]. However, there was no difference in MF to glucose before or after the intervention once they controlled for glucose disposal rate. A 12-week training program restored MF in obese Type 2 diabetic males to the level of the obese controls [7]. This program also improved IS significantly in T2D subjects and improved mitochondrial function in both groups, with the T2D subjects returning to control levels. 24 obese adults underwent a 12 week exercise intervention [86]. An OGTT was performed to group them by their prediabetes status: IFG, IGT, and IFG+IGT (n=8 for each group). MF was significantly increased after the intervention in the IFG and IGT groups. Some of these subjects joined additional obese, prediabetic adults to participate in another 12 week exercise intervention study [87]. This study also found that after the intervention, MF was significantly improved, and MF negatively correlated with fetuin-A, a hepatokine that may be linked to the development of T2D.

Type 2 diabetics who have a dodecanedioic acid (C-12) meal prior to exercise have a reduction in muscle fatigue and an increase in lipid oxidation [88]. Insulin secretion is not stimulated by C-12. After the C-12 meal, the amount of glucose oxidized was less and the amount of C-12 plus lipids oxidized was more than after the glucose meal. The authors thought that C-12 could potentially restore MF "by reverting the oxidative pathways to a quasi-physiological condition, as observed in controls." However the benefit seems to only extend to using C-12 during exercise. More research is needed to further examine exercise and training effects on MF.

As mentioned in the introduction, not all studies measure ΔRQ when discussing metabolic flexibility. For Bergouignan et al. [16, 89], MF is determined by the variance in RQ and insulin. A subject that is metabolically flexible would have a large variance in RQ and a small variance in insulin. Data was combined from two studies to examine the effects of physical activity level (PAL) on MF. The subjects included trained men that detrained for 1 month, sedentary lean and obese men that trained for 2 months, and active women that underwent strict bed rest or strict bed rest with exercise for 2 months. Increases in PAL resulted in an increased MF (decreased insulin variance, increased RQ variance), and PAL was positively correlated with RQ variance for all subject groups. Detraining resulted in a significantly lower variance in RQ and a significantly larger variance in insulin, whereas training tended to increase RQ variance and decrease insulin variance, but was not statistically significant.

MF has also been interpreted as switching to increased fat oxidation during sustained exercise [90]. Using this definition, a group of young, normal BMI women were evaluated during a 45 min exercise period (at 65% VO_2max) by their ratio of abdominal to lower body fat [91]. The women with a lower abdominal to lower body ratio had significantly higher fat oxidation rates during exercise and therefore a higher MF than the women with a higher ratio. There was also a positive correlation between the ratio and lipid oxidation rates. There was no difference at rest. The women with a higher ratio also had significantly higher RER during exercise.

Prior et al. evaluated MF when transitioning from fasting/resting to exercise in addition to the response when transitioning from fasting to an insulin-stimulated state using hyperinsulinemic-euglycemic clamp technique [13]. Overweight/obese subjects (men and women) participated in a 2 hour OGTT, and using the American Diabetes Association criteria, it

was determined that 13 had NGT and 10 had IGT. The IGT subjects had a significantly lower MF than NGT subjects during insulin stimulation via the hyperinsulinemic-euglycemic clamp. During treadmill exercise at 50% and 60% VO2max, the IGT subjects also had a significantly lower MF than NGT subjects. There was no correlation between the insulin-stimulated MF and the exercise MF. The two groups had similar energy expenditure during the exercise; however, the IGT subjects used more fat than the NGT subjects at each intensity. Once again, metabolic inflexibility was present prior to subjects developing T2D. During the exercise, glucose intolerance was negatively correlated with RQ.

A different group defined metabolic inflexibility as oxidizing less carbohydrate (using more fat) and using less muscle glycogen during exercise [92, 93]. For overweight women divided into IS and IR groups, it was found that the IR group used less carbohydrate and less muscle glycogen when walking on a treadmill at 45% VO_2max than the IS group, but blood glucose uptake was not different between the groups [92]. Additionally, during exercise, men and women with IFG+IGT used less muscle glycogen and had higher fat oxidation than a group of men and women with IGT alone [93]. The IFG+IGT group also had significantly higher blood glucose and NEFAs.

In summary, various topics related to metabolic flexibility has been addressed, but more research is needed to establish a quantitative index or identify a “normal” range. It would also be helpful if a more streamlined definition of how to measure MF is used. The term has been used as a description of many metabolic functions. We believe that it is time to come to a consensus about the most appropriate method to analyze this variable.

Chapter 2: Metabolic Flexibility: Adaptation to Diet and Exercise Challenges

2.1. Abstract

Metabolic flexibility is commonly measured as the change in respiratory quotient (RQ) from the fasted state to the insulin-stimulated state. This measurement is performed using a hyperinsulinemic-euglycemic clamp and hood calorimetry. A study was performed to evaluate if room calorimetry alone could provide the same results and to examine how diet composition and exercise affect metabolic flexibility. Sixteen healthy males and females stayed overnight in a room calorimeter on four separate occasions. While in the calorimeter, they participated in meal and exercise challenges. The subjects took part in one of two exercise sessions on the treadmill (high intensity – short duration or low intensity – long duration). They were also provided one of two treatment beverages for lunch (high carbohydrate or high fat shake). Blood samples were collected before and 30, 60, 90, and 120 minutes after breakfast and lunch to measure glucose, insulin, and non-esterified fatty acids (NEFAs). Metabolic flexibility was calculated in a manner similar to the literature, and new metabolic flexibility variables were also considered including the rate of change of RQ (slope) following exercise or a meal and the lag in RQ following exercise. Delta RQ - calculated as the difference between a two hour average night RQ and a two hour average morning RQ - was negatively correlated to % body fat, positively correlated to VO_2peak, and trending negatively with age. The post-lunch slope of RQ was significantly larger following the high carbohydrate shake compared to the high fat shake. The slope of the increase in RQ following the start of exercise

was negatively correlated with age and % body fat and positively correlated with VO_2peak. This study shows that the non-invasive room calorimetry method can be used to measure many metabolic flexibility variables.

2.2 Introduction

The phrase "metabolic flexibility" was termed by Kelley and Mandarino [21] and is specifically defined as "the capacity to switch from predominantly lipid oxidation and high rates of fatty acid uptake during fasting conditions to the suppression of lipid oxidation and increased glucose uptake, oxidation, and storage under insulin-stimulated conditions." Typically, MF is measured as the difference between insulin-stimulated respiratory quotient (RQ) and fasting RQ; however, not all studies that discuss MF calculate ΔRQ. Some discuss MF in terms of adjusting fat oxidation [14, 15] or by the variance in insulin and RQ after a meal [16]. A hyperinsulinemic-euglycemic clamp and a hood calorimeter are commonly used to measure MF [7-11], but an indirect room calorimetry could be used as well. Unlike the hyperinsulinemic-euglycemic clamp, room calorimetry alone is noninvasive and allows the subject to be ambulatory; therefore, they can perform tasks like walking on a treadmill or eating. It also allows for a more physiological response, whereas in the clamp technique, insulin and glucose are infused via a catheter, and blood measurements would determine the amounts of glucose to infuse from outside the body. There is "noise" associated with the calorimeter that does not allow for observing the instantaneous measures of RQ. The RQ would be averaged over some time period, but that eliminates the effects of major deviations collected during the sampling period. For example, a change in RQ after eating will not be noticed in the

average, yet close examination of the data consistently reveals an increase in RQ during a meal or during exercise.

A new method has been developed that smoothes the room calorimeter data so that it can be evaluated "instantaneously" (in 1-2 minute intervals) [29]. This means that variations in RQ, O_2 consumption, and CO_2 production can clearly be observed during and immediately after meals or exercise. From this new insight into the data, it is now possible determine how quickly a subject switches fuel sources as the result of an event. For example, during exercise, a subject's RQ increases to reflect use of carbohydrates for energy, and it typically would increase almost simultaneously with VO_2 (energy expenditure). Alcohol trials have confirmed that this new method provides physiological data that is separate from any calorimeter noise (unpublished data). This method would allow for the usage of a room calorimeter to measure variables of MF to determine how the results compare to those in the literature that use other methods.

The primary objectives of this study were (1) to use room calorimetry to measure metabolic flexibility and to compare the results to those collected using other methodologies; (2) to examine the effects of diet composition and exercise interventions on metabolic flexibility variables (ΔRQ, slope of RQ, variance of RQ); and (3) to see if there are any correlations that can be established between the metabolic flexibility variables and the physical characteristics of the subjects (age, BMI, % body fat, VO_2peak, and HOMA-IR). It is hypothesized that metabolically inflexible subjects will have a slower rate of change (smaller slope) after a diet or exercise challenge. The change in RQ will be lower in metabolically inflexible

subjects. It is hypothesized that subjects with a higher VO_2peak and lower body fat will switch substrate use faster (have a steeper RQ slope) than less aerobically fit subjects. The high carbohydrate lunch shake will result in a larger RQ response and a quicker response than the high fat meal. Following exercise, the new MF lag time variable will be longer for subjects with a higher % body fat and lower VO_2peak. In addition, subjects that are more fit (higher VO_2peak, lower body fat) will have a larger variance in RQ after a meal and a lower variance in insulin.

2.3 Methods

2.3.1 Subjects

Subjects were recruited from the area around the Beltsville Human Nutrition Research Center (BHNRC) and at the University of Maryland via fliers and emails. All procedures were approved by the MedStar Health Research Institute Institutional Review Board. The University of Maryland Institutional Review Board (IRB) has an authorization agreement in place with the MedStar Health Research Institute IRB for this study. Volunteers were required to attend an information meeting to learn about the study and ask any questions. If they were interested in participating, they were asked to complete a study application and provide written informed consent before participating in any aspect of the study. Volunteers were then required to complete a health history questionnaire and come to the Center for a health screening. During this visit, their height, weight, and blood pressure were measured and body mass index (BMI) was calculated. The volunteers also provided a blood and urine sample to perform the following tests: lipid panel, comprehensive metabolic panel, complete

blood count (CBC), thyroid stimulating hormone (TSH), fasting glucose, and urinalysis. When the lab results were received, a certified nurse practitioner reviewed the subjects' charts to determine who was eligible to participate. Of the eligible volunteers, 16 (8 men and 8 women) were selected to form a homogeneous group to take part in the study (**Figure 2.1**). Volunteers were between the ages of 22 and 35, nonsmokers, and had no history of diabetes, cancer, metabolic disorders (**Table 2.1**). Subjects also required no special diets and did not have any food allergies. Subjects were advised that participation was voluntary.

Table 2.1: Ineligibility Criteria
(as listed on page 2 of the Informed Consent)

- Younger than 22 years old or older than 35 years old
- Women who have given birth during the previous 12 months
- Pregnant women or women who plan to become pregnant or become pregnant during the study
- Lactating women
- History of bariatric or certain other surgeries related to weight control
- History or presence of diabetes, kidney disease, liver disease, certain cancers, gout, hyperthyroidism, untreated or unstable hypothyroidism, gastrointestinal disease, pancreatic disease, other metabolic diseases, or malabsorption syndromes
- Smokers or other tobacco users (within 6 months prior to the start of the study)
- History of eating disorders or other dietary patterns which are not consistent with the dietary intervention (e.g., vegetarians, very low fat diets, high protein diets)
- Volunteers who have lost 10% of body weight within the last 12 months or who plan to initiate a weight loss program during the next 10 months
- Unable or unwilling to give informed consent or communicate with study staff
- Self-report of alcohol or substance abuse within the past 12 months and/or current acute treatment or rehabilitation program for these problems (long-term participation in Alcoholics Anonymous is not an exclusion)
- Other medical, psychiatric, or behavioral factors that in the judgment of the Principal Investigator may interfere with study participation or the ability to follow the intervention protocol

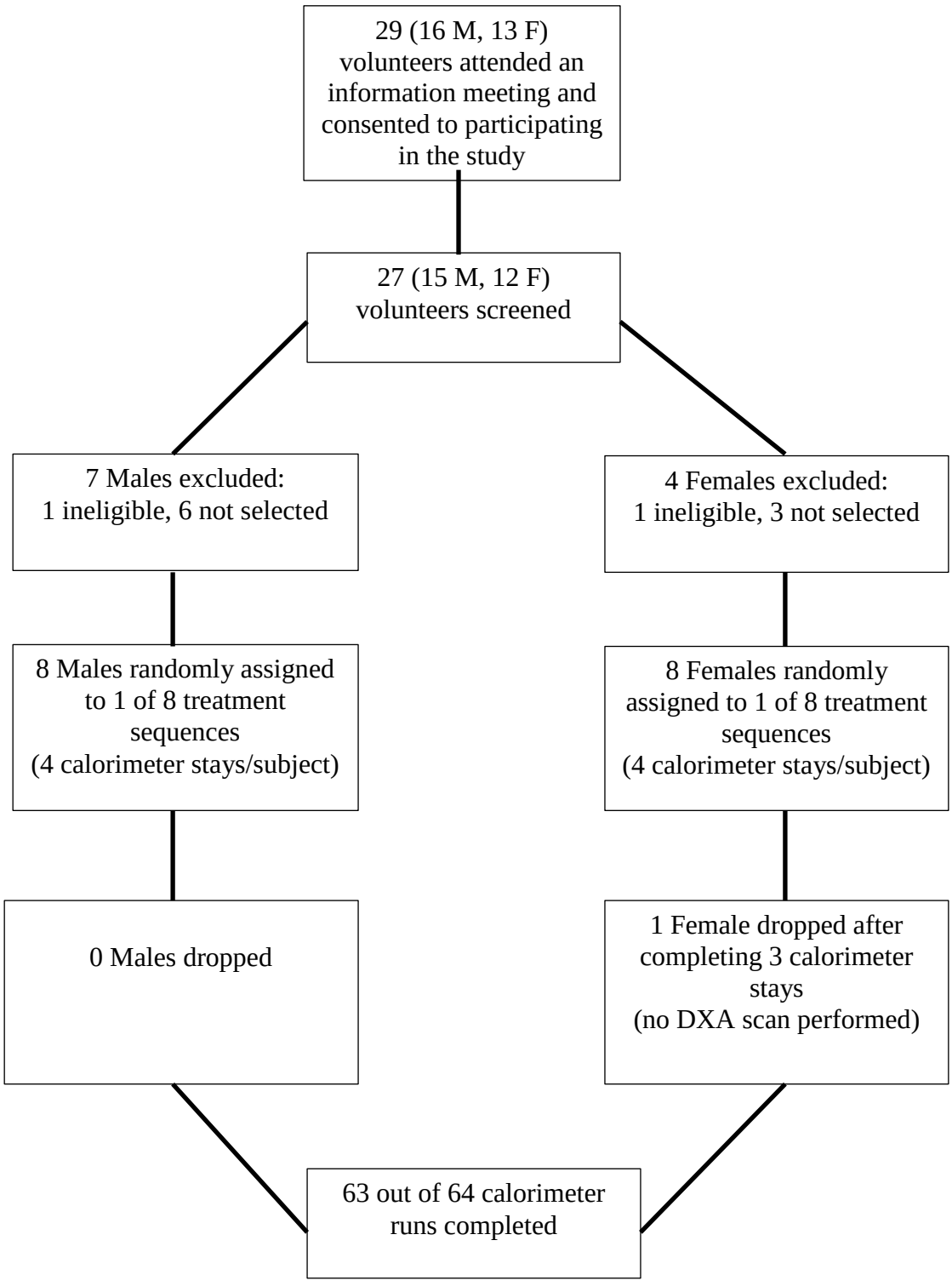

Figure 2.1: Subject selection flowchart

2.3.2 Design

The study involved volunteers staying overnight in a room calorimeter on four separate occasions with at least two weeks between each session. At each stay, they would receive one of four treatment combinations involving exercise (high or low intensity) and diet (a high fat or high carbohydrate shake for lunch). The treatment sequence was developed using two 4x4 Latin Squares for each gender. Subjects were then randomly assigned to a treatment sequence. One male and one female completed each of the 8 sequences. Subjects were compensated $150 for each calorimeter stay, and payment was received at the completion of the study.

2.3.3 Diet

On day one of the calorimeter stay, subjects were provided a base diet (approximately 58% carbohydrates, 25% fat, and 17% protein). Their kcal level was determined by estimating total energy expenditure with prediction equations (Harris-Benedict and Miflin-St. Joer). Kilocalorie levels were in increments of 200 kcal. The subjects came to the Center in the morning to consume breakfast and receive a packed lunch to take with them. Subjects were limited to 16oz of caffeinated beverages (coffee, tea, diet soda) at breakfast on Day 1 and were required to consume the same thing every morning that they came to the Center for breakfast. When they returned in the late afternoon, they consumed dinner in the calorimeter at approximately 6 PM. They had to finish dinner by 6:45PM to ensure that they were fasted for 12 hours before their baseline blood draw the following morning. The times of their first and last bites were recorded. On day two at approximately 7:15 AM, the subjects

received a standard breakfast of waffles and syrup, which contained 91g of carbohydrates (**Table 2.2**) to simulate an oral glucose tolerance test (OGTT). They had 15 minutes to consume breakfast, and first and last bite times were recorded. They also received a standard lunch at approximately 12 PM which consisted of either a high fat (HF) or high carbohydrate (HC) shake. Table 2.2 provides the composition of the shakes. The shake recipes were modified from shakes used in a previous study at the Center [94]. The HF shake was made with heavy cream, powdered egg whites, water, sugar, aspartame, and cocoa powder. The HC shake was composed of plain rice milk, non-fat vanilla coffee creamer, powdered skim milk, water, sugar, and cocoa powder. They were required to consume the shakes in 15 minutes, and first and last bite times were recorded. Subjects had to consume all food that was provided to them on the treatment days, and they could drink as much water as they wanted. When subjects were not taking part in a calorimeter stay, they could consume their typical diet at home.

Table 2.2: Day 2 Standardized Menu
Waffles & syrup was served for breakfast and either the HC or HF shake was served for lunch

	Energy (kcal)	**Carbohydrate (g)**	**Fat (g)**	**Protein (g)**
Waffles & Syrup	486	91	11	8
High CHO Shake	548	123	3	7
High Fat Shake	519	12	51	7

2.3.4 Exercise

Prior to the calorimeter stays, subjects reported to the Center to perform a VO_2peak test. When they arrived, their blood pressure was measured. The VO_2peak procedures were explained to them, and they were advised not to change their physical activity regimen during the duration of the study. Subjects were fitted with a facemask and wore a heart rate monitor (Polar Wearlink® 31, Polar Electro Inc., Lake Success, NY) during the test. A metabolic cart (TrueOne® 2400, Parvo Medics, Inc., Sandy, UT) was used to determine the oxygen consumption of the air flowing through the facemask. The metabolic cart was calibrated each time it was powered on. The VO_2peak test was performed on a treadmill **(**Trackmaster TMX425C, Full Vision, Newton, KS) using the Bruce protocol (**Table 2.3**), which involved the speed and incline of the treadmill increasing every 3 minutes. The test ended when the subject decided that he or she could no longer continue. Each subject would then perform a cool down. Thirty second averaging was done on the subjects' breaths. The VO_2peak results were used to calculate the treadmill settings for the exercise session in the calorimeter. An RQ of at least 1.1 and a comparison of their heart rate (HR) during the test to their age predicted maximum HR were used to determine if the test was successful. Evaluation of the graphs to determine if a subject's VO_2 and RQ plateaued or a rate of perceived exertion (RPE) of at least 8 (on a scale of 1 to 10) were also considered for determination of a successful test. Subjects were provided a copy of their test results upon completion of the study.

Table 2.3: Bruce Protocol

Stage	Minutes	Speed (mph)	% Grade
1	0-3	1.7	10
2	3-6	2.5	12
3	6-9	3.4	14
4	9-12	4.2	16
5	12-15	5.0	18
6	15-18	5.5	20
7	18-21	6.0	22

During the VO2peak testing, subjects start at stage 1 with the treadmill set at a speed of 1.7 mph and an incline of 10%. Every 3 minutes the speed and incline of the treadmill change. Subjects proceed through the stages until they can no longer continue.

The two exercise treatments performed on the treadmill (Smooth Fitness 7.1HR, Smooth Fitness, Mt. Laurel, NJ) in the calorimeter were high intensity – short duration (HI) and low intensity – long duration (LI). HI was performed at approximately 85% of the subjects' VO_2peak and involved four five-minute sessions on the treadmill with 5 minutes of rest in between. LI was one 40 minute session on the treadmill at approximately 65% of the subjects' VO_2peak. A standard metabolic equation was used to calculate the appropriate speed for each subject at an incline of 2 ($VO2 = (S \cdot 0.1) + (S \cdot G \cdot 1.8) + 3.5$ where S=speed (m/min) and G=grade in decimals) [95]. While in the calorimeter, subjects were instructed to only use the

treadmill when they were told to do so and to not perform any other exercises. Staff monitored the exercise sessions to record the start and stop times of the sessions and to ensure that the subjects were not struggling or having difficulty using the treadmill. Exercise occurred at approximately 10AM, about 30 minutes after the final morning blood draw. Subjects did not consume water during the exercise period to prevent any temperature fluctuations in the core temperature pill (see Ambulatory Monitoring Device section below).

2.3.5 Calorimeter

Subjects stayed overnight in an indirect room calorimeter to determine energy expenditure. They stayed in the same calorimeter on each occasion to prevent any calorimeter variations. The calorimeter measures the oxygen consumed and the carbon dioxide produced. Smoothing the calorimeter data by a method discussed previously [29] also allows for evaluation of "instantaneous" changes in the volume of oxygen consumed (VO_2) and respiratory quotient (RQ). All urine was collected during the calorimeter stay to determine urinary nitrogen for estimation of protein oxidation. VO_2, VCO_2, and urinary nitrogen values were used in the equations by Livesey et al. to calculate carbohydrate, fat, and protein oxidation. Subjects entered the calorimeter at approximately 4:45 PM and remained in the calorimeter for 23.5 hours. The calorimeters are approximately 9' by 12' and contain a Murphy bed, desk, TV, DVD player, computer, phone, treadmill, air conditioner, toilet, and sink. Meals were served through an airlock in the door. On the subjects' first stay, they were given a "tour" of the calorimeter where they received instructions on how to use the equipment in the room. The schedule of activities during their stay was also

explained. The calorimeter lab was staffed throughout the entire calorimeter stay, and the calorimeter doors were not locked in case of an emergency. Subjects were asked to leave at least one window blind open except for when they needed privacy (changing, using the bathroom) so that staff could check on them. Subjects were allowed to do low-activity tasks such as lying down, watching TV, reading, and computer/desk work when they did not have any study requirements. The time the subject entered the calorimeter (when the door closed) and exited (when the door was opened) was recorded. Prior to the subjects entering the calorimeter, alcohol lamps were burned in order to increase the CO_2 levels in the calorimeter. This allowed the calorimeter to acclimate more quickly upon the subjects' entrance. Energy expenditure, substrate oxidation, ΔRQ, post lunch RQ AUC, RQ variance, post meal RQ slopes, and exercise RQ slopes were calculated. Lag between RQ and VO2 following exercise was calculated using cross-correlation.

2.3.6 Blood Draws

On the morning of Day 2, subjects had an IV catheter placed in the antecubital area of the arm by a nurse or phlebotomist. The subjects inserted one arm through an arm port located in one of the calorimeter windows for blood draws and BG measurements. Two fasting baseline blood draws were performed prior to breakfast. Blood was then drawn 30, 60, 90, and 120 minutes after the subjects finished breakfast. Blood was then drawn immediately prior to lunch and then 30, 60, 90, and 120 minutes after the subjects finished the lunch shake. 10mL of blood was drawn at each time point for a total of 110mL during each calorimeter stay – approximately ½ cup. The catheter was removed following the last blood draw. All times of the blood

draws were recorded. For each time point, serum, plasma EDTA, and plasma NaF vacutainers were collected and inverted. The EDTA and NaF vacutainers were placed on ice until they were centrifuged. The serum tube sat for 30-60 minutes at room temperature to allow for clotting and was centrifuged at room temperature. After centrifugation, the serum and plasma vacutainers were aliquoted into the appropriate labeled cryovials and stored in a -80^{o}C freezer for analysis at a later time.

Serum insulin was measured by enzyme-linked immunosorbent assay (ELISA, EMD Millipore Corporation, Billerica, MA) on a DSX® workstation (Dynex Technologies, Chantilly, VA). Plasma glucose was measured by performing a colorimetric test using a Vitros GLU slide on a clinical chemistry analyzer (Vitros, Ortho Clinical Diagnostics, Rochester, NY). Serum NEFAs were measured by an enzymatic colorimetric assay (Wako Diagnostics, Richmond, VA) on a clinical chemistry analyzer (Vitros, Ortho Clinical Diagnostics, Rochester, NY). All blood samples were run in duplicate. Homeostatic model assessment – insulin resistance (HOMA-IR) was calculated as described previously [96]. The 2 hour post breakfast and lunch AUC was analyzed for glucose, insulin, and NEFA. The variance of insulin was calculated for approximately 2 hours post breakfast and lunch.

2.3.7 Urine Collection

Urine was collected during the entire calorimeter stay in order to analyze urinary nitrogen. Subjects were provided with a 4L urine jug in the calorimeter. Ten grams of boric acid was placed in the urine jug prior to collection to function as a preservative. The tare weight of the jug and boric acid was recorded. A second smaller jug was provided if needed. At the end of the calorimeter stay, the urine jug

was weighed, and the weight was recorded. The urine jug was shaken, and the urine was aliquoted into two 5 mL cryovials and one 15 mL centrifuge tube. The samples were stored in a -20^{o}C freezer until they were analyzed. If two jugs were used, 5% of each sample was mixed together and then aliquoted. A nitrogen analyzer (TruMac, LECO Corporation, St. Joseph, MI) was used to measure urinary nitrogen, and samples were run in duplicate.

2.3.8 Body Fat Composition

On the subjects' third or fourth calorimeter stay, a dual-energy X-ray absorptiometry (DXA) scan was performed to determine their percent body fat (Hologic QDR Discovery A, Hologic, Bedford, MA). A quality control test was performed when the DXA was turned on for the day. The scan was performed when the subjects exited the calorimeter on Day 2. Staff would go through prescreen questions with the subject to verify that there were no safety exclusions (such as having an X-ray with contrast in the last week) and that they were not wearing any metal. The subjects would then be positioned in the center of the table and informed to lie still and close their eyes. The whole body scan took approximately 4 minutes. After the scan, the staff would perform the analysis of the scan by segmenting the body and running the program. Subjects were provided a copy of their results upon completion of the study. Because this scan involved X-rays, female subjects provided a separate urine sample on the day of the DXA scan so that a pregnancy test could be performed. This sample was kept in the refrigerator and added to the urine collection jug prior to processing. A positive test result would exclude the subject from being scanned.

2.3.9 Schedule

On Day 1, subjects reported to the Center for breakfast between 6:30 and 8 AM. When they arrived, they were weighed. They then had the sensor portion of the CGMS inserted. While the sensor was "wetting", they consumed breakfast and received a packed out lunch. Diet technicians monitored their breakfast consumption and recorded their caffeinated beverage intake (type and amount). When they finished eating, the transmitter portion of the CGMS was connected to the sensor and they could leave the facility. Subjects returned to the Center at approximately 4:30 PM. They were fitted with the ambulatory monitoring device and swallowed the temperature transmitting pill. Their blood glucose was measured, and then they entered the calorimeter. **Figure 2.2** shows the approximate timeline of the activities that occurred while the subject was in the calorimeter. During each session, the subjects would participate in one of four exercise/meal treatment combinations:

- A: low intensity – long duration / high fat shake
- B: high intensity – short duration / high fat shake
- C: low intensity – long duration / high carbohydrate shake
- D: high intensity – short duration / high carbohydrate shake.

2.3.10 Statistical Analysis

The sample size was selected based on a previous calorimeter study that involved detecting differences in fat oxidation. SAS 9.4 (SAS Institute, Cary, NC) was used to analyze the data using a mixed model. Meal treatments, exercise treatments, and sequence of treatments were the fixed effects and subject nested within sequence was the random effect. Least-square means were also evaluated. When sequence was not significant, it was removed from the model statement and the analysis was repeated. Pearson's correlation coefficients were calculated to evaluate the correlations between metabolic flexibility variables (ΔRQ, slope of RQ, AUC of RQ, and variance of RQ and insulin) and the physical characteristics of the subjects (age, BMI, % body fat, VO_2peak, and HOMA-IR). Tests were significant at $\alpha = 0.05$. Results are reported as least-square means ± standard error of the mean (LSM ± SEM) unless otherwise stated.

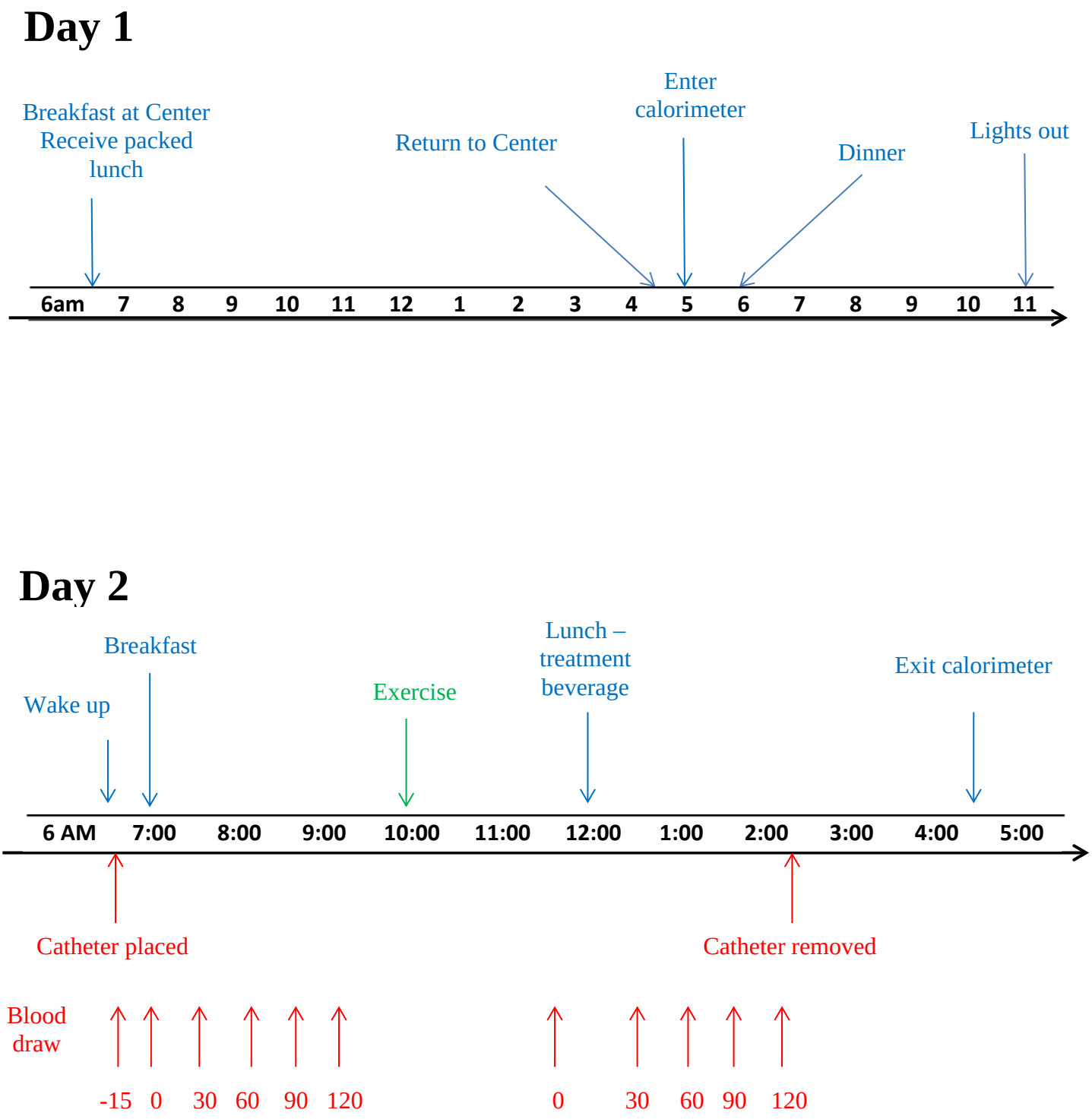

Figure 2.2: Schedule for Day 1 and Day 2 of the calorimeter session.

2.4 Results

Eight men and 7 women completed the study. One woman dropped out of the study after completing 3 out of 4 treatments and did not complete a DXA scan. Her results for the treatments that she did complete were included in the analysis. **Table 2.4** provides a summary of the physical characteristics of the selected subjects.

Table 2.4: Physical characteristics of the subjects

	Males	Females	Range
Age (years)	29 ± 2	25 ± 1^	22 - 34
BMI (kg/m^2)	25.3 ± 1.6	24.1 ± 1.2	19.3 – 31.0
VO_2max (ml/kg/min)	43.7 ± 4.8	38.9 ± 2.1	29.3 – 63.6
% Body fat*	19.5 ± 2.8	28.2 ± 2.4^	9.2 – 37.6
Fasting glucose (mg/dl)	90 ± 2	84 ± 3	71 – 99
Blood pressure (mmHg)	119 ± 5/ 70 ± 3	121 ± 7/ 70 ± 3	100 – 157 / 58 - 83
HOMA-IR	1.53 ± 0.43	1.70 ± 0.39	0.47 – 4.49

Means ± SEM
*One female subject dropped before completing a DXA scan.
^significantly different ($p<0.05$)

2.4.1 Energy Expenditure and Substrate Oxidation

Average RQ during the calorimeter run was significantly different between the meal treatments (HC: 0.86 ± 0.01 vs. HF: 0.84 ± 0.01; $p < 0.0001$) but not the exercise treatments. The average RQ was calculated between the times of 7:11 PM on Day 1 and 3:18 PM on Day 2, since this data range was available for all 63 calorimeter runs. The average RQ was negatively correlated with VO_2peak ($r = -$

0.34; p=0.0066). Energy expenditure during this same time period was also significantly different between meal treatments (HC: 1908 ± 81kcal vs. HF: 1850 ± 80 kcal; p = 0.0035) and not significantly different between the exercise treatments. The energy expenditure was positively correlated with BMI (r = 0.36; p = 0.0040) and VO_2peak (r = 0.52; p < 0.0001) and negatively correlated with % body fat (r = -0.30; p = 0.0192). Substrate oxidation (in grams) was calculated over this time period, and all macronutrients had significantly different oxidation amounts between the meal treatments (**Figure 2.3**). Exercise treatments and the exercise*meal interaction were not significant.

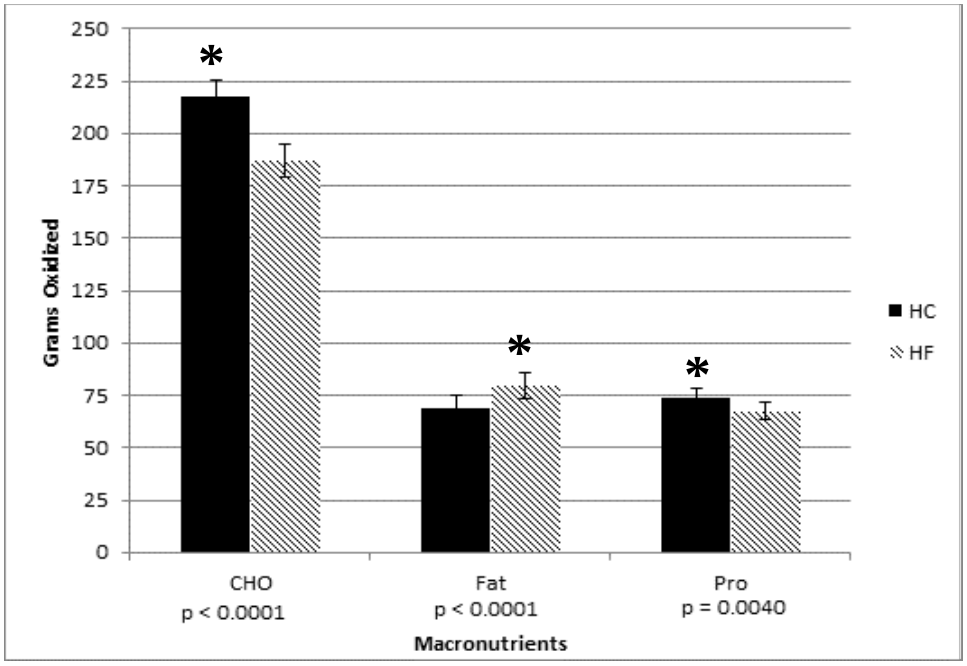

Figure 2.3: Macronutrient oxidation
The total amount of carbohydrate, fat, and protein gram oxidized during the calorimeter session. The p-value reflects the difference between meal treatments.

2.4.2 Metabolic Flexibility in Response to Meals

The first variable considered was the slope of RQ following the meals in order to determine the rate of change, how quickly the RQ changes in response to the meals. The slope of RQ following dinner and breakfast was not affected by the treatments, since the treatments were not administered until after the completion of breakfast on Day 2. There were no significant correlations between the dinner and breakfast slopes and the subjects' physical characteristics (BMI, % body fat, age, VO2peak, or HOMA-IR). However, there was a significant meal effect on the lunch slope where the high carbohydrate beverage had a larger slope than the high fat beverage (0.0049 ± 0.0004 vs. 0.0033 ± 0.0004; p = 0.0059) (**Figure 2.4**). This means that the RQ increased at a faster rate for the high carbohydrate shake. The post lunch area under the curve (AUC) of the RQ (calculated for approximately 185 minutes after lunch with the baseline value being 0.7 instead of 0) was also larger for the high carbohydrate beverage than the high fat beverage (0.024 ± 0.001 vs. 0.010 ± 0.001; p < 0.0001). The post lunch RQ AUC was negatively correlated with VO_2peak (r = -0.25; p = 0.0465).

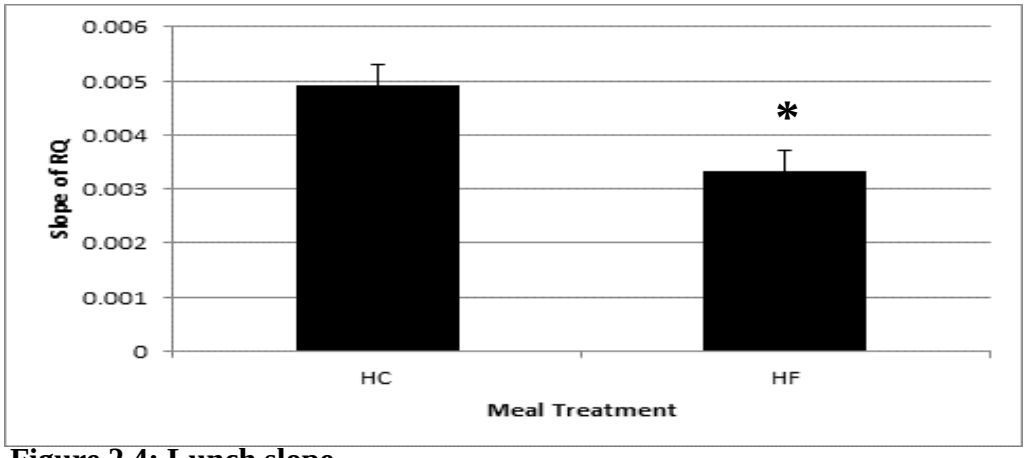

Figure 2.4: Lunch slope
Slope of RQ following the treatment lunch. P = 0.0059; HC – high carbohydrate, HF – high fat

ΔRQ was calculated in a method (Method A) that was similar to what has previously been performed in the literature using the clamp method [7-11]. The RQ data for the 30 minutes prior to breakfast was averaged and considered the fasting RQ. Two hours after breakfast was consumed, another 30 minute average RQ was calculated as the insulin-stimulated RQ. The difference was calculated as the ΔRQ. There were no significant correlations between ΔRQ and the subjects' physical characteristics. Sorting ΔRQ by gender resulted in females having a positive correlation between ΔRQ and VO_2peak ($r = 0.51$; $p = 0.0032$). Fasting RQ was negatively correlated with VO_2peak ($r = -0.32$; $p = 0.0107$).

A second method to calculate ΔRQ was also utilized (Method B). For this method, RQ between 7AM and 9AM was averaged as the insulin stimulated RQ (breakfast was served during this time) and fasting RQ was the average RQ between 2AM and 4AM. For method B, ΔRQ was negatively correlated with % body fat ($r = -0.30$; $p=0.0202$), positively correlated with VO_2peak ($r = 0.43$; $p=0.0004$), and trending negatively with age ($r = -0.25$; $p=0.0502$) (**Figure 2.5**).

The change in RQ following lunch was also considered with the pre-lunch RQ being the 30 min average RQ immediately prior to lunch and the post lunch RQ being the 30 minute average RQ 2 hours after lunch. In this case, there was a meal treatment effect on the post lunch RQ and the ΔRQ with the high carbohydrate shake resulting in a larger change in RQ (0.16 ± 0.01 vs. 0.02 ± 0.01; $p < 0.0001$). There were no significant correlations between ΔRQ and the subjects' physical characteristics even when sorting by exercise treatment or gender. The pre-lunch RQ was significantly affected by the exercise resulting in a significantly higher

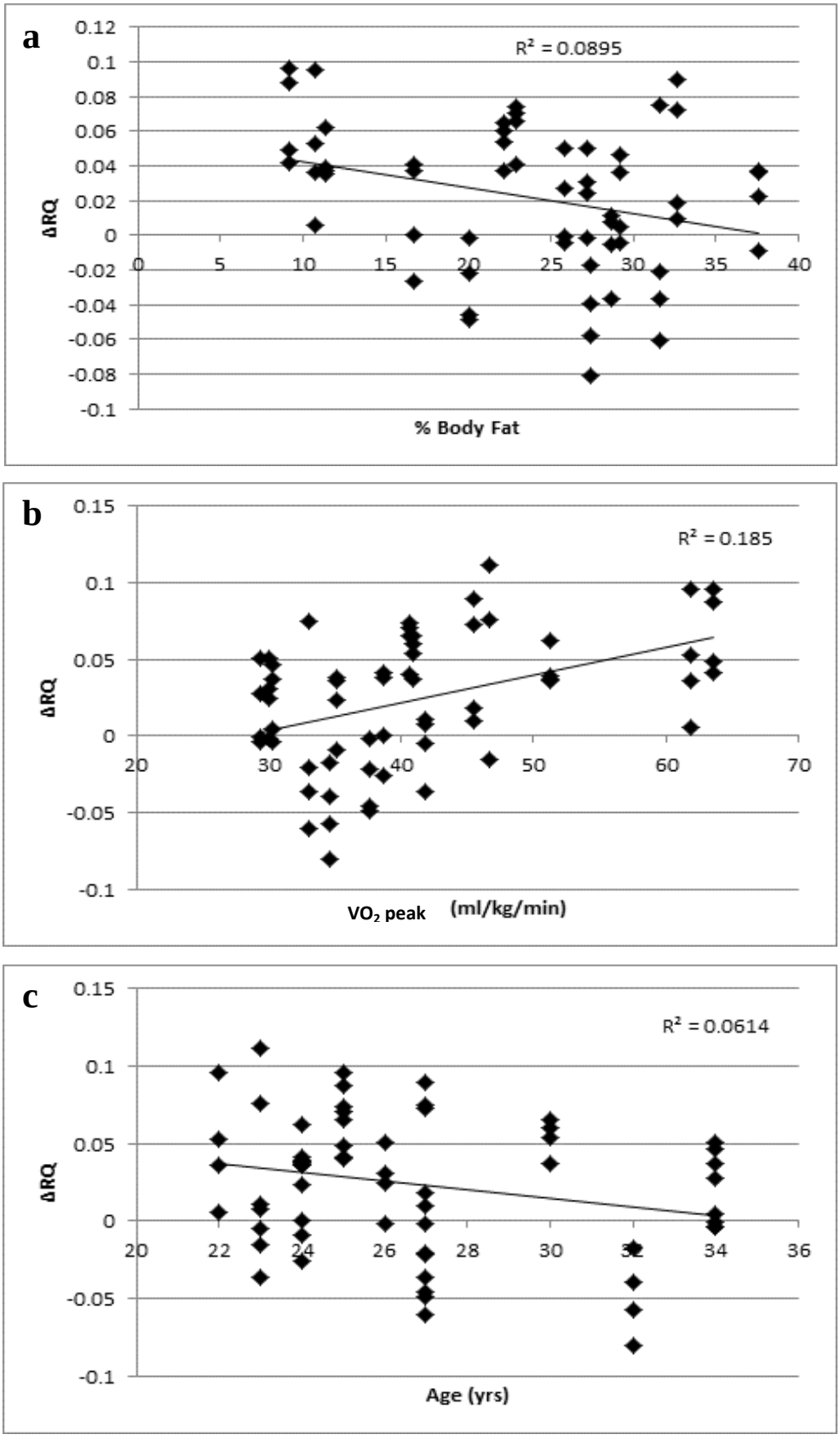

Figure 2.5: ΔRQ correlations
ΔRQ as calculated by the method A is (a) negatively correlated with % Body Fat (p=0.0202), (b) positively correlated with VO_2peak (p=0.0004), and (c) trending negatively with age (p=0.0502).

RQ following the low intensity session (0.76 ± 0.01 vs. 0.74 ± 0.01; p = 0.0130). This pre-lunch RQ was negatively correlated with VO_2peak (r = -0.26; p=0.0363). Also the fasting RQ was significantly higher than the pre-lunch RQ for both exercise treatments, demonstrating that the exercise sessions had an impact in decreasing the pre-lunch RQ (**Figure 2.6**).

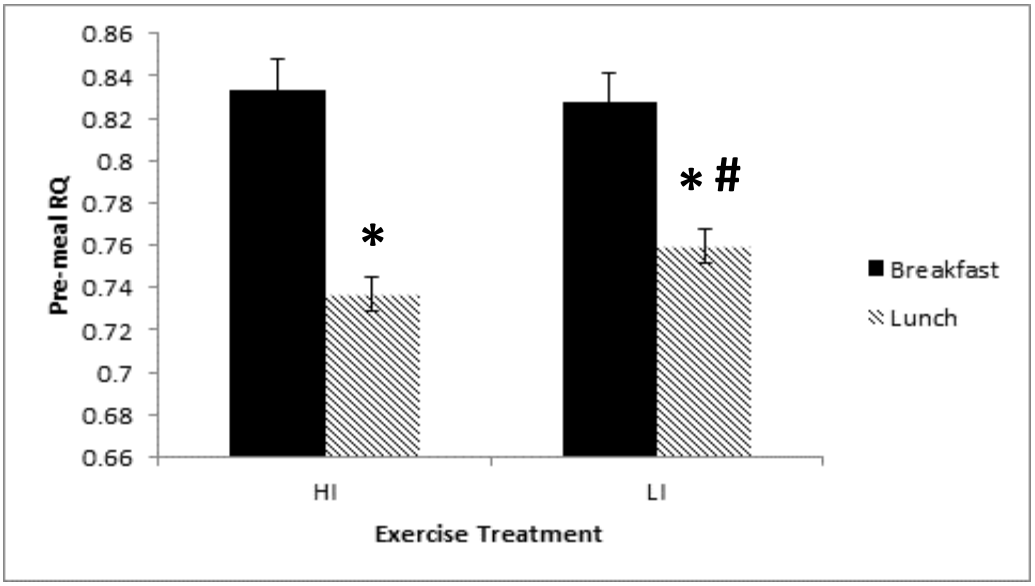

Figure 2.6: Pre-meal RQ
The 30 minute average RQ before breakfast and lunch are compared between the exercise treatments. * Breakfast pre-meal RQ is significantly different than lunch for both groups (p < 0.001). # Lunch pre-meal RQ is significantly different between treatments (p = 0.0130). HI – high intensity, LI – low intensity

When examining the variance of RQ and insulin after lunch, it was found that the meal treatment had a significant effect on the variance of insulin with the high carbohydrate shake having a significantly higher variance than the high fat shake (p<0.0001). Relevant correlations involving the variance in RQ and insulin can be found in **Table 2.5**.

Table 2.5: RQ and insulin variances
Correlation coefficients for the physical characteristics of the subjects vs. the RQ and insulin variances

	Breakfast		Lunch	
Physical Characteristics	**RQ variance**	**Insulin variance**	**RQ variance**	**Insulin variance**
Age	-0.079	0.26*	-0.029	0.15
BMI	-0.25*	0.49*	-0.18	0.24
% Body Fat	0.024	0.35*	0.009	0.29*
VO_2max	-0.093	-0.31*	-0.12	-0.21
HOMA-IR	-0.27*	0.58*	-0.16	0.23

* significant correlation ($p<0.05$)
RQ and insulin variances for 2 hours post-meal were calculated following both breakfast and lunch. For instances of missing data, the calculation was only performed if the data included baseline and 120 min blood samples.

2.4.3 Metabolic Flexibility during Exercise

For the exercise portion of the protocol, the average RQ during the session was significantly higher for the high-intensity treatment than the low intensity treatment (0.96 ± 0.01 vs. 0.93 ± 0.01; $p < 0.0001$). The average RQ was also negatively correlated with % body fat ($r = -0.26$; $p=0.0424$). There were no significant differences in the energy expenditure between the treatments. The energy expenditure was negatively correlated with age ($r = -0.39$; $p=0.0017$) and % body fat ($r = -0.64$; $p<0.0001$) and positively correlated with VO_2peak ($r = 0.86$; $p<0.001$). The slope of RQ at the start of the exercise session was also evaluated. The exercise treatment had no effect on the slope, but it was positively correlated with VO_2peak ($r = 0.67$; $p<0.0001$). The slope was also negatively correlated with age ($r = -0.31$; $p=0.0138$) and % body fat ($r = -0.55$; $p<0.0001$) (**Figure 2.7**).

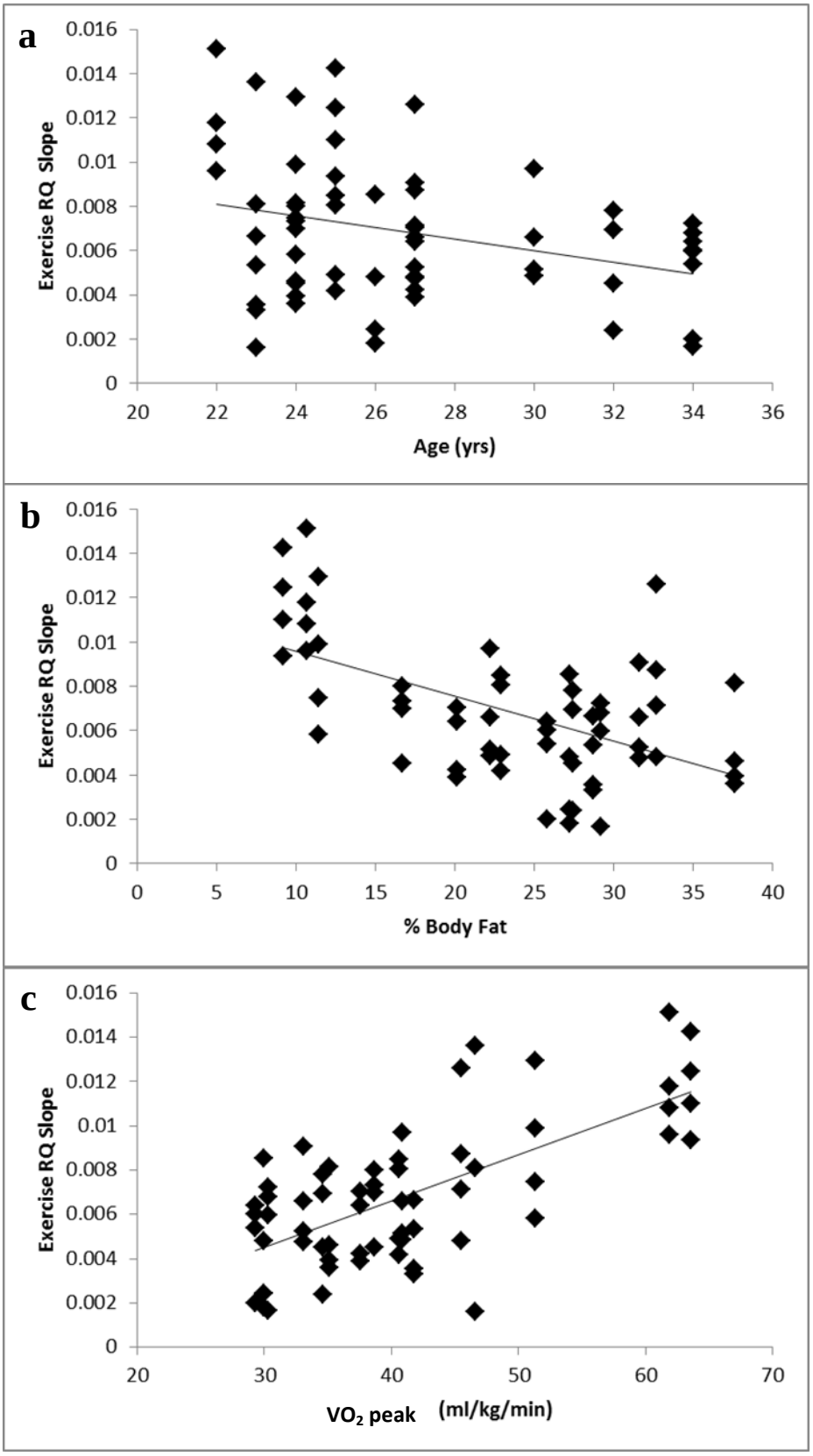

Figure 2.7: Exercise RQ slopes
The slope of RQ during exercise is negatively correlated with (a) age ($r=-0.31$, $p=0.0138$) and (b) % body fat ($r=-0.55$, $p<0.0001$) and positively correlated with (c) VO_2peak ($r=0.67$, $p<0.0001$).

Lag was calculated for the exercise session since excess CO_2 is expired by the subjects at the completion of the session. There were no exercise treatment effects on the lag; however, it was negatively correlated with VO_2peak (r=-0.30, p=0.0161) (**Figure 2.8**).

The MF during exercise was also calculated in a manner similar to Prior et al.[13]. For this calculation, the pre-exercise RQ was determined by averaging the RQ for the 10 minutes prior to the start of the exercise session. The exercise RQ was the average RQ during the final 10 minutes of the session. MF (ΔRQ) was then calculated. MF of exercise was significantly different between the exercise treatments (HI: 0.056 ± 0.012 vs. LI: 0.088 ± 0.012; p = 0.0445). However, the exercise RQ and pre-exercise RQ was significantly higher for the HI exercise. MF of exercise was positively correlated with HOMA-IR (r = 0.26; p = 0.0430); however, when examining the HOMA-IR and each exercise treatment separately, the correlation was not significant.

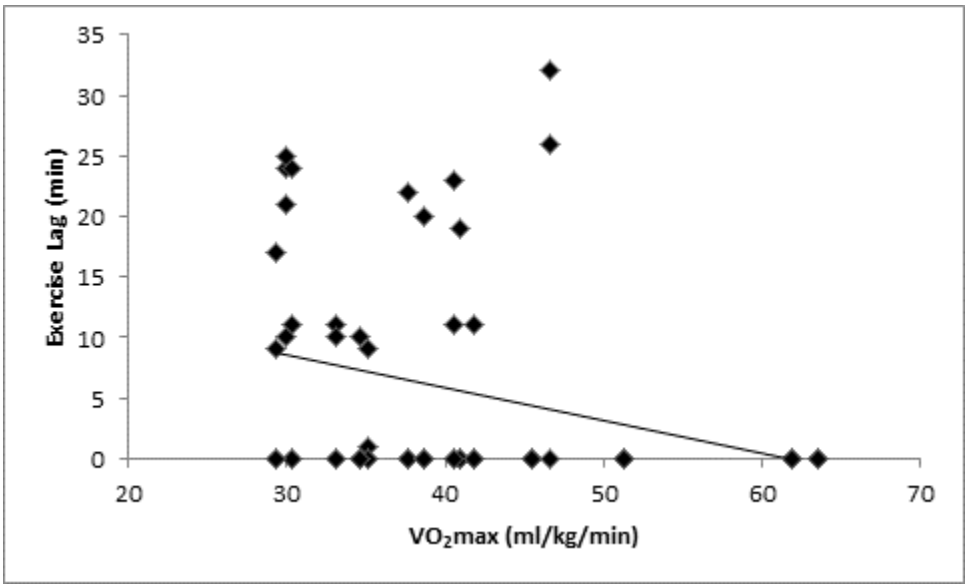

Figure 2.8: Post exercise lag time
The lag in RQ following exercise is negatively correlated with VO_2max (r = 0.30, p = 0.0161).

2.4.4 Glucose, Insulin, and NEFAs

AUC was calculated for glucose, insulin and NEFAs following the standard breakfast and the treatment lunch. For instances of missing data, the calculation was only performed if the data included baseline and 120 min blood samples. The exercise and meal treatments were not administered before breakfast; therefore, only correlations with various physical characteristics were examined. These correlations can be found on **Table 2.6**. Glucose AUC for 2 hours following lunch was significantly different between the high carbohydrate beverage and the high fat beverage (HC: 15,172 ± 405 mg/dL vs. HF: 10,156 ± 399 mg/dL; $p<0.0001$). Insulin AUC for 2 hours after lunch was also significantly different between the meal treatments (HC: 5331 ± 400 µU/mL vs. HF: 945 ± 396 µU/mL; $p<0.0001$) with the exercise treatment only having a trending effect ($p=0.0859$). There was a significant meal*exercise treatment interaction for the NEFA AUC at lunch ($p=0.0482$). **Figure 2.9** provides a graph of the significant differences of NEFA AUC for the four treatment combinations. **Table 2.6** provides the significant correlations between the lunch AUC values and the physical characteristics of the subjects.

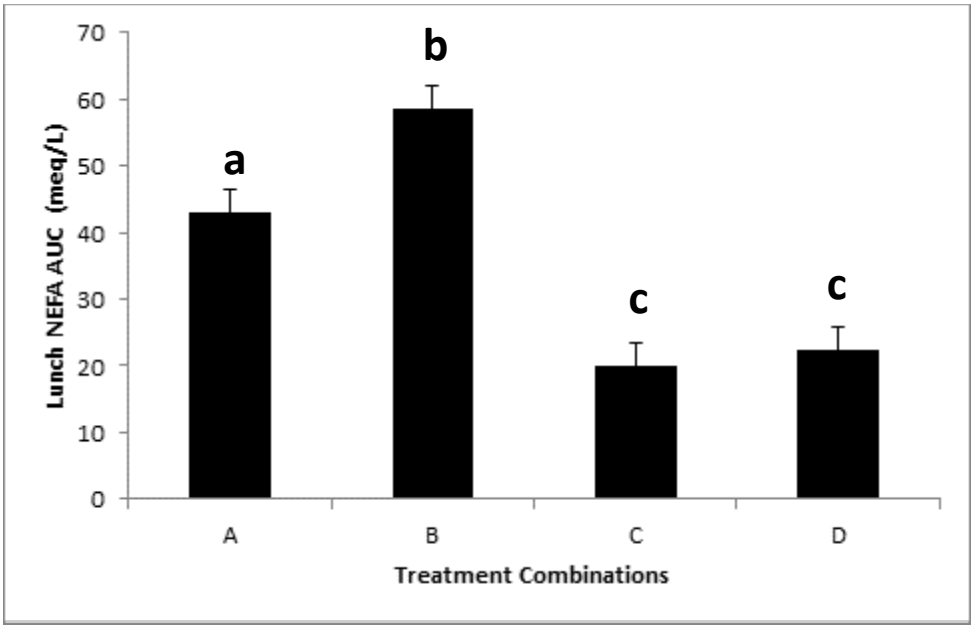

Figure 2.9: NEFA area under the curve
Non-esterified fatty acids AUC for 2 hours following lunch for each treatment. Treatments with different letter are significantly different ($p < 0.05$).

Table 2.6: Glucose, insulin, and NEFA area under the curve
Correlation coefficients for the physical characteristics of the subjects and the AUC following breakfast and lunch for glucose, insulin, and non-esterified fatty acids (NEFA)

	Breakfast			Lunch		
Physical Characteristics	**Glucose**	**Insulin**	**NEFA**	**Glucose**	**Insulin**	**NEFA**
Age	0.59*	0.38*	0.41*	0.11	0.19	-0.034
BMI	0.52*	0.63*	0.52*	0.17	0.29*	0.10
% Body Fat	0.33*	0.46*	0.16	0.33*	0.35*	0.20
VO_2max	-0.45*	-0.41*	-0.28*	-0.20	-0.28*	-0.12
HOMA-IR	0.44*	0.75*	0.56*	0.11	0.33*	0.29*

* statistically significant ($p<0.05$)

2.5 Discussion

The main objectives of this study were to (1) to use room calorimetry to measure metabolic flexibility and to compare the results to those collected using other methodologies; (2) to examine the effects of diet composition and exercise interventions on metabolic flexibility variables (ΔRQ, slope of RQ, variance of RQ); and (3) to see if there are any correlations that can be established between the metabolic flexibility variables and the physical characteristics of the subjects (age, BMI, % body fat, VO_2peak, and HOMA-IR). Some of the metabolic flexibility variables that we considered were new variables that have not been examined previously (to the best of our knowledge). We also used our data to perform the MF calculations that have been done previously in literature for comparison purposes.

The closest representation to the methods that we used was the study by Russell et al. [60] because they did not use the clamp method and used a hood calorimeter alone to evaluate MF. For their study, the subjects were passively stretched while under the hood calorimeter. The subjects then came out from under the hood to consume a glucose load and returned to the hood when they finished. They found that the control subjects were more metabolically flexible than the subjects with T2D or the subjects with a family history of T2D (there was no difference in MF between subjects with T2D and the subjects with a family history of T2D). The benefit of room calorimeter method as opposed to the hood calorimeter alone is that it allows for continuous monitoring while eating, so that the subjects do not have to leave the calorimeter to consume the food. The room calorimeter also

allows the subject more mobility so that they can perform different exercises like running on the treadmill or using resistance bands.

When we calculated ΔRQ in a similar manner to studies that used the hyperinsulinemic-euglycemic clamp (method A), we didn't observe any correlations between MF and the physical characteristics of the subjects. However, MF, as evaluated by calculating ΔRQ via method B, was negatively correlated with % body fat and positively correlated with VO_2peak which was the same results found previously for a group of young men [8, 46]. We also observed a negative trend with age as seen previously in a group of men and women [11].

The ΔRQ in response to the lunch was calculated and there was a larger difference between the pre-lunch RQ and post-lunch RQ when the subjects consumed the high carbohydrate shake. This was not surprising, because RQ is 1 when a person is only oxidizing carbohydrates and 0.71 when a person is only using fat. The pre-lunch RQ was affected by the exercise that was done prior to the lunch, and it was negatively correlated to VO_2peak. This could mean that subjects with a higher VO_2peak were better able to return their RQ to a baseline level following the exercise session. It might be worth examining the results when providing the test meal as breakfast in order to eliminate any confounding factors like the exercise effect.

We also examined MF by the variance in RQ and insulin as was done by Bergouignan et al.[16]. They classified subjects as metabolically flexible if they had a large variance in RQ but a small variance in insulin. As would be expected, the lunch beverage had an effect on the variance of insulin. The only significant correlation after lunch was that subjects with a higher % body fat had a larger

variance in insulin, meaning that subjects with more body fat had a difficult time keeping their insulin stable. When examining the RQ variance after breakfast, it was found that subjects with a larger BMI or a larger HOMA-IR value had a lower variance in RQ. This would mean that they were not experiencing a drastic increase in RQ in response to a high carbohydrate breakfast. A person that is metabolically flexible would be expected to have a larger and possibly quicker increase in RQ following a meal. In terms of insulin, subjects that were older, had a high BMI or % body fat, had a larger HOMA-IR value, or had a lower VO_2peak had a higher variance in insulin following breakfast. Their insulin control was not as good following the breakfast and one would expect it to be more stable if they were metabolically flexible. Taken with what we have seen in literature regarding the characteristics of a metabolically flexible person, these results would not be surprising.

Another method we explored was metabolic flexibility during exercise. The change in RQ during the exercise was significantly different between the exercise treatments. Prior et al. [13] calculated the change in RQ only for submaximal exercise (50 and 60% of VO_2max) and found that the obese subjects with impaired glucose tolerance had lower MF compared to those with normal glucose tolerance. They also reported that there was no correlation between MF during the clamp and MF during exercise. Looking at the LI exercise treatment alone for the current study, there were no significant correlations between the physical characteristics of the subjects and their MF during exercise. Again, these subjects were young, healthy,

and nondiabetic, and we did not assess their glucose tolerance levels. It would be interesting to evaluate this with an older population and diabetics.

The slope of the RQ in response to meals of varying diet composition was one of the new MF variables we examined. We did not observe any correlations between the physical characteristics of the subjects and the slope of RQ following dinner (mixed macronutrient meal) or breakfast (high carbohydrate). The slope of RQ following lunch was larger for the high carbohydrate shake than the high fat meaning that there was a faster rate of change in the RQ after the high carbohydrate beverage. This isn't surprising due to the carbohydrate providing a large insulin response and the RQ of using carbohydrates alone would be 1. There were no significant correlations between the lunch slope and the subjects' physical characteristics, and this was also the case when the correlations were sorted by meal type. It would be interesting to perform this analysis on a wider subject population (larger age range, Type 2 diabetics) to see if there would be a change in the rate or any correlations. I would hypothesize that type 2 diabetics would have a slower rate of change in RQ (smaller slope) than lean, non-diabetics.

Another MF variable considered was the AUC of RQ following the diet treatment lunch. The AUC of RQ was larger for the high carbohydrate shake than the high fat shake. This would be reflective of the fact that the high carbohydrate shake would cause a large rise in RQ. The negative correlation between post lunch RQ AUC and VO_2peak suggests that fitter subjects were better able to return to a baseline level following their response to lunch.

When considering MF in relation to the exercise treatments, the slopes of RQ at the start of exercise were associated with fitness level. The slope was positively correlated with VO_2peak and negatively correlated with age and % body fat. This demonstrates that subjects with a better aerobic capacity and less body fat adapted their RQ more quickly to the exercise. Again, it also shows that the older subjects adapt more slowly to the exercise.

Also, when analyzing the response in RQ to various exercise challenges, we determined that the new MF lag variable may be indicative of a subjects' fitness level and not their metabolic flexibility. The lag in RQ was caused by the excess CO_2 produced at the completion of the exercise session [97]. Lag was not affected by the type of exercise (low intensity vs. high intensity), but was negatively correlated with VO_2peak. The more physically fit a subject was, the shorter the lag in RQ following exercise. A prior study performed at this facility found that the lag after exercise was positively correlated with age, reflecting that as a person gets older, it takes longer for them to rid their bodies of the excess CO_2 [31]. This would make sense due to fitness levels declining as people age.

Area under the curve for glucose, insulin, and NEFA for 2 hours after breakfast also reflect the differences associated with fitness level and body fat. Older subjects had a larger AUC for glucose, insulin, and NEFAs. BMI and HOMA-IR were positively correlated with AUC for glucose, insulin, and NEFAs. VO_2peak was negatively correlated with AUC for glucose, insulin, and NEFAs, and % body fat was

positively correlated with insulin. Subjects who were older, less fit, and with more body fat experienced larger and/or prolonged glucose, insulin, and NEFA responses to the meal.

One limitation of this study is the small range of age. The subjects were well distributed within our age range; however, it only spanned 14 years. Based on previous examinations, it would be worthwhile to perform these calculations on an older population as well to see if the correlations remain true. The smaller sample size may have also limited our ability to see significant correlations. More subjects of both genders would have allowed us to better explore gender differences as well. Also, unlike many of the metabolic flexibility studies in the literature, we did not have any subjects that were type 2 diabetic since it was an exclusion criteria for this study.

In summary, we were able to measure MF variables without the use of the hyperinsulinemic-euglycemic clamp and observed the expected results. The room calorimeter allowed us to continuously monitor the subject RQs during the meals and exercise and obtain a more physiological response than the invasive clamp method.

Chapter 3: Conclusions and Future Work

In conclusion, we were able to measure metabolic flexibility variables using a room calorimeter and the appropriate data smoothing methods that reduce equipment noise. The significance is that we had results that were similar to the literature without using a hyperinsulinemic-euglycemic clamp. This is beneficial since the clamp method is invasive and does not give a true physiological response because the glucose levels are being controlled. In addition, the clamp would not allow for eating meals or exercising during the procedure. Using the room calorimeter and smoothing the data allows the subjects to consume actual meals and be ambulatory to exercise so that RQ response can be monitored continuously. The ability to examine small time intervals allows for the calculation of the actual rate of change in the RQ (slope) in response to a diet or exercise challenge. The slope of RQ was affected by the diet composition of the lunch shake, but there were no significantly correlations with the subjects' physical characteristics. The slope of the rise in RQ in response to exercise was not affected by the type of exercise session, but subjects that were more aerobically fit (higher VO_2peak) and had less body fat had a faster increase in RQ (larger slope of RQ). Multiple methods of calculating MF demonstrated that subjects that were more fit and had less body fat were more metabolically flexible.

This was the first calorimeter study performed at our lab where detailed data was collected to allow for analysis of these metabolic flexibility variables. Previous calorimeter studies did not have the exact meal and exercise data or start and end data of the calorimeter sessions for us to be able to perform these calculations with

accuracy using the smoothing method. We were also able to expand our data on younger subjects (under 35 years old).

Future work should involve running a similar protocol with older subjects to see if the correlations that were seen with the younger population are true for the older population as well. It would be beneficial to expand on the study of the exercise and meal slopes. For this study, having diabetes was an exclusion factor, but many studies focus on the difference between subjects with and without T2D. For comparison, it would be interesting to include a group of Type 2 diabetics when calculating these metabolic flexibility variables or take into account any family history of the disease. We have recently completed a pacing study in the calorimeter that involved a protocol that was very similar to the metabolic flexibility study. The main difference was the exercise session. The meals were the same, so we could calculate slopes of meals and other MF variables to compliment and expand on the current data.

Appendix A: Summary of Select Metabolic Flexibility Articles

Author	**Subjects**	**Objective**	**Results**
Kelley, Mandarino (2000) [21]	N/A – Review article		• Coined the term "metabolic flexibility." • Fasting – predominantly lipid oxidation; high rates of FA uptake. • Insulin-stimulated – suppression of lipid oxidation; increased glucose uptake, oxidation, and storage.
Ukropcova et al. (2005) [46]	16 young, healthy men with a range of BMIs, insulin sensitivities, and fat mass.	To evaluate whether "the capacity of myotubes to oxidize fat in vitro reflects the donor's metabolic characteristics."	• MF inversely correlated with body fat and free fatty acid (FFA) levels • MF positively correlated with IS, VO_2max, and mitochondrial DNA. • Metabolic switching reflects the metabolic characteristics of the donor. • "Metabolic switching is an intrinsic property of skeletal muscle." • Suppressability negatively correlated with MF and IS and positively correlated with body fat and fasting FFA levels. • Adaptability positively correlated with MF, IS, and aerobic capacity and negatively correlated with % body fat and fasting insulin levels.
Berk et al. (2006) [61]	Caucasian and African-American women	To establish if African-American women are metabolically inflexible.	• Caucasian women had significantly higher fat oxidation during a high fat diet and higher carbohydrate oxidation during the low fat diet, whereas African American women had no significant differences. • Delta RQ was not measured.

Ukropcova et al. (2007) [8]	Young, sedentary men (16 with a family history of diabetes and 34 without).	To determine if "defects in substrate switching in response to insulin and a HFD (high-fat diet) are linked to reduced mitochondrial biogenesis and occur before the development of diabetes."	• Sleep RQ (maximal fat oxidation) during the HFD was negatively correlated to MF. • Mitochondrial DNA content was positively correlated to MF. • Subjects with a family history of diabetes had a higher sleep RQ (lower maximal fat oxidation during HFD), a decrease in mtDNA content, and a trend towards reduced MF (p=0.07). • No differences in the amount of body fat and visceral fat.
Gaster (2007) [45]	Myotubes from lean, obese, and type 2 diabetic subjects.	To determine if "metabolic inflexibility is an intrinsic defect in myotubes established from obese and T2D subjects."	• Myotubes from all three groups had insulin-stimulated glucose and fat oxidation that was significantly different from basal. • Type 2 diabetics had significantly higher basal and insulin-stimulated glucose oxidation than leans. • Concluded that it was an "extramuscular mechanism" not an intrinsic defect.
Heilbronn et al. (2007) [59]	Controls (3 males, 5 females) Family history (2 males, 7 females)	To evaluate if the impaired ability to switch between fuel sources is a contributing factor to the development of insulin resistance and T2D or a consequence.	• After a high CHO meal, serum insulin tended to be increased in family history subjects - not significantly different from the controls in RQ • After a high fat meal, family history subjects had diminished capacity to reduce RQ compared to controls - suggests an impaired ability to switch between fuel sources. • Family history subjects had a significantly different response of the genes PGC1α and FAT/CD36 to a high fat meal.
Galgani et al. (2008) [81]	N/A – Review article		• Metabolically inflexible subjects have a higher fasting RQ and a lower RQ during the clamp. • Metabolically inflexible subjects take longer (more days) to adjust 24-h RQ to high CHO or high fat diet – eventually reach same level as flexible subjects.

Galgani et al. (2008) [10]	59 obese T2D (44 C, 12 AA, 3 H) 42 obese nondiabetic (34 C, 8 AA)	To determine if after controlling for glucose disposal rate (GDR), obese type 2 diabetics and nondiabetics have similar MF to glucose.	• MF positively correlated with insulin sensitivity (GDR). • MF negatively correlated to plasma FFA. • MF lower in T2D than nondiabetics – significant until adjusted for GDR. • After the Type 2 diabetic subjects underwent a one year lifestyle intervention to lose weight via increased physical activity and decreased caloric intake, their MF to glucose was significantly increased. • After controlling for GDF, there was no difference in MF to glucose before or after the intervention.
Sparks et al. (2009) [51]	56 young, healthy men	To examine the effect of fat cell size (FCS) and adipose tissue mass on MF.	• MF was negatively correlated with insulin-suppressed NEFAs, FCS, percent body fat, and adipose tissue inflammation markers. • MF was positively correlated with serum adiponectin levels.
Sparks et al. (2009) [9]	78 young, healthy men and women	To determine if sex differences in adipose tissue mass and characteristics affects MF.	• MF was higher in women compared to men. • MF was negatively correlated to insulin-suppressed NEFAs and visceral adipose tissue. • Women had a significantly higher alternative anti-inflammatory pathway (M2) marker and higher adiponectin levels and expression of lipid metabolism genes (PPARγ2, PCK1, SCD1, PPARα).
Meex et al. (2010) [7]	20 healthy males 18 T2D males	To evaluate if "exercise training could restore mitochondrial function and insulin sensitivity in patients with type 2 diabetes."	• 12-week training program restored MF in obese Type 2 diabetic males to the level of the obese controls. • IS improved significantly in T2D subjects. Improved mitochondrial function in both groups, with the T2D subjects returning to control levels.
Stull et al. (2010) [11]	168 men and women of various race, age, BMI, and diabetes status	To establish if race and diabetes status has an effect on MF.	• African Americans had a higher MF than Caucasians. • Non diabetics had a higher MF than diabetics. • MF was also found to be inversely correlated to age and positively correlated to IS. • Certain predictor variables were controlled.

Adamska et al. (2011) [52]	53 men and women with normal glucose tolerance 25 – lean 12 – overweight 16 - obese	To evaluate relationships between soluble TNF-α receptor concentrations, adiponectin and MF, lipid and glucose oxidation, non-oxidative glucose metabolism, and insulin sensitivity in lean and obese people.	• MF positively correlated with adiponectin • MF negatively correlated with both sTNFR1 and sTNFR2 (soluble TNF-α receptors)
Færch et al. (2011) [57]	66 men and women 20 – normal glucose tolerance (NGT) 28 – isolated impaired glucose tolerance (i-IGT) 18 – isolated impaired fasting glycemia (i-IFG)	To examine MF, non-oxidative glucose metabolism, glucose and lipid oxidation in people with NGT, i-IGT, and i-IFG.	• MF was significantly lower in both the i-IGT and the i-IFG groups when comparing them to the NGT group. • This remained true after adjusting for insulin sensitivity and BMI.
van Herpen et al. (2011) [75]	20 healthy, sedentary overweight men	To evaluate whether high-fat diets affect insulin resistance, intramyocellular lipid (IMCL), and intrahepatic lipid (IHL).	• When on a high fat diet for 3 weeks, overweight men had a decrease in MF and an increase in IHL content when compared to their low fat diet counterparts. • Insulin sensitivity and IMCL were not affected by the diet.
Chomentowski et al.(2011) [53]	40 sedentary men and women with various insulin sensitivities	To determine if metabolic inflexibility correlated with a decrease in mitochondrial content in intermyofibrillar or subsarcolemmal skeletal muscle.	• MF was significantly lower in the insulin resistant-nondiabetic group and the Type 2 diabetic group when compared to the insulin sensitive-lean control group. • MF was positively correlated to intermyofibrillar mitochondria content – this was also true when evaluated by gender. • There was no correlation between MF and subsarcolemmal mitochondria content.
Battaglia et al. (2012) [14]	22 men (18-30 yrs) 12 – lean 10 - obese	To see how exercise training affects fatty acid oxidation (metabolic flexibility) when consuming a high fat diet	• After 3 days of HFD, lean subjects increased fat oxidation but obese subjects did not. • Following the exercise training, fat oxidation and citrate synthase activity increased for both groups, but did not increase further when the HFD was implemented again.

Bergouignan et al. (2013) [16]	44 men and women from 2 studies -Men that detrained for 1 month (n=9) -Sedentary lean (n=10) and obese (n=9) men that trained for 2 months -Active women that underwent strict bed rest (n=8) or strict bed rest with exercise (n=8)	To evaluate "whether metabolic flexibility can be modulated by contrasted changes in PAL, independent of changes in energy balance."	• MF measured as the variance of RQ and insulin. • Increased PAL results in increased MF • Variance in RQ was positively correlated with PAL for all groups. • Detraining significantly lowered RQ variance and increased insulin variance. • Training tended to increases RQ variance and decrease insulin variance (no statistically significant).
Malin et al. (2013) [93]	12 sedentary, obese men and women 6 – IGT 6 – IFG + IGT	To examine "if the presence of impaired fasting glucose (IFG) affects fuel utilization during exercise in adults with IGT."	• Metabolic inflexibility defined as using more fat and less muscle glycogen during exercise. • IFG + IGT group used less muscle glycogen and oxidized more fat during exercise than the IGT group. • IFG + IGT group had significantly higher NEFAs and blood glucose.
Solomon et al. (2013) [77]	20 obese men and women (65±1 yr., mean ± SEM)	"To determine the influence of dietary glycemic index on exercise training-induced adaptations in substrate oxidation in obesity."	• MF was improved post-intervention for both diet groups. • MF was not significantly different between diet groups for either pre or post intervention.
Wijngaarden et al. (2013) [83]	26 men and women 12 – lean (2 M, 10 F) 14 – obese (2 M, 12 F)	"To investigate whether metabolic adaptations to prolonged fasting differ between lean and obese individuals and whether this is associated with changes in skeletal muscle AMPK signaling pathway.	• Fasting resulted in an increase in lipid oxidation and a decrease in glucose oxidation from baseline; however, the obese subjects had a blunted response (significantly lower increase in fat oxidation and decrease in glucose oxidation than the lean subjects) • Reduction in AMPK activity for lean subjects only.
Adamska et al. (2013) [58]	48 sedentary women 22 – lean 26 – overweight/obese	To examine the relationship of soluble E-selectin (a marker of endothelial dysfunction) with MF and insulin sensitivity.	• Negative correlation between soluble E-selectin and metabolic flexibility • Metabolic syndrome Z-score positively correlated with soluble E-selectin and negatively correlated with insulin sensitivity and MF.

Malin et al. (2013) [86]	24 obese men and women (66.7 ± 0.8 yr., mean ± SEM) 8 – impaired fasting glucose (IFG) 8 – impaired glucose tolerance (IGT) 8 – IFG + IGT	"To gain mechanistic insight to differences in insulin-stimulated glucose uptake across the prediabetes phenotypes following exercise."	• MF was significantly increased following the 12 week exercise intervention in the IFG and IGT groups only. • The IFG + IGT group had improved MF but was not significant.
Prior et al. (2014) [13]	23 sedentary, overweight/obese men and women	To determine if "metabolic flexibility during exercise" is lower in subjects with impaired glucose tolerance (IGT) compared to normal glucose tolerance (NGT)	• During the hyperinsulinemic-euglycemic clamp, IGT subjects had a significantly lower MF than NGT subjects. IGT subjects also had a significantly lower MF during submaximal treadmill exercise (50% and 60% VO2max), the IGT subjects also had a significantly lower MF. • No correlation between the insulin-stimulated MF (clamp) and the exercise MF.
Kahlhöfer et al. (2014) [12]	32 healthy men (20-37 yrs)	To evaluate if "refeeding a low GI, moderate-carbohydrate diet facilitates weight maintenance."	• MF was measured as the ΔNPRQ between the end of a refeeding diet period and the end of a caloric restriction diet period. • ΔNPRQ positively correlated with regain in both fat mass and body weight.
Malin et al. (2014) [87]	20 obese men and women (66.3 ± 0.9 yr., mean ± SEM)	"To examine the effect of exercise training on circulating fetuin-A in relation to skeletal muscle and/or hepatic insulin resistance in obese adults."	• MF significantly improved following the 12 week exercise intervention. • MF was negatively correlated with fetuin-A (hepatokine that may be linked to development of T2D).

Appendix B: Curriculum vitae

Jayme L. (Brown) Leger
Ph.D. Candidate

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Education

Doctor of Philosophy, Nutrition, Expected Summer 2015
University of Maryland, College Park, MD
Advisor: Dr. Thomas W. Castonguay

Non-Degree, 1/06 – 5/06
George Mason University, Fairfax, VA

Bachelor of Science, Chemical Engineering, Cum Laude, May 2003
University of Massachusetts, Amherst, MA

Research Experience

Graduate Research Assistant, Department of Nutrition and Food Science, University of Maryland, College Park, MD, August 2007 – December 2012
Supervisor: Dr. Thomas W. Castonguay

- Assisted with animal studies.
- Performed thorough analysis of four related animal studies.
- Assisted with an fMRI study.

Research Volunteer/Assistant, United States Department of Agriculture, Beltsville Human Nutrition Research Center, Beltsville, MD, August 2007 – present

Volunteer: 8/07-6/08
Employee (through MedStar Health Research Institute): 6/08 – 11/11
Employee (Sr. Research Assistant through Johns Hopkins University): 11/14/11 – 10/12/12
Employee (Faculty Research Assistant through University of Maryland): 10/15/12 - present
Supervisors: Dr. David Baer; Dr. William Rumpler

- Coordinated a calorimeter study for PhD project involving subjects staying overnight – duties included preparing IRB documents, recruitment, subject screenings, subject monitoring, and data collection and analysis.

- Assisted with other feeding and calorimeter studies - duties included helping with subject screenings, overseeing subjects during treatments, data collections, and blood processing.
- Perform lab duties such as: blood assays, bomb calorimetry, urinary nitrogen analysis, and HPLC analysis.
- Trained in using continuous glucose monitoring systems, running VO_{2max} tests, performing DXA scans, using physical activity monitors, and measuring endothelial function.
- Helped an intern with a project on 100-calorie snacks.

Teaching Experience

Graduate Teaching Assistant, Department of Nutrition and Food Science, University of Maryland, College Park, MD, January 2008 – May 2008; August 2008-May 2009; August 2009 – May 2010; August 2010 – May 2011

- NFSC 491(Spring 2008): helped prepare documents for lecture; graded assignments
- NFSC 380 (Fall 2008, 2009, & 2010): led one lab section; helped prepare lab assignments and quizzes; graded quizzes and lab reports; provided tours of the USDA facility
- NFSC 350 (Spring 2009): helped prepare documents for lecture; graded case studies, lab reports, projects, and exams
- NFSC 450 (Spring 2010): monitored two lab sections; helped with lab prep work; graded lab practicals and notebooks
- NFSC 460 (Spring 2011): led one lab section; graded quizzes, case studies, projects, and exams

Honors/Awards

- **NFSC Research Day Poster Contest**, May 2011: second place for Continuing Nutrition Graduate Student category
- **Graduate Program in Nutrition Poster Contest**, May 2008: winner of the First Year Student Category
- **Graduate School/AGNR Fellowship**, August 2007 – June 2008
- **Tau Beta Pi National Engineering Honor Society**

Training

- Radiation Safety – University of Maryland, College Park, MD & United States Department of Agriculture, Beltsville, MD
- Animal Handling – University of Maryland, College Park, MD

- Biosafety – United States Department of Agriculture, Beltsville, MD
- Bloodbourne Pathogens – United States Department of Agriculture, Beltsville, MD
- Collaborative Institutional Training Initiative (CITI) Program – courses completed for University of Maryland, College Park and MedStar Health Research Institute

Committees

- Served as the graduate student representative on a search committee for an Assistant Professor position in risk modeling for the Nutrition and Food Science Department and Center for Food Safety and Security Systems.
- Served as the graduate student representative on a search committee for Chair/Professor for the Nutrition and Food Science Department.
- Served as a graduate student representative on a search committee for a Coordinator position for the Nutrition and Food Science Department.
- Served as a graduate student representative on a search committee for various Faculty Research Assistant positions for the Department of Nutrition and Food Science.

Professional Societies

- Student member of the Academy of Nutrition and Dietetics

Abstracts

Leger JL, Castonguay TW, Gribok A, Rumpler WV. Room Calorimetry as a Method of Measuring Metabolic Flexibility. NFSC Research Day Poster Contest, University of Maryland, College Park (2015)

Leger JL, Schroeder N, Stevens M, Gribok A, Castonguay TW, Rumpler WV. Metabolic Flexibility: Using Room Calorimetry to Evaluate Adaptation to Diet and Exercise. NFSC Research Day Poster Contest, University of Maryland, College Park (2013).

Hudgins SM, Smaliy A, Zhan W, **Leger JL**, Gebauer S, Baer D, Castonguay TW. High Fructose Corn-Syrup Sweetened Cola and the Hypothalamus: A Dose-Response fMRI Study. FASEB J 2013; 27:1066.4. Experimental Biology Annual Meeting, Boston, MA (2013).

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Courville AB, Stote KS, Rumpler WV, Paul DR, **Leger J**, Novotny JA, Baer DJ. Effect of protein sources on glucose and insulin response in overweight and obese men. FASEB J 2009; 23:345.3. Experimental Biology Annual Meeting, New Orleans, LA (2009).

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London E, Kohli A, Renner M, **Leger J**, Castonguay TW. Effects of acute access to sucrose, fructose and glucose solutions on hepatic 11β-hydroxysteroid dehydrogenase-1 and hexose-6-phosphate dehydrogenase message in rats. Graduate Research Interaction Day, University of Maryland, College Park (2008).

Conference Paper

Buller MJ, Welles AP, Stevens M, **Leger J**, Gribok A, Jenkins OC, Friedl KE, Rumpler W. Automated Guidance from Physiological Sensing to Reduce Thermal-Work Strain Levels on a Novel Task. Body Sensor Networks Conference, MIT, Cambridge, MA (2015).

Professional Experience

Patent Examiner, United States Patent and Trademark Office, Alexandria, VA, 1/24/05– 1/20/06

- Examined patent applications to determine their compliance with the rules and legal regulations
- Researched the subject matter of the claims in the applications to determine if a new idea has been presented
- Composed reports explaining the examination and the patentability of the applications
- Assisted patent lawyers/agents by suggesting improvements to make the claims of their applications patentable

Application Engineer, Tra-Con, Inc., Bedford, MA, 6/30/03 – 4/30/04

- Supported customers in analyzing problems and determining adhesives to suit their application needs
- Maintained and managed various customer accounts
- Worked directly with lab and manufacturing personnel to meet customers' demands
- Helped manage company's monthly revenue

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