THE EFFECT OF AGING ON METABOLIC FLEXIBILITY

BY

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List of Abbreviations

Respiratory Exchange Ratio - RER

Respiratory Quotient – RQ

NPRQ – Non Protein RQ

Acetyl CoA – Acetyl Coenzyme A

Abstract

Introduction: Metabolic flexibility refers to one's ability to adjust their oxidation of carbohydrates and fats and differs with exercise training and between healthy and diseased populations. The effect of aging on metabolic flexibility is unknown.

Purpose: To examine differences in metabolic flexibility between healthy young and old adults in response to a diet challenge.

Methods: Healthy young (18-30 years old) and old (>55 years) adults came to the laboratory fasted after consuming 2 days of a defined diet. Fasted measurements of blood glucose, carbohydrate and fat oxidation were completed. A high carbohydrate meal replacement shake was then consumed, and participants were asked to remain seated while measurements were repeated at 30, 90, 120, and 180 minutes post beverage consumption.

Results: 6 young participants (4 females, 2 males) and 2 old participants (1 female, 1 male) completed the study. Effect sizes between groups for total grams of carbohydrate and fat oxidized during the 180-minute observation period were 1.27 and 1.04 respectively. Effect sizes for the total change scores in carbohydrate and fat oxidation over the 180 minutes after the meal were 0.40 and 0.51 respectively.

Conclusion: The moderate effect sizes between old and young groups for carbohydrate and fat oxidation suggest age may have an impact in altering energy metabolism with aging.

Introduction

Metabolic flexibility is defined as the ability of an individual to switch between oxidizing fats and carbohydrates in response to a challenge[1]. Young, healthy individuals adjust their oxidation of fat and carbohydrates compared to those with metabolic conditions such as diabetes. This will allow appropriate allocation of macronutrients following meals, as well as appropriate responses to exercise[2]. Currently, there are no agreed upon standards or protocols for quantifying metabolic flexibility. However, indirect calorimetry is a common method of assessment. Through indirect calorimetry, the respiratory exchange ratio (RER) can be used to determine utilization of fats and carbohydrates for fuel.

At rest, a healthy individual should have an RER closer to 0.7, implying the use of more fatty acids. Following a meal high in carbohydrates, the RER increases toward 1.0, suggesting the oxidation of more carbohydrates, before declining again. Kelley et al. found that lean individuals had fasting RER values of 0.82, and after being insulin stimulated (i.e increased in carbohydrates), the values increased to 0.95 [3]. A metabolically inflexible individual will have less variation in their RER following consumption of a carbohydrate-rich meal, and will probably hover in the middle, around 0.85[2]. Kelley et al. also found that obese individuals had fasting RER values of 0.90 during fasting and insulin stimulated conditions[4]. Impaired metabolic flexibility has been shown to be associated with obesity, detraining, diabetes, and sedentary behavior while improved metabolic flexibility has been shown to be associated with aerobic training and higher levels of physical activity[2,4–6]. Currently, the effect of aging on metabolic flexibility has not specifically been investigated.

By the year 2035, the US Census Bureau predicts that the number of people in America aged 65 and older will make up 21% of the population and outnumber the number

of people 18 and under[7]. They also predict that by the year 2060, nearly one in four Americans will be over the age of 65, and the number of people over the age of 85 will triple from where it is now in 2019[7]. Aging has been shown to be associated with increased mitochondrial dysfunction due to losses in mitochondrial enzyme activity, decreased mitochondrial biogenesis, and decreased respiratory capacity[8]. The mitochondria is the primary site of energy metabolism, both carbohydrates and fat. Further, mitochondrial dysfunction has been shown to play a significant role in the etiology of metabolic inflexbility[1]. For these reasons, it is hypothesized that metabolic flexibility declines with age. However, no studies to date have examined metabolic flexibility in response to a meal challenge between healthy young adults and healthy older adults.

The present study sought to investigate the relationship between metabolic flexibility and aging through the use of a diet challenge. Participants arrived at the laboratory in a fasted state and then consumed a meal replacement shake while having RER, carbohydrate and fat oxidation, and blood glucose levels measured. We hypothesized that older adults will be less metabolically flexible than younger adults as defined as having less variation in RER values from a fasted to postprandial state, having a higher spike in blood glucose levels with a slower recovery to baseline, and having impaired fat oxidation rates.

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Literature Review

1) Overview

Today we live in an aging America and unfortunately, as we age our metabolism becomes less efficient. Defronzo et al found that when comparing young and older adults, older adults had decreased rates of glucose metabolism and tissue sensitivity to insulin, while having similar levels of plasma insulin[9]. By the year 2035, the US Census Bureau predicts that the number of people in America aged 65 and older will make up 21% of the population and outnumber the number of people 18 and under[7]. They also predict that by the year 2060, nearly one in four Americans will be over the age of 65 and the number of people over the age of 85 will triple from where it is now in 2019[7]. Furthermore, the Center for Disease Control and Prevention (CDC) reported in 2016 that 93.3 million or almost 40% of US adults were obese. Obesity is linked to heart disease, stroke, type two diabetes and increased risk of mortality[10]. Taking into consideration the reduced metabolic efficiency with aging as well as the increasing number of aging Americans, it is particularly concerning that in 2016 men and women aged 65-74 were 40.2% and 43.5% obese respectively[10–12]. As our population continues to age, it is important that we strive to understand how our metabolism changes as we age in an effort to reduce the risk of chronic disease and mortality. Therefore, the purpose of this study was to compare the metabolic flexibility of young adults (18-29) and older adults (>55) in an otherwise healthy population in response to a diet challenge.

Metabolic Flexibility is a general term used to describe how one's body responds to a metabolic challenge. These challenges can be from a type of diet, an infusion of nutrients or insulin, an exercise-training bout, or a detraining bout. What all of these challenges have

in common is that they require the body to adapt and use different fuel sources. Metabolic flexibility refers to how well an individual is able to make these transitions between oxidizing fats and carbohydrates. Bergouignan et al suggests using an index that incorporates insulin response and respiratory quotient (RQ) to quantify metabolic flexibility[2]. A metabolically flexible individual requires a smaller concentration of insulin to result in a greater change in RQ after the challenge. This means a healthy individual will be able to switch between oxidizing carbohydrates or fat in an effort to meet the demands of the challenge. Impaired metabolic flexibility has been associated with type 2 diabetes, obesity, sedentary behavior, and detraining[3,4,6].

2) Mechanisms of Metabolic Flexibility

Philip Randle was one of the first researchers to investigate the glucose-fatty acid cycle. The Randle cycle as it came to be known, suggested that when dietary fat is increased, an increase in fat oxidation should occur[13]. This mechanism has been revisited several times over the years and new mechanisms have been added to the originally simple model[14–16]. Boden et al however suggested that rather than dietary fat increasing fat oxidation rates, an increase in ectopic storage of lipid intermediates that can impair insulin signaling may occur[17].

In our cells, our oxidative energy systems take place in the mitochondria. One factor affecting substrate oxidation capacity is the number of mitochondria we have. The more mitochondria we have, the greater capacity for substrate oxidation. We ingest energy in the form of fats, carbohydrates, or proteins and all three of these macronutrients can then be broken down to acetyl-CoA to be used in the Krebs cycle and electron transport chain to transfer energy into adenosine triphosphate (ATP)[1]. In the mitochondria, there are

mechanisms in place that regulate the formation of acetyl CoA. In her review, Muoio details the different mechanisms that can affect metabolic flexibility within the mitochondria[1]. For glucose, once acetyl CoA levels get too high, production is allosterically inhibited by pyruvate dehydrogenase and also by activating the inhibitory kinase, pyruvate dehydrogenase kinase. When fuel is plentiful, malonyl-CoA increases in production, which then inhibits a main transporter of fatty acids into the mitochondria, carnitine-palmitoyl transferase-1[18]. Malonyl CoA also can suppress lactate production If blood lactate levels are elevated, this means malonyl CoA levels are elevated and carnitine-palmitoyl is suppressed and thus fatty acids are not able to enter the cell and undergo beta oxidation[19,20]. When nutrients are no longer plentiful such as during exercise or during fasting, Malonyl-CoA levels decline, causing a decline in blood lactate levels which allows carnitine palmitoyl to bring fatty acids into the mitochondria and increase fat oxidation[6]. San Millan and Brooks affirmed this by finding that in participants with metabolic syndrome and in physically active individuals, blood lactate levels were negatively correlated with physical activity ($r = -0.97$ and $r = -0.92$ respectively)[19]. Proteins, in the form of branch chain amino acids, are regulated by branched chain ketoacid dehydrogenase which leads to acetyl CoA. When acetyl CoA levels get too high from proteins, branched chain ketoacid dehydrogenase kinase is produced to inhibit the production of more branched chain ketoacid dehydrogenase, and thus reducing acetyl CoA formation. In normal circumstances, our bodies are able to switch between these different mechanisms to meet energy demands and prevent buildup of unnecessary byproducts. However, when excess acetyl CoA is produced, reactive oxygen species and posttranslational modifications can occur which may damage the cell and disrupt the signaling pathways. These disrupted

signaling pathways can lead to metabolic inflexibility due to an inability of the cell to identify which pathways to turn off and on[1].

Insulin sensitivity has also been tied to decreased metabolic flexibility[21]. Peterson et al pointed out that it is unclear if metabolic inflexibility causes insulin resistance or rather it is another mechanism through which the process of insulin resistance and metabolic syndrome can develop[22]. If skeletal muscle is unable to respond appropriately when hormones such as insulin are present, carbohydrates are not oxidized or stored efficiently postprandial. This can lead to elevated RQ levels and increased preference for carbohydrate oxidation at rest, opposed to fat. Dube et al found that nonoxidative glucose disposal and insulin sensitivity were positively correlated with mitochondrial performance when comparing lean sedentary, lean trained, and obese sedentary individuals[5]. It was also found that increases in glucose infusion rates yield decreases in fat oxidation rates[23]. These data suggest that the increase in glucose oxidation following the infusion is at the expense of decreased fat oxidation. Substrate oxidation, insulin resistance, and metabolic flexibility are all tied together, however it is unclear exactly through which mechanisms they interact and if metabolic flexibility leads to insulin insensitivity and metabolic syndrome, or vice versa[22]. Nonetheless, it does appear that dysfunction of the mitochondria is the underlying cause for impaired metabolic flexibility.

Assessment of Metabolic Flexibility

Metabolic Flexibility has been assessed by a multitude of different methods as shown below in table I. There is a need for a more established standard in measuring metabolic flexibility in order to more easily compare results from similar studies.

Researchers	Population	Measure of Metabolic Flexibility	Measurement Time Points
Dube et al (2014)	Lean endurance trained and obese sedentary individuals	• Lipid, glucose, and insulin concentrations in blood • Body composition using DXA • Insulin sensitivity via hyperinsulinemic euglycemic clamp • Indirect calorimetry • Muscle biopsies for tissue respiration • Protein analysis of biopsies	Measurements performed at rest at baseline, start of lipid infusion, 330 minutes post and 360 minutes post
San-Millan and Brooks (2018)	Professional male cyclists, moderately active males, and males with metabolic syndrome	• Indirect calorimetry for carbohydrate and fat oxidation • Lactate concentration	An exercise test was used and measurements were compared at varying workloads
Bergouignan et al (2013)	Combined from Lipox women study and Wise2005 women	• Non protein respiratory quotient • Insulin, fatty acid and glucose concentration in blood presented graphically	Measured variance before and after either a training period of a detraining period. No continuous measurement on day of testing to compare
Galagni et al (2008)	Grouped into metabolically Inflexible individuals and metabolically flexible	• Room calorimeter for 11 hours overnight following a fast • Hyperinsulinemic euglycemic clamp	Performed 24 hour measurements over 7 days Investigated 11 hour RQ after overnight fast

Table I: Comparison of different measurement techniques and observation periods used among different studies.

a) Whole Body Measurement

One of the most common ways to assess whole-body energy metabolism is through the use of indirect calorimetry. Indirect calorimetry is a gas analysis measurement where researchers measure production of carbon dioxide (CO2) and oxygen (O2) utilization to determine energy expenditure and substrate utilization. Currently, there are no set protocols for how to use indirect calorimetry to assess metabolic rate or metabolic flexibility. A review by Compher et al however found that a 10 minute measurement period is long enough to establish a reliable measure[24]. Research suggests that the subject be allowed to rest for a minimum of 10 minutes prior to data collection and then out of the 10-minute measurement period, delete the first 5 minutes. This allows for removal of extraneous data points due to acclimation and adjustment to the mouthpiece when initially in place[24]. Researchers should then average at minimum, the remaining 5 minutes of data collection; however, extended data collection periods may further reduce variation. Many factors may affect measurements obtained via indirect calorimetry including eating, smoking, drinking alcohol, or vigorous physical activity. Participants should refrain from eating for a minimum of 5 hours, ingesting alcohol for 2 hours, using nicotine for 2 hours, ingesting caffeine for 4 hours, participating in moderate exercise for 2 hours, and vigorous exercise for 14 hours[24]. These recommendations were made by Compher and her team and are all supported by Grade II or Grade III evidence. Additional relevant recommendations are to ensure that 1) the mask or mouthpiece used is not leaking, 2) resting respiratory exchange ratio (RER) values are between 0.70 and 1.0, and 3) the participant is in a comfortable supine, sitting, or reclining position in a thermoneutral environment.

From indirect calorimetry, RER can be used to determine substrate oxidation levels. Because of this, Lam and Ravussin claim that this is one of the most valuable tools when assessing metabolic rate to understand obesity and metabolic conditions[25]. An RER value of 0.70 is associated with 100% fat oxidation and a value of 1.0 is associated with 100% carbohydrate oxidation. An RER of 0.85 suggests 50% fat and 50% carbohydrate oxidation. As values decrease toward 0.70, they indicate increased fat oxidation and as values increase toward 1.0, they indicate increased carbohydrate oxidation[2] . Kelley et al found that lean individuals had fasting RER values of 0..82 and after being insulin stimulated, the values increased to 0.95. They also found that obese individuals had fasting RER values of 0.90 during fasting and insulin stimulated conditions[4]. Bergouignan et al suggest that an index score be used that utilizes both insulin response as well as RER values. They suggest that metabolically flexible individuals should present with a larger change in RER values per unit of insulin when compared to metabolically inflexible individuals. This means that for example, after a meal metabolically fit individuals should have to produce less insulin in order to produce a larger change in RER values. RER values should form wave shape curves; fasted values should be lower and then after consuming a meal the RER value should increase before coming back down. Insulin levels should produce a similar curve but with a lower amplitude in healthy individuals[2]. Bergouignan's team showed this in their study classifying participants based on their habitual physical activity levels. They found that increases in physical activity levels lead to decreased insulin variance and increased RER variance.

Another approach to using indirect calorimetry is to calculate substrate oxidation levels in an absolute manner. Frayn established equations that can be used to determine the

amount of carbohydrate or fat oxidation that occurs over a period of time[26]. This approach will allow researchers to take into account the total amount of oxidation occurring rather than simply the ratio. This can be both a limitation as well as a strength. When comparing individuals of similar body size, these data can be used to establish that fit individuals oxidize fats at a higher rate than unfit individuals[19,27]. However, if individuals are of different sizes, the results could be skewed to show that the larger individuals are better at oxidizing fats or carbohydrates unless the values are presented relative to body mass.

b) Tissue Measurements

Similar to RER, respiratory quotient (RQ) can be calculated. Some studies will use RER and RQ interchangeably however, RER is measured through gas analysis and RQ is measured at the tissue level. RQ is a more invasive measurement that can be taken from a muscle biopsy. RQ can be used to determine fat and carbohydrate oxidation rates similarly to RER with the same scale of 0.70 indicating complete fat oxidation and 1.00 indicating complete carbohydrate oxidation. RQ values are not able to go outside of this range. However, due to acid buffering, as well as during hyperventilation, RER values may exceed 1.00.

As briefly mentioned, insulin levels can be used to determine metabolic flexibility. It is commonly accepted that with decreased levels of flexibility, insulin sensitivity decreases[28]. One of the most common methods to calculate insulin secretion is a hyperglycemic clamp and insulin sensitivity via a hyperinsulinemic-euglycemic clamp. DeFronzo et al developed this procedure in 1979[29]. During a hyperglycemic clamp, glucose concentrations are increased above basal levels by infusion of glucose and then the level is maintained by continuous infusion of glucose. This measures insulin secretion because

insulin is the hormone that disposes glucose. A hyperinsulinemic-euglycemic clamp is one in which basal levels of insulin are increased and plasma glucose levels are maintained by glucose infusion[29]. Clamps are considered the most controlled method of measuring insulin secretion and insulin sensitivity in a research setting.

Another method of investigating metabolism and metabolic flexibility levels is measuring blood lactate. San-Millan and Brooks established that blood lactate is inversely related to fat oxidation during exercise when comparing professional athletes, moderately active individuals, and patients with metabolic syndrome[19]. They used indirect calorimetry in order to determine fat oxidation. Lactate can be metabolized to malonyl-CoA, which plays a vital role in substrate oxidation rates because it is an inhibitor for a fatty acid transporter carnitine-palmitoyl transferase-1[19]. Increased levels of malonyl-CoA lead to increased levels of carnitine-palmitoyl transferase-1 and decreased levels of fatty acids inside of the mitochondria for oxidation[6,19]. Crawford et al. performed an epidemiologic study on 15,792 older adults and found that when split into quartiles, there was a strong correlation between fasted blood lactate levels and development of type 2 diabetes[30]. These data suggest that blood lactate levels may be useful in determining metabolic flexibility not only during exercise states but also during resting, fasted conditions.

Near infrared spectroscopy (NIRS) can also be used to investigate metabolic flexibility as a noninvasive alternative. Rather than using a claustrophobic mask or a blood draw, a NIRS device can be placed on the muscle to determine oxygen utilization at the muscle following a meal that induces hyperglycemia[31]. A rapid inflation blood pressure cuff is used to occlude blood flow to the muscle and the device measures how much oxygen

is used during this time and then how quickly it is replenished after the cuff is deflated. In their study comparing healthy individuals to obese individuals, Soares et al were able to successfully use the NIRS device to measure the metabolic flexibility of these individuals and added evidence to support that healthy lean individuals are more metabolically flexible than obese individuals[31].

3) Factors Affecting Metabolic Flexibility

Metabolic flexibility is influenced by several different factors such as age, diet, aerobic fitness, and level of sedentary behavior. Below, each will be described in more detail.

a) Diet

High fat diets have been used to challenge the metabolic flexibility in individuals. The change in substrate availability is used to assess changes in fat oxidation rates. Blundell et al found that people who consumed a high fat diet on a regular basis had higher fat oxidation rates after being subjected to a high fat diet provided by researchers when compared to individuals who did not regularly consume a high fat diet (RER 0.85 and 0.89 respectively). The ability of the participants to oxidize carbohydrates however was not statistically different [32]. These data suggest that there could be adaptations that occur to improve an individual's ability to increase their fat oxidation rates.

High fat diets or high carbohydrate diets can be used to challenge metabolic flexibility. The body will need to transition to oxidize the substrate being ingested in order to prevent accumulation of that substrate. During lipid infusion, metabolically flexible individuals were able to oxidize fat at a higher rate than metabolically inflexible individuals ($1.35\ \frac{mL}{kg\ FFM*min}$ and $0.54\ \frac{mL}{kg\ FFM*min}$ respectively)[5]. In the presence of a high

carbohydrate diet, metabolically flexible individuals should require a smaller increase in insulin levels that result in a faster depletion of blood glucose levels[33]. In metabolically inflexible individuals, the slower response to decrease plasma glucose levels can be a result of mitochondrial impairment that leads to insulin resistance and eventually metabolic disease[1,6].

b) Aerobic Training versus Sedentary Behavior

It has been known since the late 1960's that aerobic training improves mitochondrial performance and even leads to mitochondrial biogenesis[34]. Chronic aerobic training increases fat oxidation both at rest and during exercise. It has been suggested that this elevated fat oxidation status can lead to decreased likelihood of development of insulin resistance because less fats will be circulating to disrupt signaling[35]. Intramuscular triglycerides have been linked to insulin resistance in overweight and obese populations. Highly trained aerobic athletes have also been shown to have elevated levels of intramuscular triglycerides[36]. These data suggest that body composition and fat distribution are not solely responsible for disrupted insulin signaling. The population and reason the intramuscular fat accumulates could also play a role. In this instance, aerobically trained individuals use intramuscular triglycerides as an energy source during exercise thus increasing those stores could lead to increased availability during an exercise bout[37]. On the other hand, sedentary individuals not using triglycerides as a substrate allow fats to accumulate, interfere with insulin signaling, and lead to a decreased metabolic flexibility.

Bergouignan et al found that among differing levels of physical activity, insulin resistance and metabolic flexibility correlated with higher levels of physical activity ($R=.70$, $p<0.00036$). When the high physical activity group was given a bed rest intervention, the

researchers observed a decline in their metabolic flexibility that was represented by a smaller change in their RQ values (RER declined ~0.04). Conversely, the researchers found that implementing training in overweight individuals led to improvements in metabolic flexibility (RER increased ~0.02)[2]. These results insinuate that metabolic flexibility, just like insulin resistance can be improved or hindered by improvements or declines in physical activity respectively.

c) Aging

Aging has not been directly investigated in regards to its effect on metabolic flexibility however, several studies have shown that as we age, we become more susceptible to mitochondrial dysfunction as indicated by a loss of mitochondrial respiration capacity, declines in enzyme efficiency, and declines in mitochondrial biogenesis[1,8].

Glucose homeostasis also deteriorates as we age. Melanson et al showed that older women maintained a higher level of postprandial glucose and insulin levels (.22, 2.26 and -.04, -.01 95% CI intervals for change scores of glucose and insulin respectively) after eating both a small and a larger meal when compared to younger adults [38]. Blood glucose levels have been shown to be associated with satiety and hunger[39–44]. When younger adults experience hypoglycemia, they tend to experience more intense feelings of hunger compared to older adults[45,46]. Wolden-Hanson et al showed that when older animals were given an insulin sensitizing agent they had similar eating response after fasting as the younger animals[47]. Older adults however also have a slower recovery from hypoglycemia when compared to young adults[38,45]. These elevated levels of postprandial glucose and insulin levels as well as the slower recovery from hypoglycemia after fasting suggest that

older adults may have a decreased metabolic flexibility as prolonged elevated glucose and insulin levels could lead to an insulin resistance which can lead to other metabolic diseases.

Older adults also present with a decreased basal metabolic rate (BMR)[48,49]. Keys et al did a longitudinal study on BMR and found that participants experienced a 1-2% decrease in BMR per decade between the ages of 20 and 70[49]. Most studies found a similar drop in BMR values with aging,[48,50–54] but not all[55–57]. In their review, Roberts and Rosenberg [58] suggest these differences in findings can be explained by whether or not studies accounted for the body composition of the subjects. Roberts et al showed in another study that there was a positive correlation with fat-free mass and energy expenditure and a negative association of fat mass with energy expenditure. This suggests that as the amount of fat mass increases we could expect a decrease in energy expenditure. NHANES III showed that as the participants aged, the increases in BMI and fat mass were also linked to limitations in everyday activities[59]. This could help explain the decreased BMR; increases in fat mass and BMI could limit our ability to perform tasks and thus decrease energy expenditure. Westerterp and Meijer found that the decline in energy expenditure associated with age was independent of lean tissue and fat mass. It is well accepted that decreases in maximal oxygen consumption (VO_2 max) are associated with age[60,61]. A Surgeon General report from the CDC found that regardless of training or activity level, VO_2 levels declined by about 50% from age 20 to 80[62]. Declines in VO_2 max and declines in BMR can be associated with mitochondrial dysfunction. In their extensive review, Sun et al explain mitochondrial impairments associated with aging. Telomere dysfunction associated with aging has also been linked to declines in mitochondrial biogenesis[8]. Aging has also been shown to be accompanied by declines in mitochondrial enzymes, respiratory capacity of

individual mitochondrial, and increases in reactive oxygen species[8]. Metabolic flexibility is largely determined by the ability of the mitochondria to switch between different carbohydrate and fat oxidation. If mitochondrial dysfunction is associated with aging, studies should be done on the effect of aging on metabolic flexibility.

Conclusion

With the rise of older adults in the United States as well as an increase in obesity and metabolic disorders, it is imperative that we continue research in both aging and metabolic disorders[10–12]. Metabolic flexibility has been shown to be associated with insulin resistance, obesity, physical activity levels and mitochondrial function[2,6,29,54]. While metabolic flexibility has not been specifically investigated in regards to aging, age has been shown to have a negative effect on mitochondrial function, decreased lean mass, and decreased physical activity[51,54,56].

Purpose/Hypothesis

The purpose of this study was to determine if age has an effect on metabolic flexibility by comparing a sample of otherwise healthy young and older adults. We hypothesize that the older adults will have a decreased metabolic flexibility when compared to the young adults as defined by (1) decreased fat oxidation rates both at rest and during the observation period, (2) having a less variable RER, and (3) a steeper increase in blood glucose levels with a more gradual decline.

Methods

Participants

Participants in the present study were healthy and free of other chronic disease on a self-reported basis so as to investigate age alone as a factor to affect metabolic flexibility. Inclusion and exclusion criteria are detailed in table I below. Participants also provided written informed consent as approved by the Wake Forest Institutional Review Board. We collected data on 6 young adults (4 women, 2 men) and 2 old adults (1 woman, 1 man). Participants were recruited using emails to the department of Health and Exercise Science Department of Wake Forest University, flyers, and word of mouth.

Inclusion	Exclusion
• Aged 18-25 or >55 years old • BMI range of 18-25 kg/m^2 • Young women – regular menstrual cycle • Old women – post menopausal • Must be able to consume study foods • Must speak English • Must obtain transportation to visits	• Self-reported diagnosis of chronic disease such as diabetes, cancer, cardiovascular disease, or COPD • Tobacco users • Febrile or chronic infection • Aversion to testing procedures following familiarization • Diagnosis of hypertension, hypo/hyper thyroidism • Use of caffeine during study period • Use of medication or supplements that affect metabolic rate • Pregnancy • Competitive athletes or sedentary behavior

Table II: Details inclusion and exclusion criteria for participation in our study.

Study Design

The present study was a cross-sectional design with comparisons in outcomes between young and old participants. Participants attended two laboratory visits separated

by 3-7 days. In the first visit individuals completed a screening questionnaire, signed the informed consent, underwent familiarization procedures, and were provided with food logs. Leading up to the second laboratory visit, participants logged their food intake for three days on the food logs and were then given two days worth of food. This gave researchers five total days of known food consumption for each participant. During the three days of self-reported intake, the participants were asked to maintain their normal diet. The two days of provided meals were a combination of milk and cereal for breakfast, a lean cuisine chicken panini and chicken Caesar salad for lunch, a lean cuisine chicken parmesan for dinner, an apple, a banana, a granola bar, a bag of trail mix, pretzels, carrots and ranch for snacks. The number of calories assigned were determined based upon United States government recommendations for age, sex, and activity levels and ranged from 1800-2800 calories with a macro nutrient breakdown of about 52% carbohydrate, 17% protein, and 31% fat. To meet these caloric recommendations the number of items provided was adjusted.

The second laboratory visit was the main data collection visit. Participants were asked to report to the lab in the morning between 6 and 9 am after an overnight fast of at least 8 hours. Baseline measurements were then collected and are detailed below. After completing baseline measurements participants were given a high carbohydrate beverage that included two servings of the original formula Slimfast® meal replacement shake mixed with 16 ounces of fat free milk for a total of 400 or 390 calories for the chocolate and vanilla formula, respectively, with a macronutrient breakdown of 9% Fat, 68% carbohydrate, and 23% protein. Participants chose chocolate or vanilla based on their taste preference. Participants were asked to drink the formula within 5 minutes. When the meal

replacement shake was finished, a timer was started and all measurements were collected again at 30, 90, 120, and 180 minutes post beverage consumption. The app "seconds®" was used to create a custom timer and ensure all measurements were performed on time and in a controlled and repeatable manner. Participants were asked to stay seated in a semi-recumbent position during the entire testing period. Participants were allowed to engage in non-stimulating activities such as reading or listening to music between measurement periods.

Indirect Calorimetry

Indirect calorimeter (Cosmed K5 (Rome, Italy)) assessed resting metabolism by measuring oxygen consumption and carbon dioxide production. Respiratory exchange ratio (RER) was calculated from these measures. RER is equal to carbon dioxide consumption (VCO_2)/ oxygen consumption (VO_2). Before each visit, the K5 was calibrated in accordance with manufacturer's recommendations. Subjects were fitted with the proper size mask and head strap at the first lab visit in order to ensure that no air leaks occurred during data collection. Fat oxidation and carbohydrate oxidation rates were assessed in accordance to Frayn's methodology[26]. The number of grams oxidized from fat and carbs were used to calculate the total number of calories oxidized in comparison to the total number of calories burned. This was done by multiplying the number of grams of fat by 9 and the number of grams of carbs by 4.

$$FATox\ (g * min^{-1}) = 1.67VO_2(L * min^{-1}) - 1.67VCO_2(L * min^{-1})$$

$$CHOox\ (g * min^{-1}) = 4.55VO_2(L * min^{-1}) - 3.21VCO_2(L * min^{-1})$$

Blood Measurements

Blood glucose levels were determined using a HemoCue 201® (Brea, CA) using capillary blood obtained by a finger stick. Manufacturer guidelines were followed to calibrate the devices and obtain blood samples. Blood measures were collected at baseline, 30, 90, 120 and 180 minutes post consumption of the beverage.

A change score was determined for the change in RER values over the five time points and assigned to each participant. Oxygen consumption and carbon dioxide production measures were averaged over ten minutes after an initial five minutes were collected and then discarded to reduce the variation within each subject. RER was then calculated for these averages. Change scores were also applied to blood glucose levels.

Statistical Analysis

Due to variation in reporting of data across several different studies, the present study reported RER, carbohydrate oxidation, fat oxidation, at each time point. All of these values are calculated based off of the subject's VO_2 and VCO_2 values found in the appendix. Our sample size did not allow for standard statistical analyses to be conducted. Instead, effect sizes were calculated for change scores between the young and old groups for carbohydrate oxidation, fat oxidation, blood glucose, and RER. Because these values were expected to increase and decrease over the 180 minutes, an absolute value change score was made. Effect sizes were also determined for the total grams of carbohydrate and fat oxidized in the total 50 minutes measured and estimated for the total 180 minute observation period. The estimation was made by averaging all 5 of the 10 minute measurements to get a 50 minute average. This value was converted to a minute by minute

value and then multiplied by 180 minutes. Date are presented as means (S.D.) and effect sizes were calculated using Excel.

Results

The participants' demographics can be found in table I. The basal metabolic rate for each group was measured from baseline values and are presented in Figure 1. RER, carbohydrate and fat oxidation values are calculations based off of participant's VO_2 and VCO_2 values. Please see appendix for individual participant data and raw values.

To answer our first hypothesis, the carbohydrate and fat oxidation rates in grams per minute at the five time points for young and old adults are shown in Figures 2 and 3. These figures are average values from the two groups. Figures 4 and 6 show the amount of carbohydrate and fat estimated to be oxidized over the 180-minute observation period. Figures 5 and 7 show the amount of carbohydrate and fat measured to be oxidized during the 50 minute measurement period. The 180-minute estimates were found by averaging the total grams measured to be oxidized in 50 minutes for each group and multiplying by a 180-minute multiple. Effect sizes for carbohydrate oxidation, fat oxidation, blood glucose levels, and RER are found in Table 3. The total change score in Table 3 was calculated by summing the change scores from 0-30, 0-90, 0-120, and 0-180 minutes.

Cohen's D effect sizes for total grams of carbohydrate and fat oxidation for 50 and 180 minutes are reported in Table V. The 50 minute values are a sum of the total grams oxidized during the 5 separate 10 minute measurement periods. The 180 minute values are an estimation made by dividing the 50 minute sum by 50 to obtain a per minute value and multiplying it by 180 to calculate the total across the 3 hour data collection period. The effect sizes for carbohydrate and fat oxidation at 50 minutes were large at 0.80 and 0.85 respectively. The effect sizes increased at 180 minutes for carbohydrate and fat oxidation to 1.27 and 1.04 respectively.

	Old (n=2)	Young (n=6)
Height (cm)	169.88	171.38
Weight (kg)	73.71	68.78
BMI (kg/m^2)	25.33	23.49
Age (yrs)	71.5	24.17
Males	1	2
Females	1	4

Table II: Depiction of participant demographics in the present study.

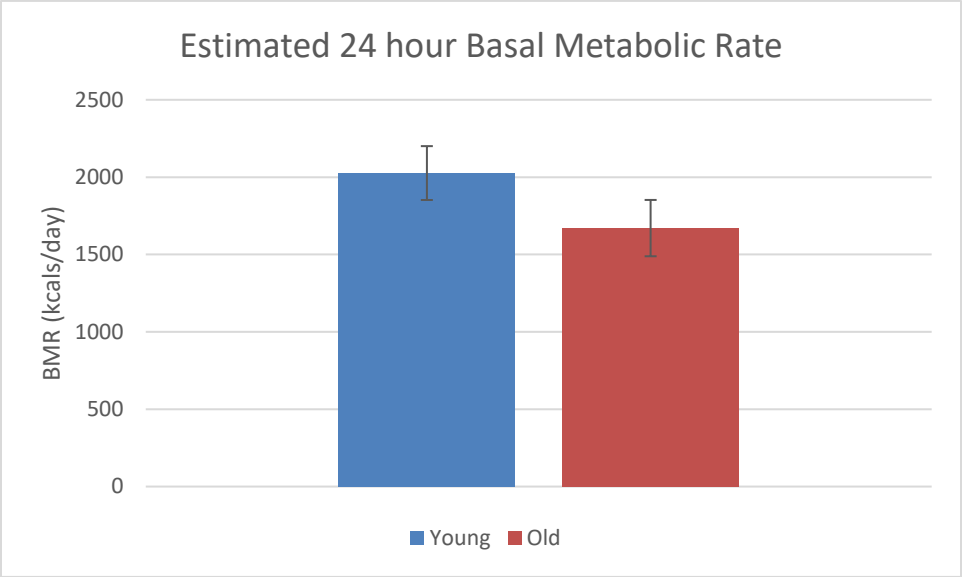

Figure 1: Estimated basal metabolic rate for young and old adults in kilocalories per day. (BMR – basal metabolic rate)

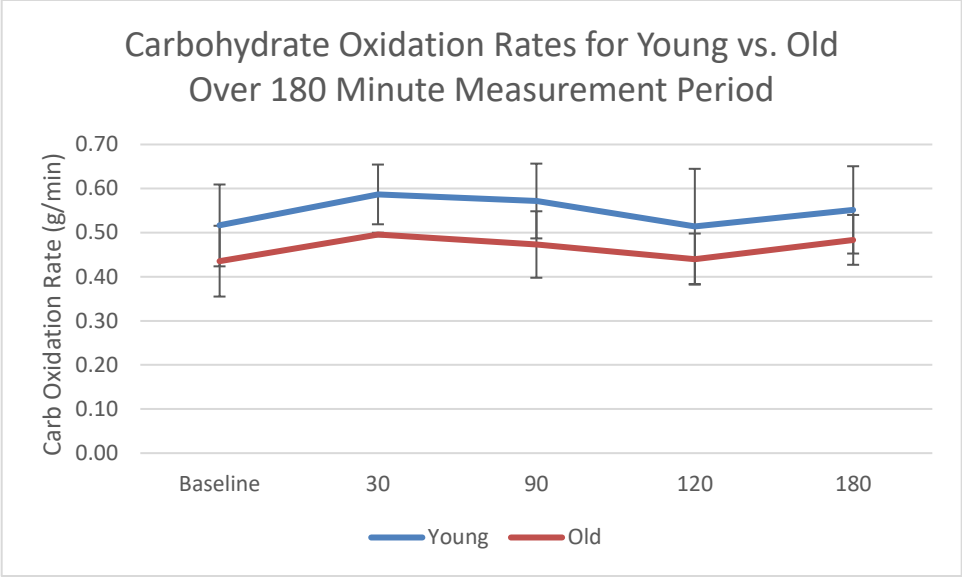

Figure 2: Carbohydrate oxidation rates for each group over the 5 measurement periods. (Carb – carbohydrate)

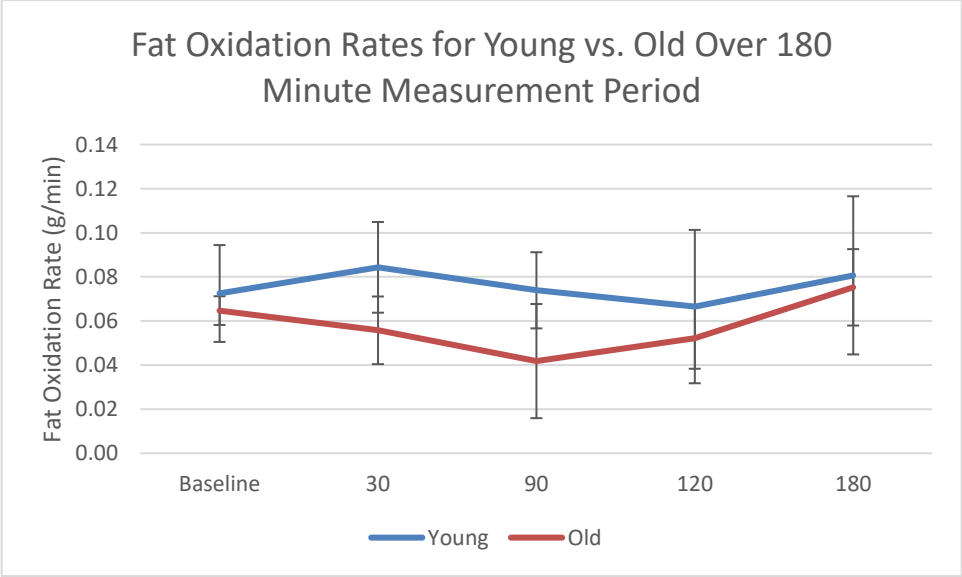

Figure 3: Fat oxidation rates for each group over the 5 measurement periods.

Cohen's D Effect Sizes for Absolute Value of Change Scores Between Young and Old					
	0 - 30 mins	**0 - 90 mins**	**0 - 120 mins**	**0 - 180 mins**	**Total Change**
Carb Oxidation	0.17	0.15	1.64	0.24	0.40
Fat Oxidation	0.00	0.29	0.38	0.80	0.51
Blood Glucose	-0.32	-0.97	0.31	0.29	`-0.28
RER	-1.30	0.40	-0.12	0.50	0.26

Table III: Cohen's D effect sizes for carbohydrate oxidation, fat oxidation, blood glucose levels, and RER for change scores from 0 minutes to the various follow-up periods (30, 90, 120 and 180 minutes). The total change column is the effect size for the sum of all the change scores for the previous time points when comparing young and old adults. (Carb – carbohydrate, RER – respiratory exchange ratio).

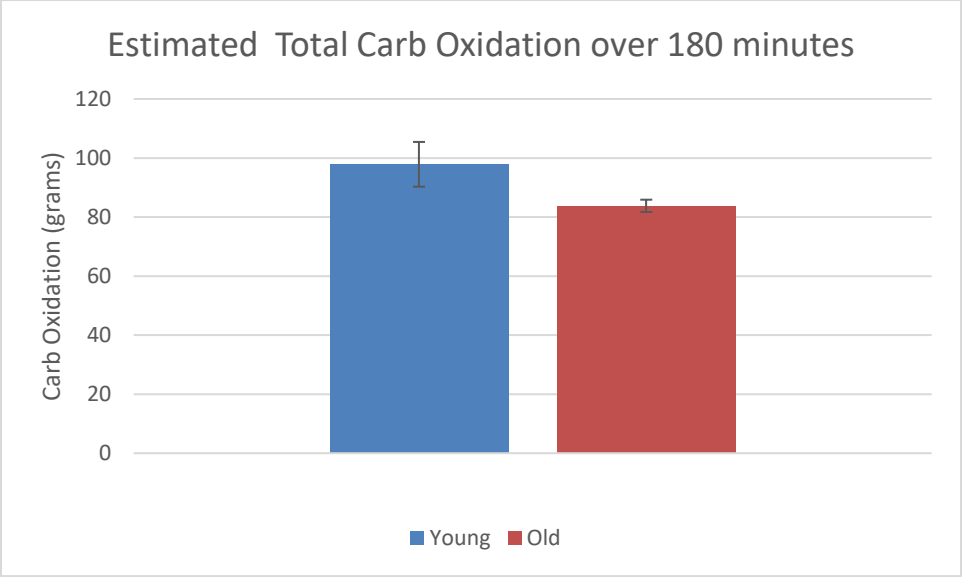

Figure 4: Estimated total grams oxidized from carbohydrates over the 180 minute measurement period. (Carb – carbohydrate)

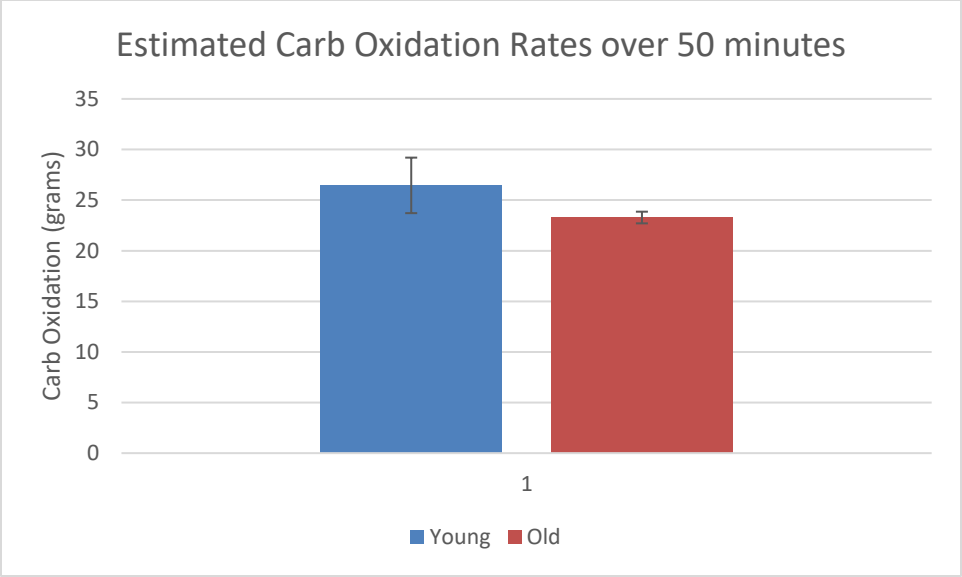

Figure 5: Measured total grams oxidized from carbohydrates over the 180 minute measurement period. (Carb – carbohydrate)

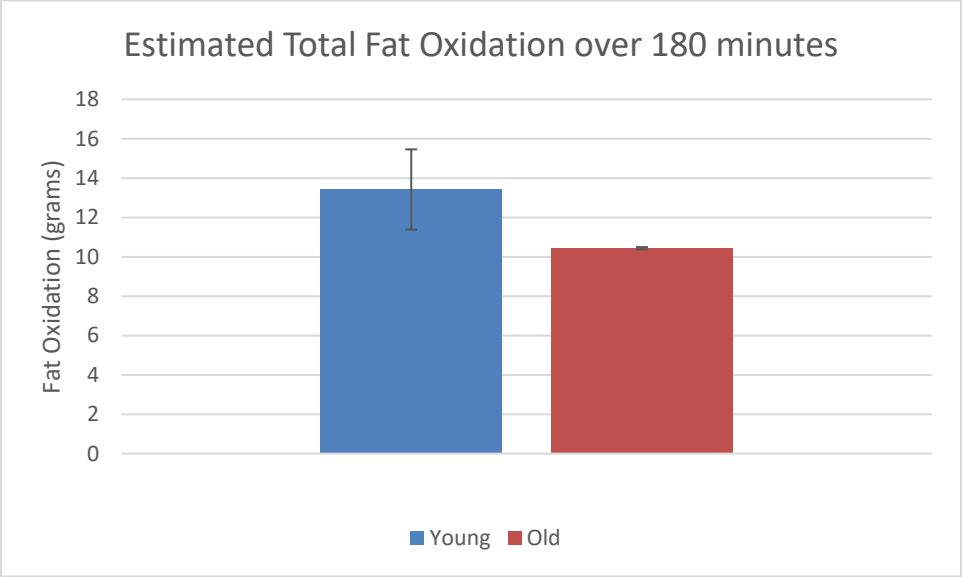

Figure 6: Estimated total grams oxidized from fats over the 180 minute measurement period.

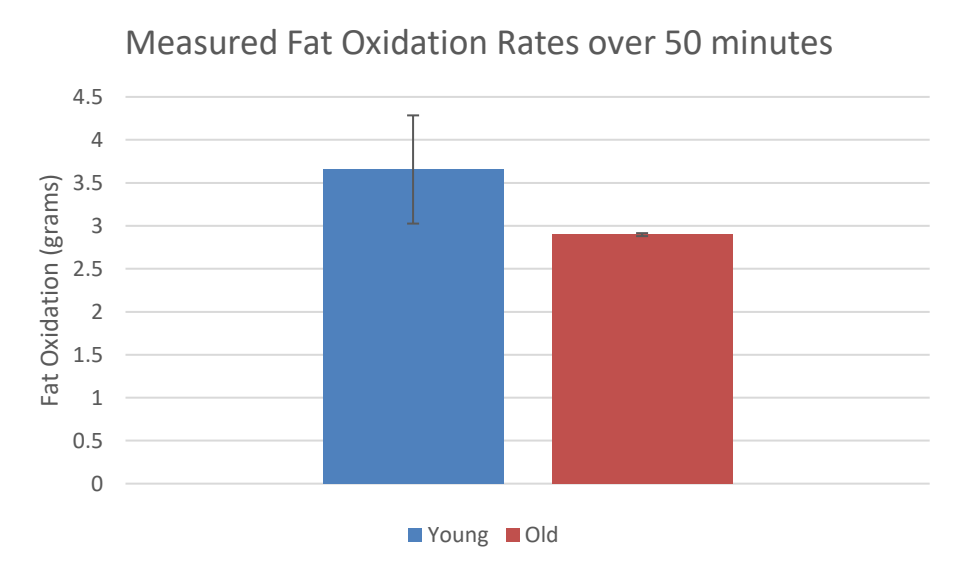

Figure 7: Measured total grams oxidized from Fat over the 180 minute measurement period.

Cohen's D Effect Sizes for Total Grams Oxidized Between Young and Old		
Carb Oxidation	Total Grams 50 mins	0.80
	Total Grams in 180 mins	1.27
Fat Oxidation	Total Grams in 50 mins	0.85
	Total Grams in 180 mins	1.04

Table V: Cohen's D effect sizes for carbohydrate and fat oxidation during the total 50 minutes of gas exchange collection as well as estimated values for 180 minutes when comparing young and old adults. (Carb – carbohydrate)

To answer our second hypothesis, the trends for average RER values are found in Figure 8. These values are a ratio of VCO_2/VO_2. RER can be used to determine how much fat and carbohydrate are being oxidized (values closer to 1.0 indicate carbohydrate oxidation and values closer to 0.70 indicate fat oxidation). Table IV shows the actual percentage values for young and old adults are each of the measured time points. These values were determined by converting the total grams of each substrate to calories and then calculating the ratio of calories from each substrate to the total number of calories. For our third hypothesis, blood glucose levels over time are found in figure 9. Cohen's D effect sizes are found in Table III.

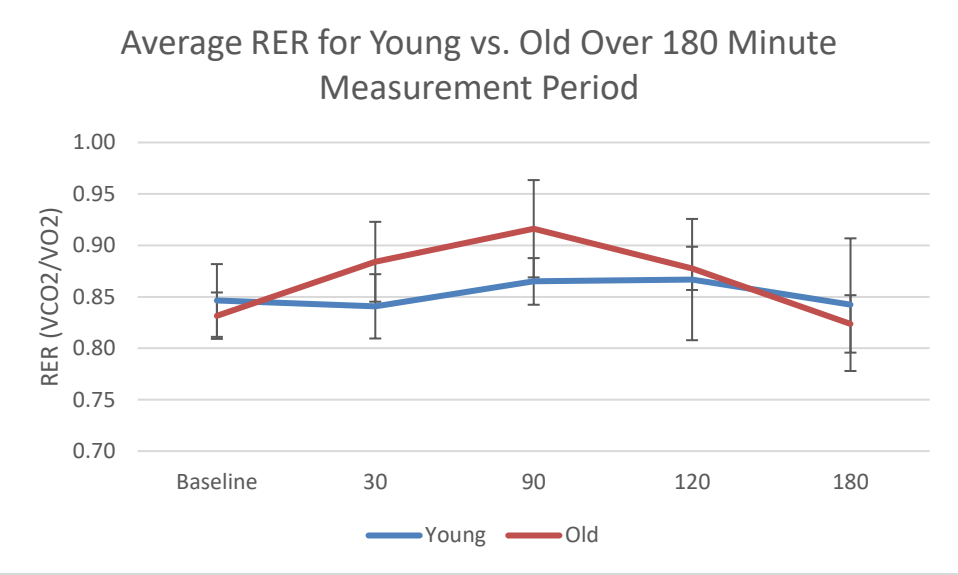

Figure 8: RER values over the 180 minute measurement period for young and old adults. (RER – respiratory exchange ratio)

Percentage of Total Calories From Fat					
	Baseline	**30 mins**	**90 mins**	**120 mins**	**180 mins**
Young	23.99	24.43	22.53	22.55	24.76
Old	25.06	20.19	16.58	21.08	25.93
Percentage of Total Calories From Carbohydrate					
	Baseline	**30 mins**	**90 mins**	**120 mins**	**180 mins**
Young	76.01	75.57	77.47	77.45	75.24
Old	74.94	79.81	83.42	78.92	74.07

Table IV: Shows percentage of total calories from both fats and carbohydrates at various time points for young and old adults.

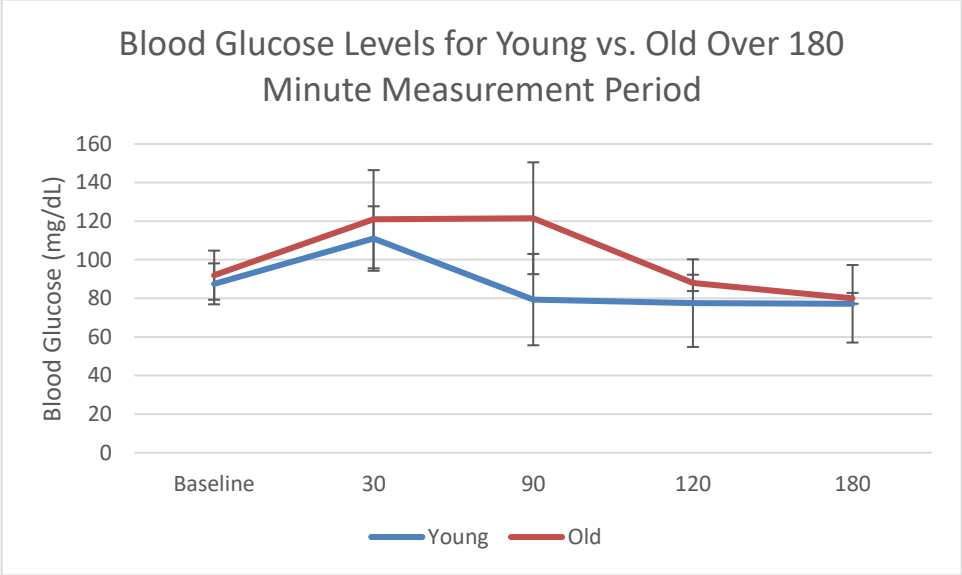

Figure 9: Blood glucose levels for both groups over the 180 minute measurement period for young and old adults.

Discussion

Investigation of our first hypothesis, whether age was associated with decrements in carbohydrate and fat oxidation, was investigated using effect sizes and graphical data. Carbohydrate and fat oxidation over the 180 minute measurement period have moderate effect sizes at 0.40 and 0.51 respectively. This difference in absolute oxidation levels of fat and carbohydrates could be explained by differences in metabolic rate. It has been found that individuals experience a 1-2% decrease in BMR per decade between the ages of 20 and 70[49]. With a lower BMR, older adults would oxidize less carbs and fats per unit of time as the young adults with the same RER. This is consistent with the RMR findings from the current study as BMR values for young adults were 2026 kcal/day and 1670 kcal/day for the older adults, even though body weight was higher in the older than lean individuals. The age associated decline in BMR is associated with a decline in mitochondrial enzymes such as citrate synthase, a decline in the respiratory capacity of the mitochondria and an increase in reactive oxygen species production[8]. Decreases in fat oxidation however have been shown to lead to increased likelihood of ectopic fat storage[65]. If older adults are not able to oxidize the fats consumed at the same rate as the young adults, the fat not oxidized will be stored and more reactive oxygen species will be produced in an attempt to keep up with demand due to an inability to process the excess acetyl CoA[1]. This suggests older adults gain body fat more easily than young adults and that they have a more difficult time losing the weight. A possible suggestion would be for older adults to eat multiple smaller meals throughout the day in order to not have the increased likelihood of ectopic fat storage

due to their decreased fat oxidation and reduce the likelihood of an influx of a large amount of macronutrients to be oxidized at once.

For our second hypothesis, whether or not age was associated with a less variable RER response to the meal, graphical data and effect sizes were used again. Upon visual inspection, the old adults in the current study appeared to have more variability in their RER values over the measurement period compared to the young adults in our study with an effect size of .26 over the total observation period. Typically, more variability in RER values after a challenge such as the meal replacement shake used in this study is associated with greater metabolic flexibility. The selection criteria for the study was apparently healthy and free of metabolic diseases. If the individuals were healthy enough to qualify for the study, they may not represent the typical older population and thus may be more metabolically flexible. Metabolic flexibility has been associated with diseases like obesity and diabetes so the individuals that make it to old age and are still free of chronic disease and in a healthy weight range may be more metabolically flexible than those with lifestyle-related conditions[21,66,67].

Our final hypothesis was that the older group would have a sharper increase in blood glucose levels and a delayed decline to normal following the challenge meal. The blood glucose levels observed in the present study followed a similar trend to one that compared a population of healthy glucose responders to impaired glucose tolerance[18]. The effect size for baseline to 30 minutes post consumption of the beverage was -1.30. This shows that there was a large effect and that the mean of the old group was above that of the young group. These data are consistent with Corpelejin who compared healthy individuals to that of impair glucose tolerance[18]. They found no significant difference for

blood glucose between the groups at baseline but following the meal, the group with impaired glucose tolerance had a steeper increase in blood glucose levels followed by a slower recovery to baseline[18]. The current study found the older adults to have decreased glucose tolerance and insulin sensitivity but future studies should seek to investigate blood insulin levels as well.

Our study attempted to control for the influence of habitual dietary intake on RER by providing the participants with food for two days prior to the data collection visit. Studies have shown that variability in daily dietary intake alters RER[68]. To ensure that the observed RER values were due to metabolic changes and not due to variability in dietary intake each participant was provided with the recommended amount of calories based upon their age, sex, and physical activity level[69]. We did not however require the participants eat the food at the study site but relied on the integrity of the participants to adhere to the study protocol and only consume the provided food. Some studies have required participants stay at the testing site to eat food from a metabolic kitchen and then complete a one-day stay in a room calorimeter[21].

As there is no set standard for how to assess metabolic flexibility, it is difficult to compare results from one study to the next as different measurement techniques are used. Even within the same measurement technique, there are variations. Even after obtaining the measurement values, studies report the values differently for assessing metabolic flexibility. Some studies report the area under the curve of RER or RQ over time as a score. Additionally studies report the difference between the peak and low values, and others refer to an index score combining RER and insulin sensitivity[2,5,67,70]. This is a challenge to this

field and if this area is going to progress and attempt to be used as a clinical tool or evaluation, more consistency and an accepted standard protocol is needed between studies. The metabolic challenge chosen (exercise, diet, detraining etc.) also presents with variability. Within the same challenge, a number of variables can change. The diet provided to the participants can vary widely from study to study, making it difficult to compare directly. Without a metabolic kitchen, or an agreed upon, premade meal that can universally be provided to participants, it is difficult to replicate the exact diet challenge as a previous study to attempt and replicate results.

One of the major limitations of our study included an inability to observe participants during the time they consumed the study food. Any variation from the provided diet could have driven the participants above or below their eucaloric state. This state was estimated however and was not calculated. Future studies could perform a baseline BMR measure to precisely prescribe the amount of calories needed to obtain a eucaloric state. The current study also did not measure BMR for the entire measurement period and instead opted for a single 15 minute bout. Having a total daily energy expenditure would further enhance the accuracy of the eucaloric condition. For the comfort of our participants and to decrease the likelihood of aversion to our measurements, we allowed the mask to be taken on and off at regular intervals. To more thoroughly examine specific tissue oxidation, a skeletal muscle biopsy would allow for the determination of muscle fiber type and measure RQ at the tissue level. This would allow researchers to account for the amount of type 1 fibers that play a more active role in aerobic metabolism and metabolic flexibility. The current study also could have utilized an euglycemic-insulin clamp. Measuring blood glucose levels is useful however, insulin is the main hormone regulating glucose levels.

Measuring insulin concentrations would have allowed the researchers to investigate insulin response between the two groups. The study also presented with a small sample size. The effect sizes that were calculated however did provide evidence that further data collection could allow for confirmation of our hypothesis, that older adults have a decline in their metabolic flexibility when compared to young adults in an otherwise healthy population. It is important to note that the inclusion criteria of being healthy was based upon self-report and did not involve a check of medical records nor did the study employ a physician to give participants an exam or check conflicting medications.

While the current study was not without limitations, there were also strengths. The study did follow the best practice recommendations for RER measurement[24]. For each of the 10 minute measurement periods, measurements occurred for 15 minutes. Per the recommendation, the first 5 minutes were omitted to allow for adjustments to the mask and equipment to be worked out. The remaining 10 minutes from each stage was then averaged to minimize any variation in each measurement. The measurement period of the study was also 3 hours which is a common amount of time for studies to observe individuals. Even though the mask was taken on and off, a customized timer was used in order to make sure the timing was consistent and happened at regular intervals. This was a better approach that simply taking pre and post measurements as it allowed researchers to attempt and observe trends over the 5 time points even though there was not a continuous measurement. The researchers also attempted to account for physical activity. Competitive athletes and sedentary individuals were excluded. Physical activity levels have been shown to affect metabolic flexibility so both extremes of physical activity levels were excluded in order obtain a more homogenous sample[2].

Recommendations to future studies would be to find a more efficient way to recruit healthy older adults. The present study had difficulty finding eligible, healthy, older adults. The older adults recruited in this study both came from a clinical research center that focuses on weight loss and specializes in stress tests for clinical populations. For this reason, there is a disproportionate number of diseased individuals compared to healthy. The researchers in the present study were hesitant about the 180 minute measurement period. Subjectively, participants tolerated the time very well and future studies could possible extend the time period to 240 minutes. Future studies should also seek to collect muscle biopsies in order to investigate oxygen consumption at the muscle rather than using indirect calorimetry.

Moving forward these data can be used to further explore metabolic flexibility in aging. This was the first study to investigate aging alone for its effect on metabolic flexibility. Previous studies have investigated either physical fitness, disease status, or sedentary behavior or obesity. More studies investigating the effect of aging on metabolic flexibility are needed, especially with the projection that by 2035, number of US citizens aged 65 and older will outnumber the number of citizens under the age of 18[7]. The number of elderly Americans is not only increasing but Americans are also getting older as the number of people over the age of 85 will triple by 2060 from 2019[7].

While it was not possible to test for a statistical difference between groups, the effect sizes found are promising. Further data will be collected and if the trends continue the way they are now, aging will have been shown to be associated with decreased metabolic flexibility. Further studies will be needed to make this definitive. Future studies

should also seek to employ standardized measurements to increase consistency between results and make direct comparison easier.

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Appendix

VO2 Values (mL/min)					
Young	**Baseline**	**30**	**90**	**120**	**180**
1	218.8	337.1	350.4	327.6	302.8
2	283.7	290.4	293.1	238.4	299.3
3	353.4	315.9	324.4	313.8	296.7
4	272.1	282.4	266.7	265.7	266.8
5	245	327		222	270
6	315.7	348.8	369.1	362.5	340.1
average	281.5	316.9	320.7	288.3	296.0
Old	**Baseline**	**30**	**90**	**120**	**180**
1	196.3	276.5	306.6	237	240.8
2	267.8	303.4	279.5	269.9	265.2
average	261.3	267.1	273.2	250.0	265.3

Table VII: Raw VO_2 values for young and old in mL/min.

VCO2 Values (mL/min)					
Young	**Baseline**	**30**	**90**	**120**	**180**
1	189.3	294.7	302.8	296.6	241.4
2	231.6	244.7	253.1	193.4	244.7
3	315.3	257.3	269.4	261.7	240.33
4	235.2	245.2	238.2	235.3	224.8
5	207	279		212	262
6	250	277.8	319	292.1	272.6
average	238.1	266.5	276.5	248.5	247.6
Old	**Baseline**	**30**	**90**	**120**	**180**
1	160.3	236.6	270.6	211.6	203.1
2	226.3	276.5	265.4	232.7	212.8
average	193.3	256.6	268.0	222.2	208.0

Table VIII: Raw VCO_2 values for young and old in mL/min.

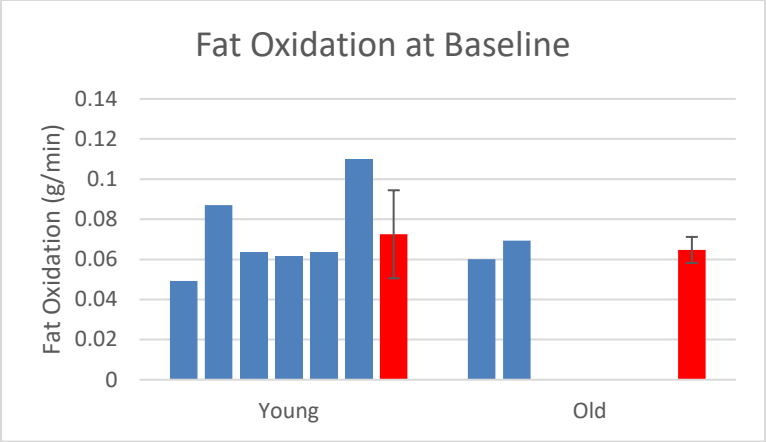

Figure 10: Fat oxidation rates for young and old at baseline. Blue bars are individuals and red bars are means of each group

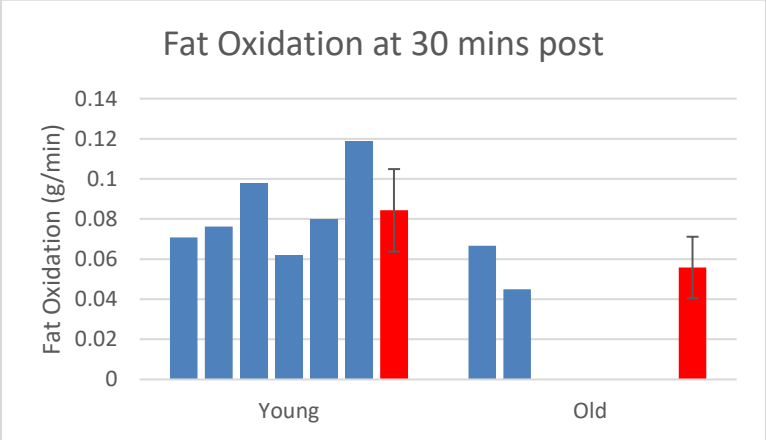

Figure 11: Fat oxidation rates for young and old at 30 minute post. Blue bars are individuals and red bars are means of each group

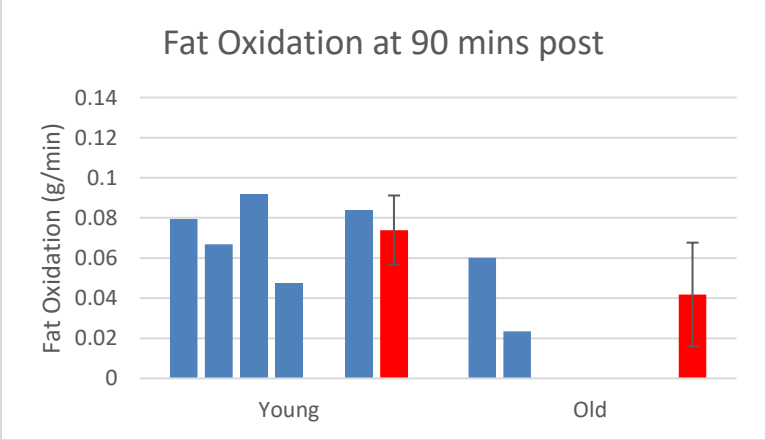

Figure 12: Fat oxidation rates for young and old at 90 minutes post. Blue bars are individuals and red bars are means of each group

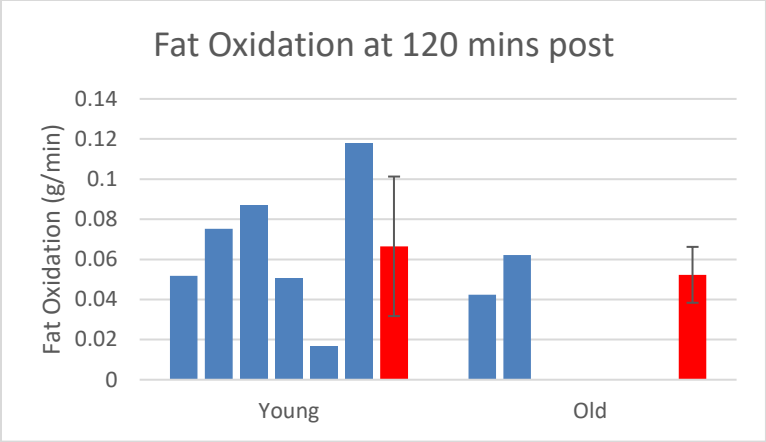

Figure 13: Fat oxidation rates for young and old at 120 minutes post.. Blue bars are individuals and red bars are means of each group

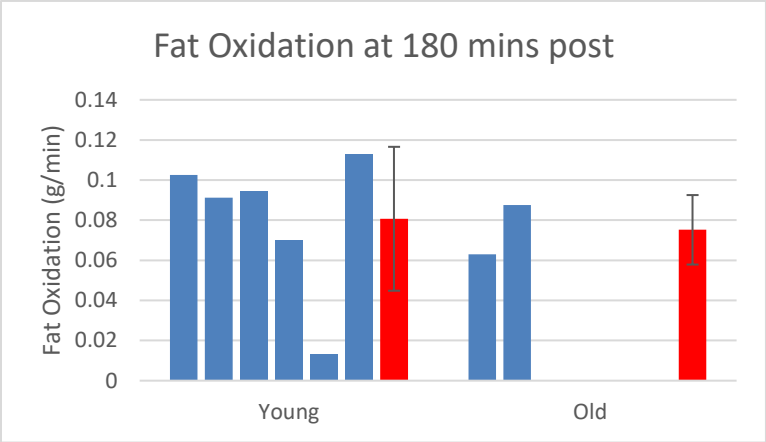

Figure 14: Fat oxidation rates for young and old at 180 minutes post. Blue bars are individuals and red bars are means of each group

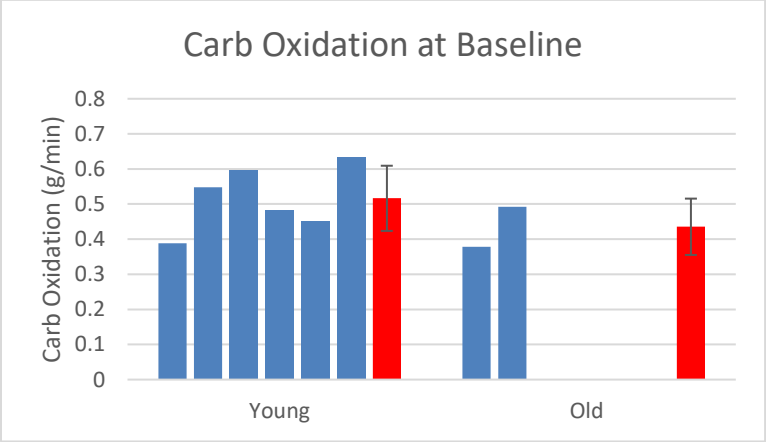

Figure 15: Carbohydrate oxidation rates for young and old at baseline. Blue bars are individuals and red bars are means of each group (Carb – Carbohydrate)

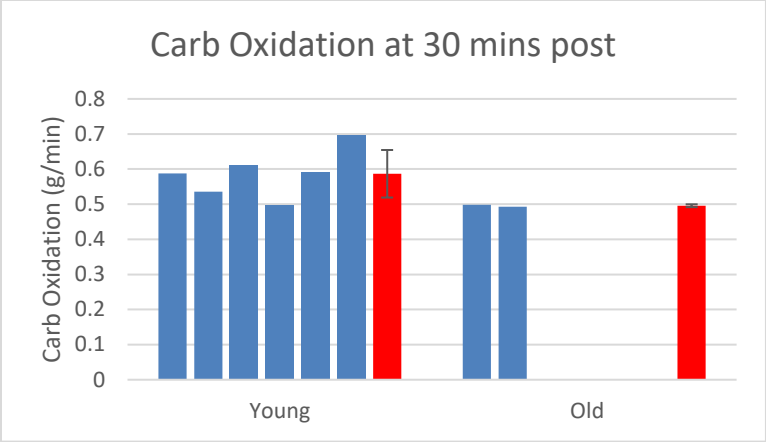

Figure 16: Carbohydrate oxidation rates for young and old at 30 minutes post. Blue bars are individuals and red bars are means of each group (Carb – Carbohydrate)

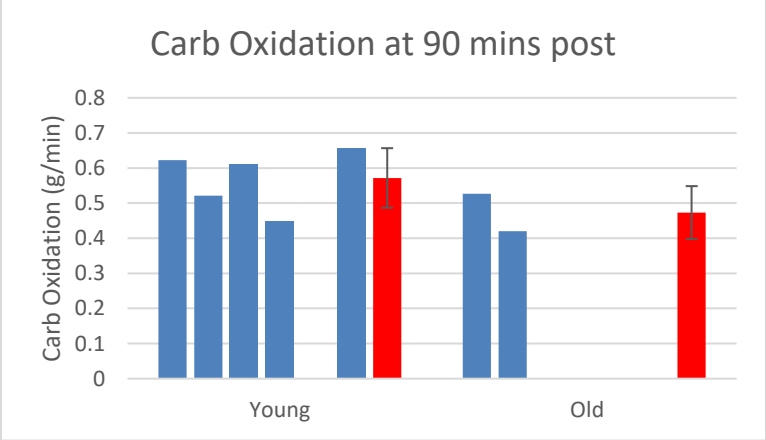

Figure 17: Carbohydrate oxidation rates for young and old at 90 minutes post. Blue bars are individuals and red bars are means of each group (Carb – Carbohydrate)

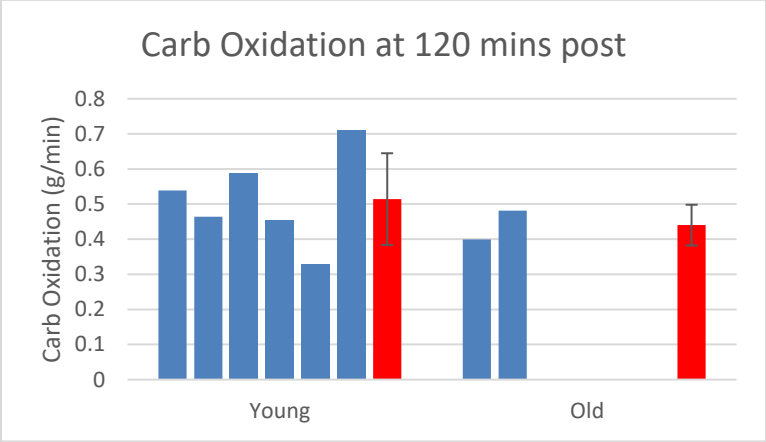

Figure 18: Carbohydrate oxidation rates for young and old at baseline. Blue bars are individuals and red bars are means of each group (Carb – Carbohydrate)

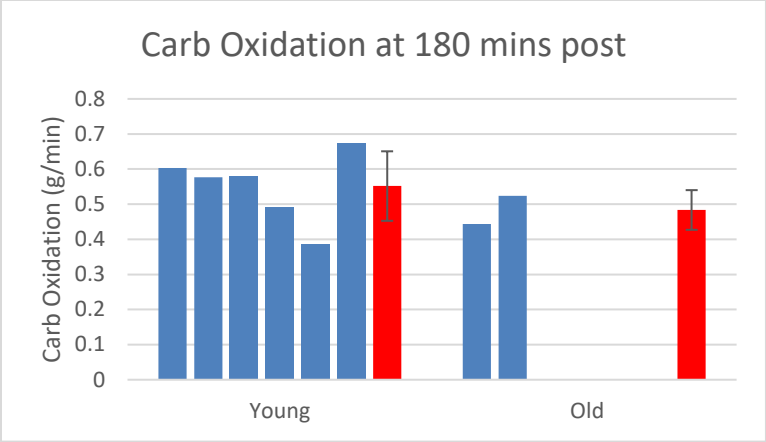

Figure 19: Carbohydrate oxidation rates for young and old at 180 minutes post. Blue bars are individuals and red bars are means of each group (Carb – Carbohydrate)

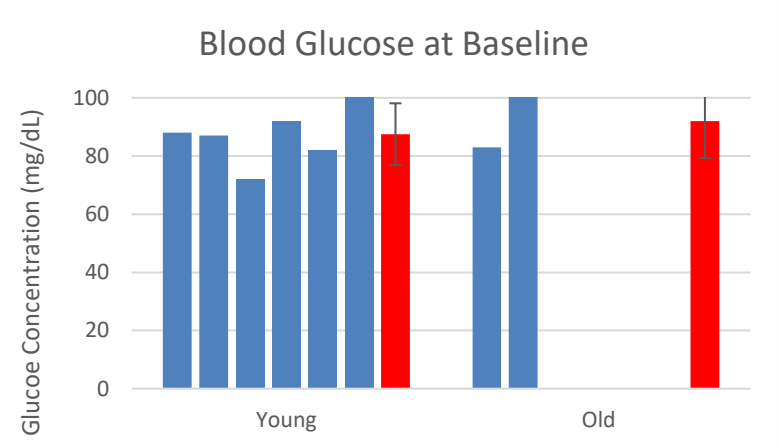

Figure 20: Blood glucose levels for young and old at baseline. Blue bars are individuals and red bars are means of each group

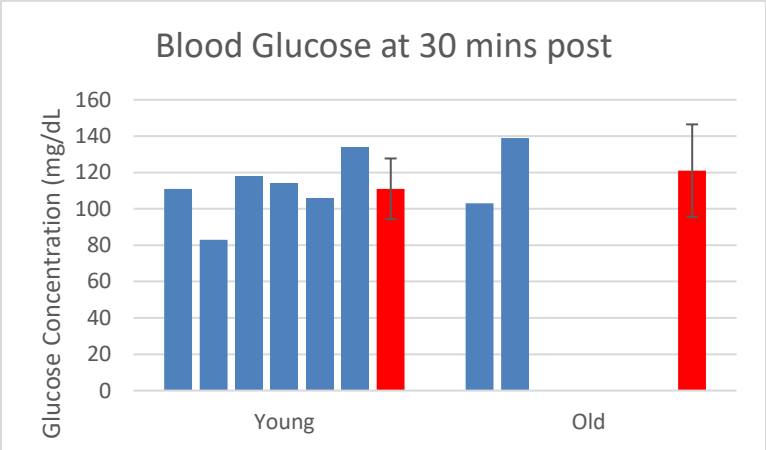

Figure 21: Blood glucose levels for young and old at 30 minutes post. Blue bars are individuals and red bars are means of each group

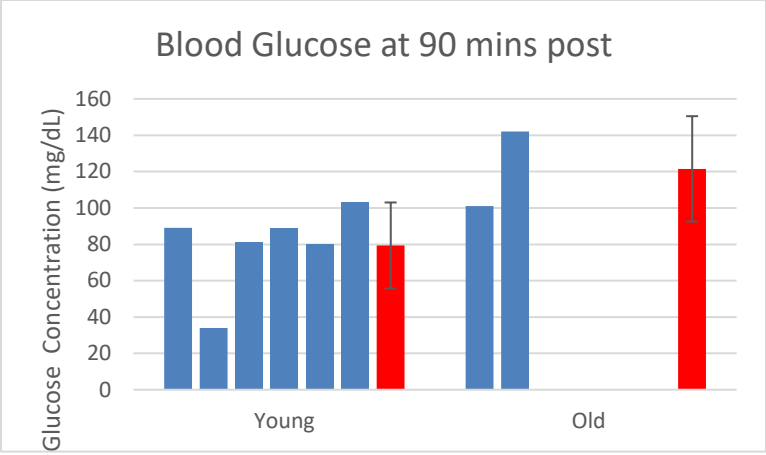

Figure 22: Blood glucose levels for young and old at 90 minutes post. Blue bars are individuals and red bars are means of each group

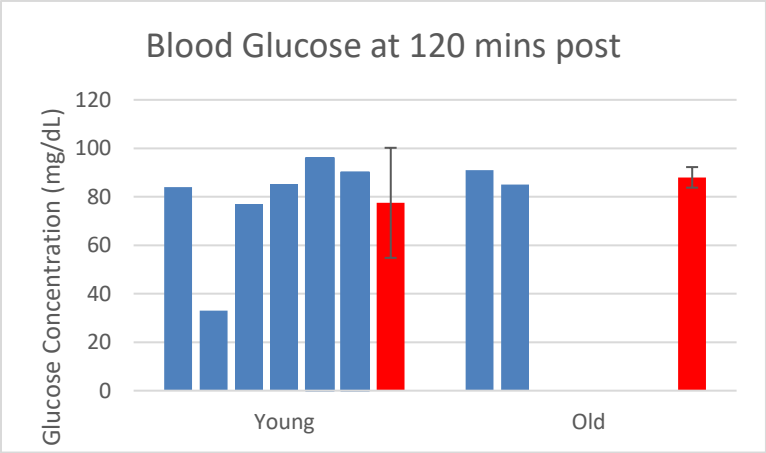

Figure 23: Blood glucose levels for young and old at 120 minutes post. Blue bars are individuals and red bars are means of each group

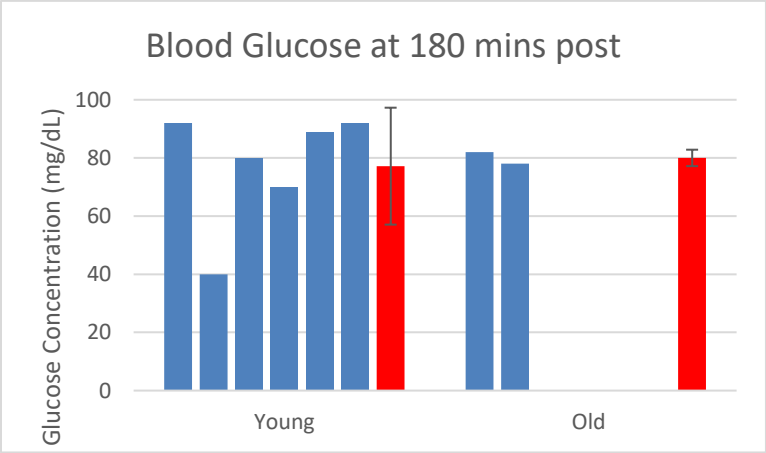

Figure 24: Blood glucose levels for young and old at 180 minutes post. Blue bars are individuals and red bars are means of each group

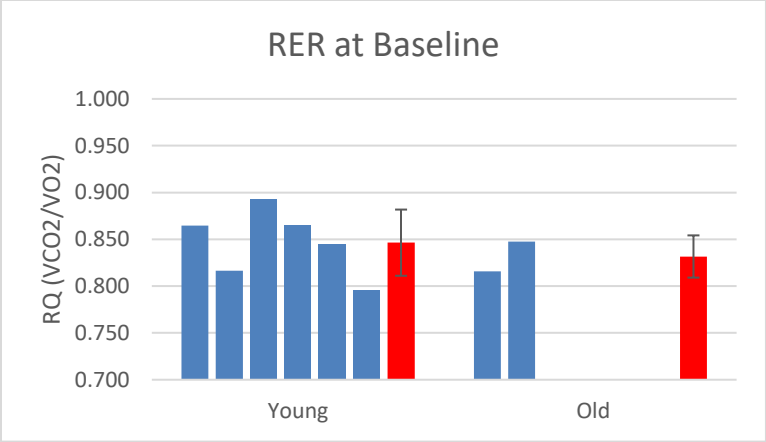

Figure 25: RER values for young and old at baseline. Blue bars are individuals and red bars are means of each group (RER – respiratory exchange ratio)

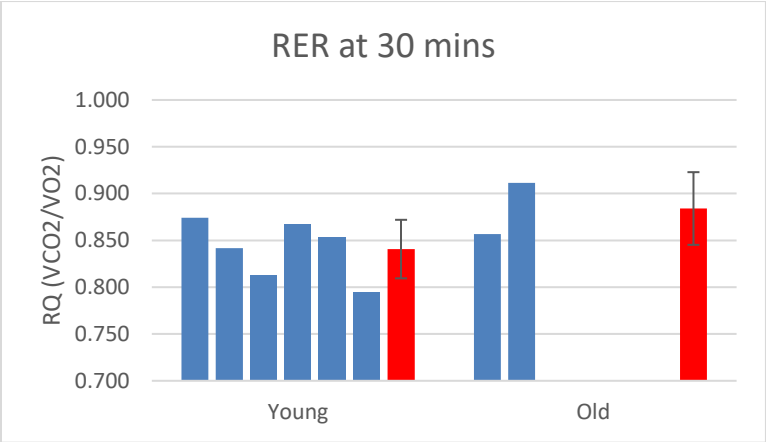

Figure 26: RER values for young and old at baseline. Blue bars are individuals and red bars are means of each group (RER – respiratory exchange ratio)

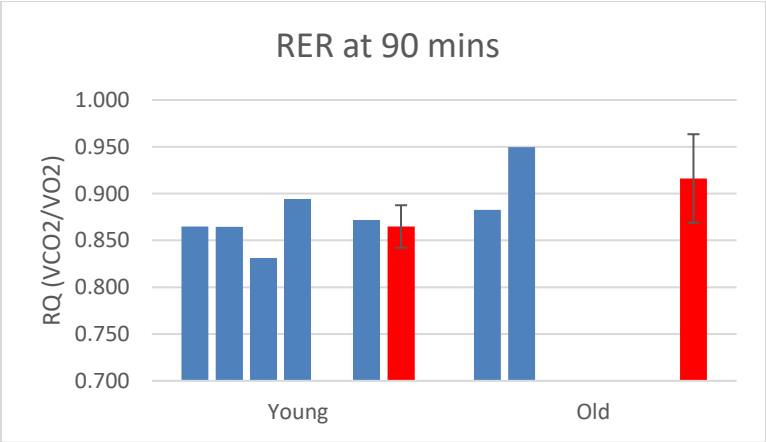

Figure 27: RER values for young and old at 90 minutes post. Blue bars are individuals and red bars are means of each group (RER – respiratory exchange ratio)

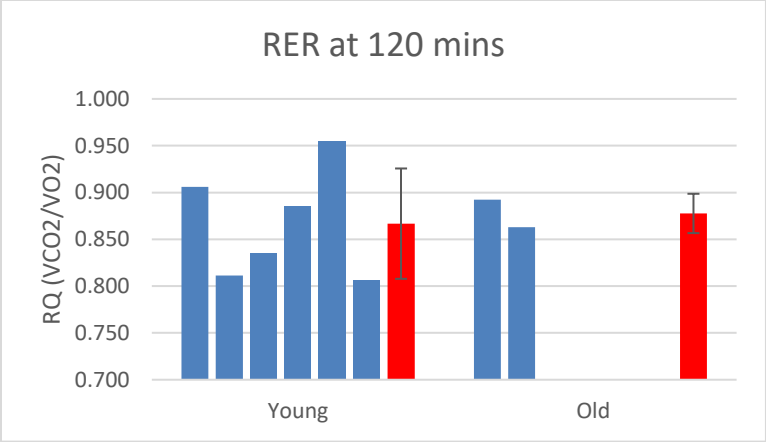

Figure 28: RER values for young and old at 120 minutes post. Blue bars are individuals and red bars are means of each group (RER – respiratory exchange ratio)

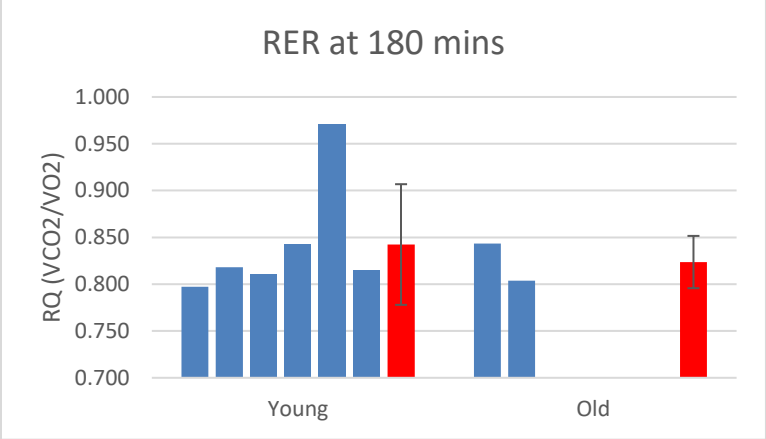

Figure 29: RER values for young and old at 180 minutes post. Blue bars are individuals and red bars are means of each group (RER – respiratory exchange ratio..

Curriculum Vitae

Caleb G. Jones, MS (5/2019)

PROFESSIONAL EXPERIENCE

HEALTHY EXERCISE AND LIFESTYLE PROGRAMS LAB COORDINATOR
August 2018 to Present
Wake Forest University Department of Health and Exercise Science – Winston-Salem, NC
Supervise participants in a medically based chronic disease prevention program. Responsibilities include:

- Monitored patients' medical status during exercise through EKG telemetry, assessment of vital signs, and regular check-ins for ~850 hours
- Responded to patient emergency situations by treating on-site or notifying emergency medical services
- Performed roughly 175 physician-monitored EKG stress tests on participants and developing corresponding exercise prescriptions
- Provided strength training and Silver-Sneakers classes for participants
- Trained first year graduate students in EKG prep, anthropometric measurements, informed consent process, exercise blood pressures and GXT protocols on treadmill, recumbent bike, and airdyne
- Managed lab supply inventory

GRADUATE RESEARCH ASSISTANT
October 2017 to Present
Wake Forest University Department of Health and Exercise Science – Winston-Salem, NC

- Weight Loss and Exercise for Communities with Arthritis in North Carolina (P.I. Dr. Stephen Messier, funded by NIH)
  - Lead interventionist for diet and exercise arm: trained undergraduates and ensure safe practices during exercise
  - Perform educational phone calls for the Nutrition and Health Arm and maintain participant contact records
- Nitrate and Exercise Performance in Middle to Older Aged Adults (P.I. Dr. Michael Berry, funded by Isagenix International, LLC)
  - Monitored patients' health status during 15 baseline maximal exercise tests using EKG telemetry and vital assessments
  - Performed 45 submaximal exercise tests on participants that qualified for participation in the study

- Effect of Protein Supplementation on ACL Repair (P.I Dr. Gary Miller, no external funding)
  - Helped develop data collection sheets, randomization tables, and wrote the informed consent form
- The Effect of Aging on Metabolic Flexibility (P.I. Dr. Gary Miller, no external funding)
  - Wrote informed consent, created data collection forms, wrote methods for study.
  - Performed all screening visits, administered informed consent, performed data collection, and organize data

GRADUATE TEACHING ASSISTANT AND COURSE INSTRUCTOR

August 2017 to Present

Wake Forest University, Department of Health and Exercise Science – Winston-Salem, NC

Instructor for HES 101 Exercise and Health. Responsibilities Include:

- Co-taught 12 sections of 32 students in HES 101 with the goal of improving students' knowledge of exercise so they could better incorporate it into their lives to reduce the risk of chronic disease.
- Was responsible for grading the work of 16 of the 32 students in class
- Led weekly class and lab sections to teach both the scientific and practical basis of exercise.
- Completed six formal graduate and post-doctoral student teaching workshops through the WFU Teaching and Learning Center (TLC)

PERSONAL TRAINER

Wake Forest University Health and Wellness Center – Winston-Salem, NC

July 2018 to April 2019

Fit4Life – McGee's Crossroads, NC

February 2017 to June 2017

- Designed exercise programs for clients on a one on one basis
- Led consults with clients to identify weaknesses and create goals
- Maintained a safe exercise environment and provided feedback on technique
- Educated clients on importance of exercise for physical and mental health

VALENCELL INTERN AND RESEARCH ASSISTANT

May 2016 to May 2017

Valencell Inc. – Raleigh, NC

- Performed validation testing on commercially available heart rate monitors as well as prototypes
- Conducted a 6-week training study on 10 untrained individuals
- Performed pre and post VO2 max testing with lactate measurements and created individualized exercise prescriptions
- Coordinated all data organization and collection
- Led subjects through both aerobic and strength training exercises during study

PRESENTATIONS AND PUBLICATIONS

- Master's Thesis: The Effect of Aging on Metabolic Flexibility. Publisher: Wake Forest University..Forthcoming ……..May 2019
- Guest lecture to "The Leaders of Winston-Salem" on The Importance of Body Composition...……February, 2019
- Assessment of Step Accuracy Using the Consumer Technology Association Standard (Published in the *Journal of Sport Sciences)*…….*June 2018*
- Southeast American College of Sports Medicine, poster presentation ……..……*February, 2018*
- Wiggins Memorial Library Academic Symposium, oral presentation…….....…….*March 30th, 2017*
- Southeast American College of Sports Medicine, Thematic Poster Presentation for Tiffany Spears……...*February 17th, 2017*
- Southeast American College of Sports Medicine Poster Presentation…….…….*February 17th, 2017*
- State of North Carolina Undergraduate Research & Creativity Symposium, poster presentation……....*November 5th, 2016*

EDUCATION

Master of Science: Health and Exercise Science (forthcoming)

August 2017 to May 2019

Wake Forest University – Winston-Salem, NC

- Emphasis on exercise testing and randomized clinical trials

(Primary advisor: Dr. Gary Miller, PhD.)

- Award recipient of full-ride scholarship.
- Course work: Epidemiology, clinical research methods, cardiac pathophysiology, special populations exercise testing and prescription, exercise physiology, health psychology, and biomechanics.

Bachelor of Science in Kinesiology

August 2013 to May 2017

Campbell University – Buies Creek, NC

- Minors in Biology and Spanish

CERTIFICATION AND SKILLS

- ACSM Certified Personal Trainer (Exp: 12/2020)
- ACSM Certified Clinical Exercise Physiologist (**Pending: 05/2019**)
- AHA BLS CPR/First Aid Certified (Exp: 8/2019)
- EKG stress testing
- Patient Informed Consent Process
- IRB/CITI Training Certified
- Blood Borne Pathogens Certified
- Indirect Calorimetry Experience
- Exercise Prescription Experience
- Data Organization Skill\