Investigating Predictors of the Transition from Fat to Carbohydrate Oxidation in Overweight Individuals during Submaximal Exercise

By

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ABSTRACT

Purpose: This study is important for knowing the mechanisms behind fuel transitions from fat to carbohydrate oxidation, so that we can further understand what the main predictors are in this crossover point in a group of people who are currently overweight and are at risk of obesity. Understanding what factors are associated with the transition from fat to carbohydrate oxidation will help us better recognize who could be at risk for metabolic diseases later in life. **Methods:** Up to 60 subjects (29 ± 7 yr) men and women classified as overweight (26.4 ± 1.8 kg/m2) were recruited and participated in this observational study. Resting blood pressure and blood metabolites were measured after an overnight fast. Resting lactate was also measured before and after a three-day eucaloric high-fat (70 %) diet. Visceral adipose tissue mass was determined via dual X-ray absorptiometry. A 65% VO2 max test was then administered to assess the transition point from fat to carbohydrate oxidation. A 10-lead ekg was also attached to the participant for continuous monitoring which is essential throughout the test. The participant was then instructed

to maintain a steady cadence of 50-70 revolutions per minute (RPM) throughout the exercise phase. Once the individual's 65% of their VO2 max was achieved, the test then transitioned to a cool-down phase. This allowed for adequate time for blood pressure and heart rate to return to resting levels. Once the values returned to normal levels, the test was then terminated. **Results:** To investigate the associations between various physiological, metabolic characteristics and the primary variable of transition time, a series of Pearson correlations were performed. The level of significance of each variable was assessed using the corresponding P-value (significance level) and R-value. Among all the variables tested, carbohydrate oxidation (CHOX), fat oxidation (FAOX), age, and body mass index (BMI) emerged as the statistically significant predictors ($p < 0.05$) of transition time, each demonstrating a meaningful level of significance. CHOX displayed the strongest connection with a p-value of 0.01 and an r-value of -0.36. FAOX was also a statistically significant predictor with a p-value of 0.04. Another variable that was significant was age, which yielded a p-value of 0.02 with an r-value of 0.35. The last variable we tested that showed significance as predictor of transition time was BMI ($p= 0.02$, r-value= -0.33). Several variables (fat mass, lactic acid, % body fat, REE, among others) failed to demonstrate statistically significant relationships with the transition time from fat to carbohydrates, and their significance was minimal. **Discussion**: This study identified carbohydrate oxidation, fat oxidation, age, and BMI as significant predictors of the transition from fat to carbohydrate oxidation during submaximal exercise. With substrate oxidation patterns, BMI, and age being significant indicators of transition time, traditional fitness markers such as VO_2 max, resting energy expenditure, and time to exhaustion did not show a significant enough correlation. Future research of this transition should aim to validate these findings in larger and more diverse populations.

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A Thesis

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Chapter 1: Introduction

Introduction

Metabolic flexibility refers to the body's ability to adjust the utilization of fuel, primarily switching between fat and carbohydrate oxidation, based on changes in metabolic demands, such as exercise. This ability to change and transition is essential for maintaining energy homeostasis and is tightly linked to mitochondrial function, insulin sensitivity, and overall metabolic health. The concept of metabolic flexibility, first introduced by Kelley and Mandarino (2000), highlights how insulin-sensitive individuals efficiently shift between fatty acid oxidation in the fasted state and carbohydrate oxidation after feeding. During rest or low-intensity exercise, fat is the preferred substrate due to its high energy yield per molecule, whereas carbohydrates are the primary energy source during high-intensity physical activity due to their rapid conversion to ATP. This flexibility allows for an adapting energy production depending on the physiological state of a person, as seen in endurance pro athletes, whose sustained fat oxidation at moderate exercise intensities, conserving glycogen stores for high intensity efforts. Research by San-Millán and Brooks (2018) emphasizes the differences between populations with varying degrees of metabolic flexibility during an acute bout of exercise. Individuals who are obese are characterized by metabolic inflexibility, display an impaired fat oxidation and a dependence on carbohydrate oxidation even at low exercise intensity. This metabolic rigidity is a pinnacle of insulin resistance, which is prevalent in people with obesity. Metabolic flexibility is inversely related to blood lactate accumulation, a marker of metabolic stress. Further research and studies have indicated that the transfer from fat oxidation to blood lactate in people with obesity occurs at a faster rate compared to moderately active individuals, and at a much significantly faster rate

compared to individuals who are athletes (Goodpaster, 2017). In conditions like metabolic syndrome and type 2 diabetes, metabolic inflexibility is a catalyst in the inability to utilize fat efficiently, leading to heightened reliance on carbohydrates and increased risk of metabolic disease progression. Metabolic flexibility is a key factor of both athletic performance and metabolic health, influenced by substrate availability and insulin sensitivity. While much of the existing literature has focused on individuals with obesity or those with diagnosed metabolic disorders, there remains a gap in understanding how these metabolic processes function in people who are overweight but not yet obese. Overweight individuals (BMI 25.0–29.9 kg/m²) often display early signs of metabolic dysfunction, including elevated fasting insulin, increased inflammatory markers, and reduced mitochondrial efficiency (Bergman et al., 2011).Additionally, Goodpaster et al. (2001) showed that overweight individuals often possess ectopic fat storage and decreased skeletal muscle oxidative capacity, both of which contribute to metabolic inflexibility. Lavie et al. (2009) point out that overweight individuals often have distinct cardiometabolic risks from both obese and normal-weight individuals, but they are rarely analyzed as a standalone group. Studies have even shown that overweight individuals show early signs of metabolic disease. Even without meeting obesity criteria, overweight individuals often display insulin resistance, inflammation, or lipid abnormalities. Wildman et al. (2008) noted that up to 23.5% of overweight adults are metabolically abnormal despite not being obese. Since the transition point is an important marker of metabolic flexibility and has been shown in previous research to play a crucial role in determining metabolic health, knowing specifically what metabolic health factors are associated with the transition from fat to carbohydrate oxidation will help us better recognize who could be at risk for metabolic diseases later in life.

Research Question

What metabolic health factors associate with the time to transition from fat to carbohydrate oxidation during submaximal exercise?

Purpose

Examine metabolic health factors that might be related to transition time in adults who are classified as overweight.

Hypothesis

We hypothesize that factors associated with metabolic health and fitness will predict the transition time from fat to carbohydrate oxidation in overweight individuals.

Significance

This study is important for determining metabolic health parameters that are associated with fuel transitions from fat to carbohydrate oxidation, so that we can further understand what the main predictors are in this crossover point in a group of people who may be at risk for developing obesity.

Chapter 2: Literature Review

I. Introduction

Obesity, metabolic flexibility, insulin sensitivity, and resting blood lactate are interconnected components of metabolic health that are often examined within the broader context of metabolic diseases such as type 2 diabetes and cardiovascular disorders According to the National Institute of Diabetes and Digestive and Kidney Diseases (NIH) the overall prevalence of obesity among U.S adults is about 42.4%. This is an extremely high number and has been trending upwards for the past several decades. From a general health point of view, obesity is also linked to other chronic conditions such as type 2 diabetes, high blood pressure and cardiovascular disease. According to the CDC, 58% of adults with obesity also have high blood pressure. Financially, obesity puts a strain of 1.4 trillion dollars annually on the U.S economy (Obesity Medicine Association). This figure includes costs from obesity treatment obesity related conditions. The Obesity Medicine Association also suggests that the total cost of obesity will continue to trend upwards in the coming years. According to Goodpaster (2001), metabolic inflexibility is linked to disorders such as obesity, type 2 diabetes, and metabolic syndrome. These individuals experience impaired fat oxidation and an over-reliance on carbohydrates, even at lower exercise intensities, leading to increased risk for insulin resistance, hyperglycemia, and metabolic complications. Another aspect that will be discussed is insulin sensitivity. Insulin sensitivity is a measure of how effectively the body responds to insulin. Insulin is a hormone that regulates

blood glucose levels by promoting the uptake of glucose into cells for storage or energy. During submaximal exercise, insulin sensitivity is an important factor in the transition from fat to carbohydrate oxidation. Insulin sensitivity is a crucial component in determining how efficiently the body switches between fuel sources, which impacts exercise endurance, energy production, and overall metabolic health. Another molecule that has been implicated in metabolic health is blood lactate. Blood lactate is a molecule produced by the body during intense exercise when oxygen supply is limited, acting as an indicator of the transition from primarily fat oxidation to carb oxidation as the primary energy source (Emhoff & Messonnier 2023). This transition is associated with an increase in blood lactate levels. High blood lactate levels indicate a greater dependency on carbohydrate metabolism. This transition is important because it helps us understand metabolic flexibility, and the body's ability to switch between fat and carbohydrate utilization efficiently (Broskey & Houmard 2020). A detailed literature review of these elements reveals a complex web of physiological mechanisms and adaptations that impact health outcomes.

II. Obesity

The accumulation of excess fat is what constitutes what obesity is (Fruh et al., 2017). This fat can accumulate anywhere in the body but usually in males and females, this fat accumulates in certain places. For males, this fat is mainly accumulated in the midsection to the neck of the body. This is called “android” fat distribution as the body is apple shaped due to the amount of fat above the waist such as the stomach, chest, shoulders and up to the neck. For females, this fat is mainly accumulated below the waist and into the lower body such as the thighs, legs, and glutes. This is called “gynoid” fat distribution as the body is pear shaped due to the amount of fat

below the waist and in the lower body. Between these two though, the “android” body type is more dangerous as most of this fat is visceral fat, which is fat around our organs, and in this case fat around the liver, kidney, heart, and other important internal organs. This excess visceral fat in people with obesity has been linked to conditions such as impaired glucose and lipid metabolism, and insulin resistance. The same individuals are also at a higher risk of colon, prostate cancer and prolonged hospital visits (Shuster & Patlas, 2012). Due to this excess fat around organs in people with obesity, the risk of ischemic heart disease and hypertension is increased as well. During bouts of acute exercise, people with obesity display several physiological responses compared to fitter counterparts. This is due to differences in metabolic function, cardiovascular health and body composition. Research has shown that heart rate and stroke volume are elevated during exercise in obese individuals, leading to a greater oxygen cost (Wiklund et al., 2016). The Goodpaster and Kelley studies (2001) also investigated how intramuscular triglyceride (IMTG) fat stores are utilized and oxidized in individuals with obesity compared to fit individuals. They found that IMTG serves as an easily accessible energy source that can be effectively utilized and burned during acute exercise in fit individuals. Meanwhile in people with obesity, the ability to use and oxidize these fat stores during acute exercise is significantly impaired (Goodpaster & Kelley, 2001). The inability to properly oxidize these fats during acute exercise in people with obesity shows a dysfunction in how the body metabolizes fat which contributes to the reduced fat burning capacity and capabilities during acute exercise bouts. During acute exercise, the transition from lipid to carbohydrate oxidation occurred at lower relative work rates in people with obesity or who were overweight (Arad & Basile, 2020). Obesity can be caused by several factors including caloric intake, genetics, and lifestyle choices. Having a caloric surplus without those calories being burned through exercise leads the body to storing the excess calories as fat,

leading to weight gain overtime if no action is taken. Genetics can also play a role in the formation of obesity by affecting appetite, satiety, metabolism, food cravings, body-fat distribution, and the use of eating as a coping method to deal with stress (Harvard Medical School, 2019). Lifestyle choices are another significant factor that can determine if someone develops obesity or not. Diet and exercise are the major lifestyle choices someone can make whether they develop obesity or not. Knowing the mechanisms of obesity and why obesity develops can give us better insight into how people may develop metabolic disorders later in life.

III. Metabolic Flexibility

Metabolic flexibility refers to the body's ability to efficiently switch between fuel sources, primarily carbohydrates and fats. In a healthy individual, this transition is a smooth one allowing for the efficient oxidation of fat during prolonged physical activity and the rapid shift to carbohydrate utilization when glucose becomes readily available. However, in metabolic disorders like obesity and type 2 diabetes, metabolic flexibility is negatively affected, leading to metabolic inflexibility. Metabolic inflexibility is a condition where the body has a hard time adapting to changes in energy supply (San-Millán & Brooks, 2018). Knowing the intricacies of metabolic flexibility is critical for grasping the broader implications of metabolic health and disease for the general population. During low-intensity exercise, fat oxidation becomes the dominant energy source. In fit individuals, insulin plays a key role in promoting metabolic flexibility by signaling cells to increase glucose uptake (San-Millán & Brooks, 2018). However, when insulin sensitivity is lowered as often is the

case in obesity, this transition becomes impaired and metabolic flexibility is lost. Instead of seamlessly transitioning between fuel sources, individuals with metabolic inflexibility show a blunted response to both carbohydrate intake and fat oxidation during a resting state or at exercise (Kelley et al., 1999). Obesity is one of the main conditions in which metabolic inflexibility is observed. Research has shown that individuals with obesity have an impaired ability to switch between fat and carbohydrate oxidation, even during a rested state or during insulin stimulation (Kelley et al., 1999). This is mainly in part due to insulin resistance, which impairs the body's ability to respond to carbohydrate intake. The studies also state that the rate of lipid oxidation is increased in obesity during insulin stimulated conditions. Metabolic flexibility is a key indicator of metabolic health, allowing the body to efficiently adapt to changes in energy availability and demands. In healthy individuals, this adaptability supports optimal fuel utilization and energy balance, reducing the risk of metabolic disorders. However, in conditions such as obesity and type 2 diabetes, metabolic flexibility becomes impaired, leading to metabolic inflexibility. This condition is characterized by an inability to efficiently switch between fat and carbohydrate oxidation, which exacerbates insulin resistance and contributes to the progression of metabolic disease.

IV. Insulin Sensitivity

Insulin sensitivity is the body's response to insulin, which is a key hormone to regulating glucose metabolism. When cells in insulin sensitive tissues such as liver, muscle, and fat respond

effectively to insulin, they efficiently take in glucose from the bloodstream and use it for energy or store it for future usage. Insulin sensitivity is important for maintaining normal blood glucose levels and metabolic health. On the other hand, when insulin sensitivity declines, this is known as insulin resistance. Insulin resistance is a precursor to metabolic disorders such as type 2 diabetes, cardiovascular diseases, and obesity (Freeman & Acevado, 2023). Understanding how insulin sensitivity works and the factors that can impact it can be critical to addressing the worldwide rise in metabolic diseases. The primary role that insulin achieves is to facilitate glucose uptake, particularly in skeletal muscle and adipose tissue. It does this by binding to the insulin receptor on cell surfaces, which triggers a signaling involving the phosphorylation of insulin receptor substrate (IRS) proteins. This signaling promotes the translocation of glucose transporter type 4 (GLUT4) to the plasma membrane, allowing glucose to enter the cell (Saltiel & Kahn, 2001). In muscle and liver tissue, glucose is then either oxidized for energy usage or stored as glycogen. While in adipose tissue, glucose can be stored as fat. Insulin also impairs the production of glucose in the liver (gluconeogenesis) and inhibits the breakdown of fats (lipolysis), ensuring that glucose remains the primary fuel source when there is a need for energy (Brooks & Mercier, 1994). In fit and healthy individuals, insulin sensitivity makes sure that blood glucose levels remain in homeostasis. After eating, insulin levels rise to facilitate glucose uptake and storage which in turn lowers blood sugar levels to standard ranges. During a fasting state or during exercise, insulin levels drop and the body transitions to using stored fat as a source of energy, which helps from a glucose homeostasis standpoint. Insulin resistance occurs when cells in certain tissues such as skeletal muscle, liver, and adipose tissue don't efficiently respond to insulin. Due to this, insulin resistance puts a strain on glucose metabolism which then leads to elevated blood sugar levels known as hyperglycemia. Early on, the pancreas can

generate and produce enough insulin to overcome this resistance. However, over time as the insulin resistance worsens, the beta cells in the pancreas become unable to keep up with the increased demand, leading to a lowered glucose tolerance which ends up resulting in type 2 diabetes (DeFronzo et al., 2015). From a cellular perspective, insulin resistance relates to a derangement in the insulin signaling pathway. Insulin resistance in the liver further exacerbates the situation by allowing excessive glucose production which contributes to the ongoing issue of hyperglycemia. The ability of insulin to compromise the process of lipolysis in adipose tissue is also negated which leads to elevated levels of free fatty acids (FFA) in the bloodstream which can worsen insulin sensitivity (Boden, 2011). One of the key and central mechanisms linking obesity and insulin resistance is chronic inflammation. During chronic inflammation, visceral fat becomes inflamed as it expands in obesity. This inflammation is driven by macrophages, which release pro-inflammatory cytokines that disrupt insulin signaling (Shoelson et al., 2006). In addition to inflammation and its connection with visceral fat, systemic inflammation also plays a role in insulin resistance. Higher levels of circulating inflammatory markers such as C-reactive protein (CRP) and IL-6, are common in individuals with insulin sensitivity and obesity (Pradhan et al., 2001). These inflammatory markers not only display the presence of insulin resistance but also may contribute to the continued progression of metabolic dysfunction by promoting chronic inflammation and oxidative stress on the metabolic system.

V. Blood Lactate

In the past several years, resting blood lactate levels have garnered significant interest as a marker of efficiency and metabolic health. Blood lactate has traditionally been regarded as a by-product of the anaerobic metabolism process, which accumulates during physical

activity such as high-intensity exercise when the body's energy needs outpace the availability of oxygen. Despite this, recent research has dramatically changed our understanding of blood lactate and its importance. Today, blood lactate is known as an important metabolic substrate and a potential marker for metabolic health and disease risk. Resting blood lactate levels, which are defined as blood lactate levels that is observed without recent exercise or strenuous physical activity, can offer valuable information into an individual's metabolic status, as well as their insulin sensitivity, and cardiovascular health (Reljic & Frenk, 2023). Historically, lactate was viewed as a waste product of metabolism, but it is now understood as a vital energy source that is actively used by various tissues including the heart, liver, and even the brain. In a resting state lactate production is occurring but on a low scale, as it is continuously reused or oxidized for energy usage (Richter & Nottelmann, 2004). Resting blood lactate concentrations are typically between 0.5 to 2.2 mmol/L in healthy individuals (Rasmussen et al., 2009). The role of resting blood lactate as an indicator of metabolic flexibility has also been explored. Metabolic flexibility refers to the ability of an organism to efficiently switch between fuel sources, primarily between fats and carbohydrates. Elevated resting lactate levels suggest that an individual may be less capable of switching to fat oxidation during fasting or low-energy states, relying instead on carbohydrate metabolism, and producing excess lactate as a byproduct (Goodpaster & Sparks, 2017). Due to this finding, high resting lactate levels can be seen as an indicator of metabolic inflexibility. Research has also shown that there is a significant relationship between resting blood lactate levels and insulin sensitivity. Insulin resistance is a condition in which cells become less responsive to insulin. People who are insulin resistant may predispose that individual to metabolic disorders such as obesity and type 2 diabetes. Elevated lactate levels at rest have

been observed in individuals with insulin resistance, suggesting a link between impaired glucose metabolism and the accumulation of lactate (Juraschek et al., 2015). From an athletic perspective, endurance athletes usually tend to have lower resting lactate levels compared to sedentary or less active individuals. Lower resting lactate levels in this case are indicative of a greater capacity for oxidative metabolism and reduced reliance on anaerobic glycolysis at rest (Weltman et al., 1990). This lower resting lactate also displays enhanced lactate clearance, as trained and fit individuals have an increased capacity to utilize lactate as a fuel source in various tissues such as the heart and muscles.

VI. The Transition

In individuals without overweight and obesity, substrate utilization during exercise is much more flexible compared to individuals who are overweight or have obesity. This flexibility allows for a more effective transition between fat oxidation during acute exercise bouts (Romijn et al., 1993). At rest and during bouts of low-intensity exercise, fat oxidation is the main source of oxidation which provides a steady supply of energy without depleting glycogen stores. As the exercise bout continues and intensity increases, there is a gradual transition to carbohydrate oxidation which eventually becomes the primary energy source after a small timeframe of a back and forth of fat and carbohydrate oxidation fluctuation (Brooks & Mercier, 1994). This shift from fat to carbohydrate oxidation is mediated by changes in hormonal signals (Holloszy & Coyle, 1984). However, in overweight and individuals with obesity, this metabolic flexibility is compromised. Studies have shown that overweight individuals have a diminished capacity for fat oxidation during both rest and exercise, resulting in a greater reliance on carbohydrate metabolism, even at relatively low exercise intensities (Kelley et al., 1999). The impaired ability

for the body to oxidize fat is often referred to as "metabolic inflexibility" (Brooks & Mercier, 1994). Another common characteristic of overweight individuals is insulin resistance which further impairs the changeover from fat to carbohydrate oxidation. In individuals who are healthy and fit, insulin sensitivity leads to efficient glucose uptake during acute exercise leading to a efficient transition between fuel sources. In overweight individuals who are insulin resistant, this process can be impaired and not as efficient resulting in greater fluctuations in blood glucose and insulin levels, which affects substrate switching (Storlien et al., 2004). In overweight individuals, the transition from fat to carbohydrate oxidation during acute exercise is often impaired due to the decreased activity of metabolic enzymes, and insulin resistance. Understanding and knowing the mechanisms behind this transition can give us better insight as to why people with obesity have a propensity to develop metabolic disorders.

Chapter 3: Methods

Participants:

This study had a total of 60 individuals compromised of both men and women with age ranges from 20-48 years old. The individuals were healthy but overweight (BMI 25.0-29.9 kg/m2). The individuals also reported living a sedentary lifestyle.

Protocol:

Screening:

After utilizing various recruitment strategies such as the ECU Announce List, Pirate 411, Brody Graduate School, Pitt Community, School of Allied Health and the School of Nursing at ECU, participants respond indicating interest in participating in the study. Once the subjects made their interest known, prescreening via telephone was conducted to establish if they met the inclusion criteria such as having overweight which is classified as having a BMI of 25.0-29.9 kg/m2, weight stable for the last 3 months, not currently following a specific meal plan and do not have any health conditions such as cancer, uncontrolled hypertension or Gastrointestinal disease, psychiatric disorders, or Type 1 or Type 2 diabetes. Medications such as those that help with anxiety were also part of the exclusion criteria. The subjects who met the inclusions criteria then came into the Human Performance Lab at East Carolina University where they signed an informed consent form and filled out several different questionnaires. Height (m) and weight (kg) were acquired to calculate BMI.

Metabolic Testing

Up to 60 subjects (29 ± 7 yr) men and women classified as overweight (26.4 ± 1.8 kg/m2) were recruited and participated in this observational study. Blood pressure and blood metabolites were measured after an overnight fast. Lactate will be also measured before and after a three-day eucaloric high-fat (70 %) diet. The homeostatic model assessment for insulin resistance (HOMA-IR, fasting insulin (mlU/L)*fasting glucose (mg/dL)/405) was calculated as a measure of insulin resistance. Visceral adipose tissue mass will be determined via dual X-ray absorptiometry.

Maximal Testing

Using the LODE protocol, the participants then performed a VO2max test on a cycle ergometer, and indirect calorimetry was used to obtain results of aerobic capacity. The cycle seat and the mouthpiece were adjusted for the subjects' comfort while a member of the research staff explained the test procedure. The subject was hooked up to a 10-lead EKG which captured the subject's heart rate and EKG data. Prior to the start of the test, resting heart rate and blood pressure were recorded. Once the test started, the baseline of gas exchange was measured, and the workload was programmed into the computer. The subject was instructed to keep their revolutions per minute to approximately 50-70 and was notified about 10 seconds before moving on to the next stage. Near the end of each stage, the Rate of Perceived Exertion on the Borg 6-20 scale and heart rate and blood pressure were taken and recorded. The subjects went through each stage until maximum effort was accomplished or until the subject was cycling at 40 or below

revolutions per minute. Maximum exercise was determined by meeting two of the three criteria: RER of 1.10, RPE greater than 17 on the Borg Scale, and or peak heart rate within +/- 5% of age-predicted max heart rate. Once the test was completed, heart rate and blood pressure were recorded immediately, and the subject entered the active recovery phase to bring heart rate and blood pressure down. The mouthpiece was removed at this time. Heart rate was recorded every minute and blood pressure every two minutes during recovery. The cycle ergometer was set to zero watts during the active recovery phase. Once the heart rate and blood pressure were close to the subjects resting state, the test was terminated. The VO2 max from this test was used to calculate 65% of the participants VO2 max.

Submaximal exercise bout

A 65% VO2 max test was also administered to assess the transition point from fat to carbohydrate oxidation. This test is also similar to the maximal test; however, the test was terminated once the individual reaches 65% of their VO2 max. To conduct a 65% VO2 max submaximal exercise test on a cycle ergometer, age, height, weight, and resting heart rate is first collected from the individual and the cycle ergometer was calibrated for accuracy. The seat's height was adjusted so that the participant's leg is slightly bent when the pedal is at the lowest point, ensuring proper form and comfort during the test. A 10-lead ekg was attached to the participant for continuous monitoring which is essential throughout the test. Using the data from the previous maximal test, the participants were informed of their 65% of their VO2 max from that test. Therefore, the purpose of the VO2 max test was solely to determine this workload

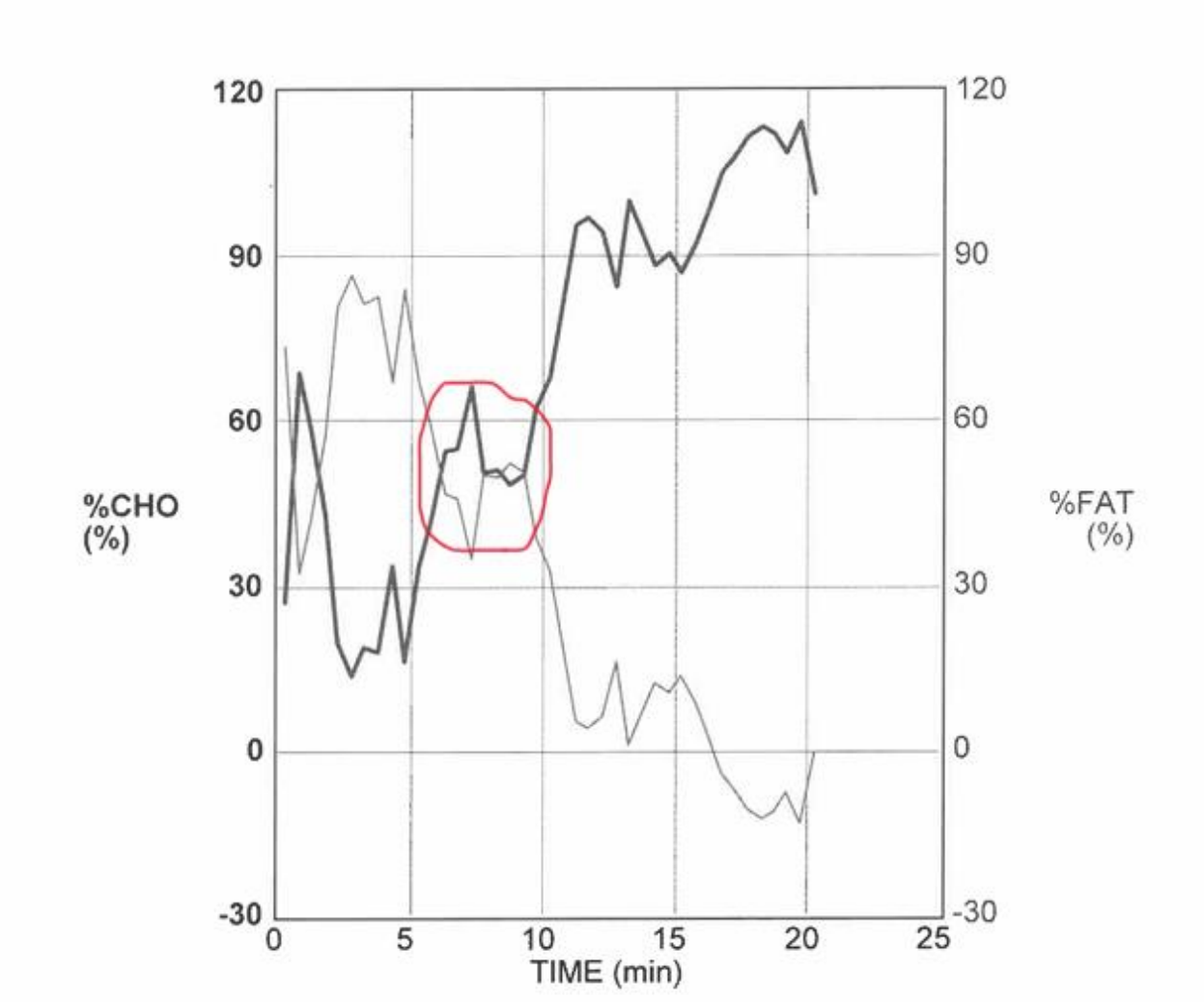
intensity. No additional VO2 max-related variables were used in statistical analyses. The participant was asked to maintain a steady cadence of 50-70 revolutions per minute throughout the exercise phase. The test duration was between six and twelve minutes, allowing the participant to reach a steady-state heart rate. Throughout the test, heart rate and their perceived exertion (RPE) is recorded at the end of each stage using the Borg scale (6-20) to gauge the individuals level of intensity of each stage. Once the individual's 65% of their VO2 max was achieved the test will then transition to a cool-down phase. During this stage, the resistance was decreased on the ergometer and the participant will cycle at a low intensity for several minutes. This allowed for adequate time for blood pressure and heart rate to return to resting levels. Once these values returned to normal levels, the test was then terminated. Heart rate, %FAT, and %CHO were the main variables that were collected from this test. .

TIME	WorkR	RPM	VE STPD	VO2 STPD	VCO2 STPD	RER	FEO2	FECO2	HR	FATmin	CHOmin	%FAT	%CHO
min:sec	W		L/min	L/min	L/min		%	%	bpm	g/min	g/min	%	%
0:32	0	2	9.34	0.35	0.27	0.78	17.37	2.96	76	0.13	0.11	73	27
1:00	0	1	5.79	0.19	0.17	0.90	17.68	3.03	74	0.03	0.15	32	69
1:32	0	0	4.08	0.14	0.12	0.87	17.65	2.98	69	0.03	0.09	42	58
2:01	0	0	5.93	0.20	0.17	0.83	17.66	2.85	70	0.06	0.10	58	43
2:33	0	0	6.00	0.22	0.17	0.76	17.47	2.81	70	0.09	0.05	81	19
3:00	0	0	6.42	0.24	0.18	0.74	17.36	2.85	70	0.10	0.04	87	14
3:32	26	57	12.17	0.49	0.37	0.76	17.09	3.11	95	0.20	0.11	81	19
4:02	30	65	13.66	0.62	0.47	0.76	16.64	3.46	94	0.25	0.12	82	18
4:31	30	67	11.66	0.50	0.40	0.80	16.85	3.44	95	0.17	0.19	67	33
5:03	30	66	13.27	0.67	0.50	0.75	16.15	3.83	100	0.28	0.12	84	16
5:33	30	65	13.98	0.68	0.55	0.80	16.24	3.96	102	0.23	0.26	67	33
6:02	30	66	13.77	0.68	0.56	0.82	16.19	4.09	98	0.20	0.32	59	42
6:32	30	64	15.10	0.70	0.60	0.86	16.42	4.04	99	0.17	0.44	46	54
7:02	30	64	16.58	0.75	0.64	0.86	16.56	3.92	99	0.17	0.48	46	55
7:30	30	66	13.58	0.56	0.50	0.89	16.88	3.75	97	0.10	0.43	35	66
8:02	30	65	14.98	0.68	0.58	0.85	16.54	3.89	97	0.17	0.40	50	51
8:30	30	64	14.81	0.68	0.58	0.85	16.46	3.96	97	0.17	0.40	50	51
9:02	30	63	14.63	0.69	0.58	0.84	16.37	4.01	96	0.18	0.38	52	48
9:32	60	63	16.75	0.78	0.66	0.85	16.45	3.96	96	0.20	0.45	51	50
10:02	67	66	17.59	0.78	0.69	0.88	16.59	3.97	108	0.16	0.57	39	62
10:32	67	66	17.96	0.83	0.74	0.90	16.43	4.18	114	0.14	0.66	33	68
11:01	67	65	21.09	0.98	0.92	0.94	16.33	4.42	120	0.10	0.93	20	81
11:31	67	64	21.02	0.92	0.91	0.98	16.57	4.35	121	0.02	1.05	5	96
12:01	67	64	22.45	0.99	0.97	0.99	16.55	4.38	125	0.02	1.14	4	97
12:31	67	65	22.65	1.02	1.00	0.98	16.46	4.44	128	0.03	1.14	6	94
13:02	67	63	23.30	1.07	1.02	0.95	16.37	4.42	127	0.09	1.07	16	84
13:30	67	65	24.30	1.02	1.02	1.00	16.73	4.24	125	0.00	1.22	1	100
14:00	67	66	22.54	0.97	0.95	0.98	16.64	4.26	127	0.03	1.09	6	95
14:32	67	63	23.82	1.05	1.01	0.96	16.56	4.28	123	0.07	1.10	12	88
15:00	67	64	23.88	1.02	0.99	0.97	16.67	4.19	127	0.06	1.10	10	90
15:30	100	63	26.04	1.14	1.09	0.96	16.61	4.21	132	0.08	1.17	14	87

Substrate Utilization Across an Incremental Exercise Test

Legend:

This table displays respiratory gas exchange and substrate oxidation data collected during a graded cycling protocol. Key metabolic variables include oxygen consumption (VO_2, L/min), carbon dioxide production (VCO_2, L/min), respiratory exchange ratio (RER), and rates of fat and carbohydrate oxidation (FATmin, CHOmin; g/min). The %FAT and %CHO columns represent the relative contribution of each substrate to total energy expenditure at each timepoint. The red box highlights the crossover in substrate usage becomes apparent which is indicated by a decreasing %FAT and increasing %CHO as exercise intensity rises.



Substrate Utilization Crossover During Incremental Exercise

Legend:

During the submaximal test, indirect calorimetry was used to assess real-time substrate oxidation rates. The transition time was defined as the timepoint during the submaximal test when carbohydrate oxidation (CHO g/min) completely and persistently surpassed fat oxidation

(FAT g/min). This point was determined both numerically and visually using a graph that plotted %CHO and %FAT on the Y-axis and time on the X-axis. The crossover was recorded as the minute when FATmin first dropped below CHOmin, indicating a shift in primary substrate use.

LODE Bike VO_2 Max Test Protocol (Leg Cycle Ergometer)

Minutes	Stages	Workload (Watts)
1	1	0
2		0
3	2	30
4		30
5	3	60
6		60
7	4	90
8		90
9	5	120
10		120
11	6	150
12		150
13	7	180
14		180
15	8	210
16		210
17	9	240

18		240
19	10	270
20		270

LODE Cycle Ergometer VO_2 Max Test Protocol

Legend:

This table shows the LODE protocol used during the VO_2 max test performed on a cycle ergometer. Participants began with a 2-minute resting period at 0 Watts, followed by 2-minute stages that increased by 30 Watts each time. The workload started at 30 Watts and continued until the participant reached exhaustion or was unable to keep up with the required pedaling cadence.

LODE Bike Submaximal Test Protocol (Leg Cycle Ergometer)

Stage	Time	rVO2	Watts	*HR	*RPE	Comments
1	Minute 0 – 2	Rest	0			
	**Minute 2 – 3	Rest	0			
2	Minute 3 – 8 **6 min Assess VO_2**	20%				
	**Minute 8 – 9					
3	Minute 9 – 14 **12 min assess VO_2**	35%				
	**Minute 14 – 15					
4	Minute 15 – 20 **18 min assess VO_2**	50%				
	**Minute 20 – 21					
5	Minute 21 – 26 **24 min assess VO_2**	65%				
	**Minute 26 – 27					
Recovery	Minute 0 – 3	Active/Cool				
Recovery	Minute 3-6	Seated/Rest				

Submaximal rVO_2 Cycling Protocol for VO_2 Assessment

Legend:

This test is similar to the maximal test; however, the test was terminated once the individual reaches 65% of their VO2 max. Using the data from the previous maximal test, the participants were informed of their 65% of their VO2 max from that test which was used to determine workload intensity. The participants began with a 3-minute resting period (Stage 1), followed by five exercise stages that gradually increased in intensity. Each stage lasted 6 minutes, with VO_2 targets set at 20%, 35%, 50%, and 65% of the individual's VO_2 max for Stages 2 through 5.

Visit 1 (Screening)	Visit 2 (VO2 Max Test Day)	Visit 3 (65% VO2 Max Test Day)

Consent, height/weight, BMI, questionnaires, resting blood draw (for metabolites, insulin, lactate), DXA scan	VO2 Max test, Resting Energy Expenditure (REE)	65% VO2 submaximal exercise test to determine crossover point

Overview of Study Visits and Procedures

Legend:

This table outlines the sequence of events the study visits completed by each participant. Visit 1 served as the screening session and included informed consent, participant measurements, health and activity questionnaires, a fasting blood draw for metabolic markers, and a DXA scan to assess body composition. Visit 2 involved a maximal exercise test on a cycle ergometer to assess VO_2 max, and resting energy expenditure (REE) measurements. Visit 3 consisted of the submaximal test at 65% of each participant's VO_2 max to determine their metabolic crossover point.

Statistical Analysis

Pearson product-moment correlation were used to measure the strength of the association between the crossover transition time and the metabolic factors. The statistical significance were set a priori at a probability level of $P < 0.05$. Statistical analyses were performed utilizing Prism 4.0 software (GraphPad Software, Inc.). Multiple regression analyses were performed using JMP, Version 17 (SAS Institute Inc.)

Chapter 4: Results

To investigate the associations between various physiological, metabolic characteristics and the primary variable of transition time, a series of Pearson correlations were performed. Each variable that will be discussed was analyzed in relation to the independent value of transition time. The level of significance of each variable was assessed using the corresponding P-value (significance level) and R-value . Among all the variables tested, carbohydrate oxidation (CHOX), fatty acid oxidation (FAOX), age, and body mass index (BMI) emerged as the statistically significant predictors ($p < 0.05$) of transition time, each demonstrating a meaningful level of significance (see table 2). CHOX displayed the strongest connection with a p-value of 0.01 and an r-value of -0.36 (see figure 1). FAOX was also a statistically significant predictor ($p= 0.04$; see figure 2), as a dependent variable. While the variance was slightly lower than CHOX, this variable is still significant to the transition time. Another variable that was significant was age, which yielded a p-value of 0.02 with an r-value of 0.35 (see figure 3). The last variable we tested that showed significance as predictor of transition time was BMI ($p= 0.02$, r-value= -0.33 ; see figure 4). As mentioned in the literature review, elevated BMI is often correlated with increased adiposity, reduced cardiorespiratory fitness, and metabolic inflexibility. Several variables approached statistical significance ($p < 0.05$; see table 2) but were not meaningful and significant enough to make an association. Total body weight in kilograms also approached significance ($p = 0.10$, r-value= -0.24 = ; see table 2). While not statistically significant, this finding suggests that general body mass composed of both lean and fat components, may play a factor in transition time. Several variables failed to demonstrate

statistically significant relationships with the transition time from fat to carbohydrates, and their significance was minimal (see table 2).

Variable	Mean	Std Dev
Fat Mass (kg)	26.15	6.15
Insulin (ulU/mL	9.54	5.01
Lactic Acid (m	8.87	4.31
VAT Mass (g)	403.51	138.09
Lean Mass (kg	54.91	12.29
% Body Fat	31.82	8.26
VO2 Max (mL/	31.48	8.12
%Type 2 Fiber	65.34	13.49
Body Weight (l	81.18	12.77
Time to Exhau	13.02	3.31
REE (Kcal/day	1657.06	340
CHOX (g/day)	169.29	108.25
FAOX (g/day)	100.78	53.05
Age (Years)	24.49	7.96
BMI (kg/m2)	27.55	1.85
Participants		
Males	24	
Females	27	
Total	51	

Table 1. Participant Descriptive Statistics

Legend:

Mean ± standard deviation values for metabolic, and physiological variables collected from study participants (N = 51). Variables include body composition (e.g., fat mass, lean mass, visceral adipose tissue), metabolic markers (insulin, lactic acid), aerobic capacity (VO_2max), fiber-type distribution, and exercise performance indicators (e.g., time to exhaustion, substrate oxidation rates, resting energy expenditure).

Variable	R-Value	P-Value	β-Value
Fat Mass (kg)	-0.1	0.48	-0.1
Insulin (ulU/mL	-0.1	0.5	-0.1
Lactic Acid (m	0.04	0.79	0.04
VAT Mass (g)	-0.16	0.28	-0.16
Lean Mass (kg	-0.21	0.15	-0.21
% Body Fat	0.05	0.74	0.05
VO2 Max (mL/	0.19	0.2	0.19
Body Weight (l	-0.24	0.1	-0.24
Time to Exhau	0.14	0.35	0.14
REE (Kcal/day	-0.04	0.77	-0.04
CHOX (g/day)	-0.36	0.01	-0.36
FAOX (g/day)	0.3	0.04	0.3
Age (Years)	0.35	0.02	0.35
BMI (kg/m2)	-0.33	0.02	-0.33

Table 2. Variable Results Predicting Transition Time

Legend:

Pearson Correlations examining the relationship between individual physiological and metabolic variables and transition time from fat to carbohydrate oxidation. Beta coefficients (β), R- values, and P-values are reported. Significant predictors ($p < 0.05$) include carbohydrate oxidation (CHOX), fat oxidation (FAOX), age, and BMI. We were able to collect information on 24 male participants, and 27 female participants for a total of 51 participants.

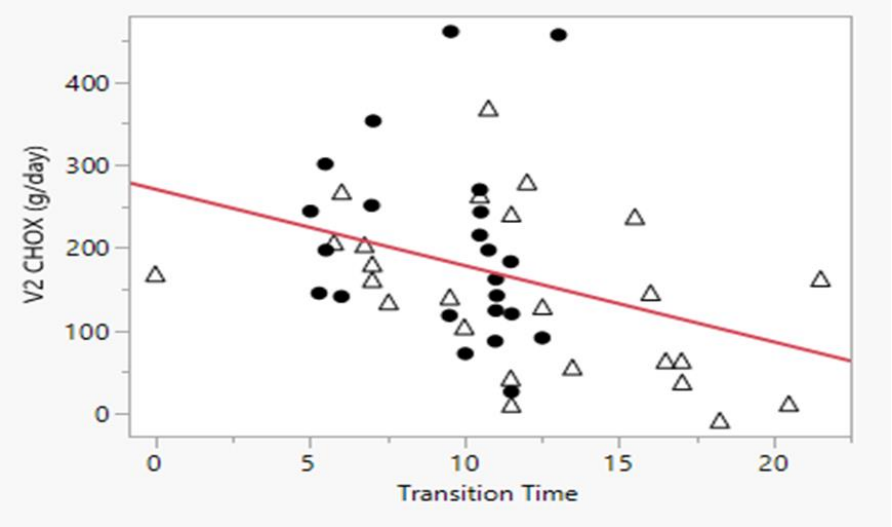


Figure 1. Relationship Between Carbohydrate Oxidation and Transition Time

Legend:

The relationship between carbohydrate oxidation (g/day) during submaximal exercise and transition time (minutes). Each point represents an individual participant, with ● = male and Δ = female. The red regression line indicates a significant negative correlation (p = 0.01), suggesting that higher carbohydrate oxidation is associated with an earlier crossover point.

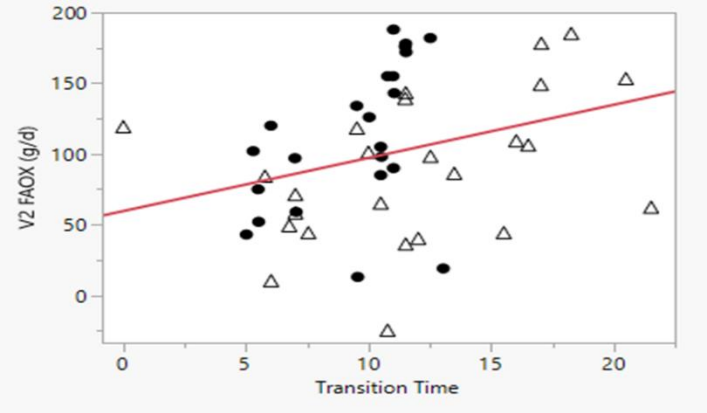


Figure 2. Relationship Between Fat Oxidation and Transition Time

Legend:

The association between fat oxidation rate (g/day) during submaximal exercise and transition time (minutes). Each data point represents an individual participant (● = male, Δ = female). The red regression line indicates a statistically significant positive correlation (p = 0.04), suggesting that higher fat oxidation is associated with a later transition from fat to carbohydrate oxidation.

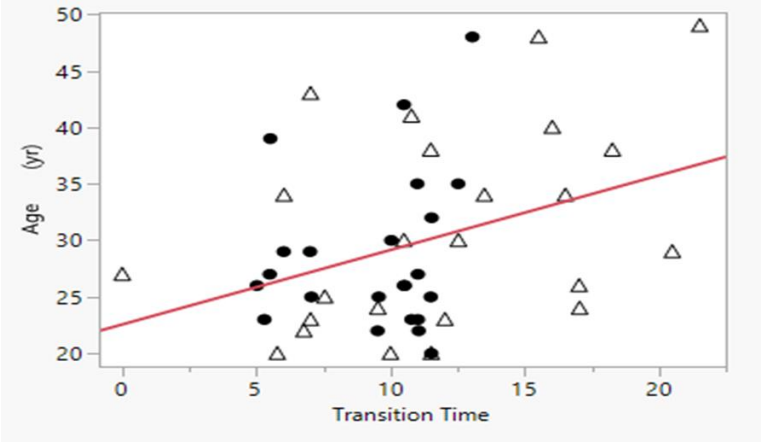


Figure 3. Relationship Between Age and Transition Time

Legend:

The association between participant age (years) and transition time (minutes) from fat to carbohydrate oxidation. Each point represents an individual participant (● = male, ∆ = female). The red regression line indicates a statistically significant positive correlation (p = 0.02), suggesting that older individuals tend to transition later.

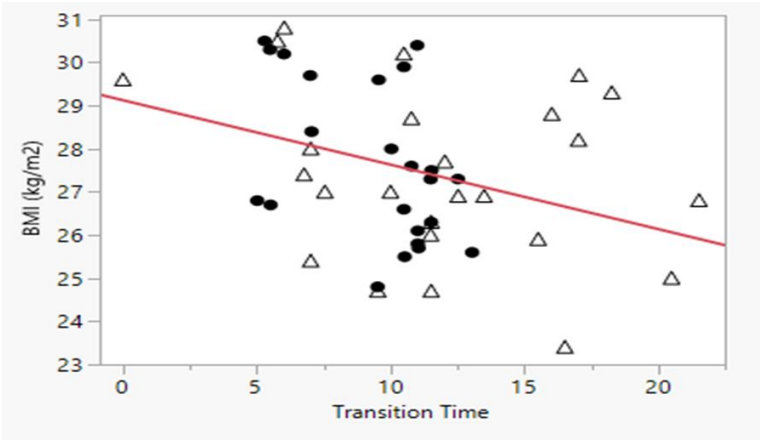


Figure 4. Relationship Between Body Mass Index and Transition Time

Legend:

The association between body mass index (BMI, kg/m²) and transition time (minutes) from fat to carbohydrate oxidation during submaximal exercise. Each point represents one participant (● = male, ∆ = female). The red regression line shows a statistically significant inverse relationship (p = 0.02), indicating that individuals with higher BMI tend to transition earlier

Chapter 5: Discussion

The goal of this study was to explore what factors predict the transition from fat to carbohydrate oxidation during submaximal exercise in individuals who are classified as overweight but otherwise healthy. This transition point is an important marker of metabolic flexibility and has been shown in previous research to play a crucial role in determining metabolic health, exercise efficiency, and long-term disease risk. Our hypothesis was that factors associated with metabolic health and fitness will predict the transition time from fat to carbohydrate oxidation in overweight individuals, and some of the data we collected and analyzed provided support for that. We found that carbohydrate oxidation (CHOX), fatty acid oxidation (FAOX), body mass index (BMI), and age were significantly correlated with transition time, while other metrics like VO_2 max, insulin, and even fat mass did not reach significance. These findings suggest that how someone utilizes their energy may be more informative for predicting metabolic flexibility than traditional aerobic performance or body composition measures. CHOX had the strongest correlation with transition time ($p = 0.01$, r-value= -0.36, $\beta = -0.36$; see figure 1), which makes sense given the nature of the crossover point is taking into account these changes in substrate use during exercise. Individuals who transitioned to carbohydrate oxidation earlier during submaximal exercise also had higher rates of CHOX overall, which supports the idea that they were relying more on carbohydrate metabolism even at lower intensities. This pattern mirrors what San-Millán and Brooks (2018) found when comparing trained athletes to less-fit individuals

which they found that those with lower metabolic flexibility shifted to carbohydrate metabolism sooner and more often, while athletes and other fit individuals showed better fat utilization and a more gradual transition. Similarly, FAOX was also a significant predictor ($p = 0.04$, r-value= 0.3, $\beta = 0.30$; see figure 2) for this transition. As mentioned in the literature review, elevated FAOX rates may indicate greater metabolic flexibility and reliance on lipid metabolism under a submaximal exercise setting. The combined significance of both CHOX and FAOX highlights the relevance of utilization efficiency in shaping the outcome measure of transition time .FAOX tends to dominate during rest and low-intensity exercise. Goodpaster and Sparks (2017) stated, those who are metabolically flexible can maintain fat oxidation longer before switching to carbs. In our data, individuals with higher FAOX values during rest tended to have later transition points suggesting that greater dependence on fat metabolism may reflect better metabolic flexibility, even in a population that isn't highly trained. The results for CHOX and FAOX display a key theme about metabolic flexibility which is how efficiently the body can switch between them when energy demands change such as at rest or in this case, during submaximal exercise. As a result, this balance seems to matter more than absolute performance metrics when predicting and understanding transition time. While fitness and metabolic markers are often emphasized and investigated, it's worth noting that two of the most basic characteristics we measured, BMI and age, were also significant predictors of transition time. BMI showed a significant relationship ($p = 0.02$, r-value= -0.33, $\beta = -.33$; see figure 4), suggesting that higher BMI may be linked with earlier crossover time. This could reflect reduced fat oxidation that has been documented in individuals with excess adiposity (Goodpaster et al., 2001; Shuster & Patlas, 2012). The accumulation of visceral adipose tissue has been specifically implicated in promoting systemic inflammation and disrupting normal lipid and glucose metabolism, ultimately impairing

metabolic flexibility (Shoelson et al., 2006). The significance of BMI in this analysis implies a potential link between total body composition and the utilization of fats and carbohydrate energy sources, aligning with previous research showing that individuals with greater adiposity demonstrate impaired intramuscular fat oxidation and reduced metabolic flexibility during exercise (Goodpaster & Kelley, 2001; Wiklund, 2016). Age also showed a significant connection ($p = 0.02$, r-value= 0.35, $\beta = 0.35$; see figure 3). This result suggests that age-related physiological adaptations or declines may play a major role in the transition between fat to carbohydrate oxidation transition time. As an individual ages, changes in insulin sensitivity, body composition, and aerobic capacity are documented and are expected (DeFronzo et al., 2015). With the oldest participant in the study being 49, the results suggest that these age-related declines appear to affect fuel transitions even in adults under 50. Which suggests that metabolic flexibility may begin diminishing earlier than previously expected. Several well-known indicators of fitness and health were not significant predictors in this research. VO_2 max ($P = 0.20$, r-value= 0.19, $\beta = 0.19$) and time to exhaustion ($P= 0.35$, r-value= 0.14, $\beta = 0.14$; see table 2) were not significant. According to the crossover concept proposed by Brooks and Mercier (1994), substrate utilization during exercise reflects an intricate balance between lipid and carbohydrate oxidation that shifts with exercise intensity. Earlier crossover to carbohydrate usage as observed in some participants, may indicate reduced flexibility in substrate selection at submaximal workloads. Other nonsignificant variables included fat mass, lean mass, insulin, and lactic acid. The lack of significance in these variables suggests that neither absolute tissue mass nor basal metabolic indicators strongly influenced transition time in this analysis. While elevated lactate levels have been tied to metabolic inflexibility (Broskey & Houmard, 2020), resting lactate alone may not be sensitive enough to predict dynamic energy transitions during

exercise. What makes these results and data insightful is that they suggest metabolic dysfunction may begin earlier than we typically expect. All our participants were overweight but not obesity, and all were free of chronic disease. Despite this, we still found significant differences in how they transitioned between fat to carbohydrate oxidation during submaximal exercise. The study had several strengths that were shown throughout, from literature review to data collection. The first of these strengths was that submaximal substrate transition times were examined within a population that was relatively healthy but overweight. This substrate changes and transitions to be examined within this specific population. The use of submaximal exercise mirrors how people typically move in day-to-day real life. Which makes the findings more applicable outside of just a lab setting. Another strength was the fact that several variables including body composition, aerobic fitness, and metabolic markers were all examined and incorporated into the factors that might influence transition time. Despite the study's strengths, there were several limitations as well. This study is an observational analysis, meaning the data was collected at a single point in time allowing us to see correlations between variables. While reasonably large enough for this research and to draw conclusions, the sample size of 51 participants is small and there could have been more variance with a larger participant pool. Although indirect calorimetry is the gold standard tool for assessing substrate oxidation, no direct measures of mitochondrial function, insulin resistance, or muscle enzymatic activity were collected during the study. Collecting these data points would have provided additional insights into the factors that determine transition time. Despite the numerous limitations, this study offers substantial evidence that substrate oxidation rates, BMI, and age are meaningful predictors of transition time and metabolic flexibility in a group of individuals potentially at risk for developing obesity. Future research of this transition should aim to validate these findings in larger and more diverse populations. This

includes individuals with obesity, different ethnic backgrounds, and varying levels of physical activity. This would be of great benefit for continuing research in the future.

Conclusion

Worldwide, metabolic diseases such as insulin resistance, type 2 diabetes, and obesity, continue to be a major issue to society and recent trends and research show no sign of that stopping. These metabolic disorders are often attributed to inefficient metabolic flexibility during exercise, which is the body's ability to switch between fat and carbohydrate oxidation at rest or during exercise. This study identified carbohydrate oxidation, fat oxidation, age, and BMI as significant predictors of the transition from fat to carbohydrate oxidation during submaximal exercise. Moreover, the findings suggest that both substrate utilization patterns and basic physiological characteristics can have significant influence on an individual's metabolic flexibility. It was hypothesized that the combination of metabolic indicators and traditional fitness markers would predict the transition time from fat to carbohydrate oxidation during submaximal exercise. After collecting and analyzing our data, we can conclude that the results partially supported this hypothesis. While resting substrate oxidation patterns, BMI, and age

were significant indicators of transition time, traditional fitness markers such as VO_2 max and time to exhaustion did not show a significant enough correlation (see table 1). These findings suggest that metabolic flexibility is influenced more by physiological factors such as body composition and whole-body resting substrate oxidative capacity rather than peak aerobic and exercise performance. These outcomes highlight the need to consider a broader range of factors and variables to consider when assessing transition time and overall metabolic health, which leads into future research of this topic. In conclusion, this study discovers and highlights valuable evidence that basic and simple non-invasive indicators such as BMI, age, and substrate oxidation rates are primarily the main predictors for substrate transition during submaximal exercise in overweight individuals.

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APPENDIX A: IRB Approval Memo

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board
Willis Building · Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office **252-744-2914** · Fax **252-744-2284** ·
rede.ecu.edu/umcirb/

Notification of Continuing Review Approval

From: Biomedical IRB
To: Joseph Houmard
CC: Elizabeth Gates
Date: 1/22/2025
Re: CR00010678
UMCIRB 19-000914
Metabolic inflexibility is related to elevated muscle anaerobic glycolysis

I am pleased to inform you that at the convened meeting on 1/22/2025 12:15 PM of the Biomedical IRB, this research study underwent a continuing review and the committee voted to approve the study. Approval of the study and the consent form(s) is for the period of 1/22/2025 to 1/21/2026.

The Biomedical IRB deemed this study Greater than Minimal Risk .

As the Principal Investigator you are explicitly responsible for the conduct of all aspects of this study and must adhere to all reporting requirements for the study. Your responsibilities include but are not limited to:

1. Ensuring changes to the approved research (including the UMCIRB approved consent document) are only initiated with UMCIRB review and approval except when necessary to eliminate an apparent immediate hazard to the participant. All changes (e.g. a change in procedure, number of participants, personnel, study locations, new recruitment materials, study instruments, etc.) must be prospectively reviewed and approved by the UMCIRB before they are implemented;

2. Ensuring that only valid versions of the UMCIRB approved, date-stamped informed consent document(s) are used for obtaining informed consent (consent documents with the IRB approval date stamp are found under the Documents tab in the ePIRATE study workspace);

3. Promptly reporting to the UMCIRB all unanticipated problems involving risks to participants and others;

4. Applying for continuing review and receive approval of continuation of the study prior to the study's current expiration date. Application for continuing review should be submitted no less than 30 days prior to the expiration date. Lapses in approval (i.e. study expiration) should be avoided to protect the safety and welfare of enrolled participants and liability to the University; and

5. Submission of a final report when the study meets the UMCIRB criteria for closure. Study approval should not be allowed to expire simply because the study is completed, rather the UMCIRB should be formally notified of study completion via the final report process.

The approval includes the following items:

Document	Description
Adipose Tiissue Sampling Protocol(0.01)	Study Protocol or Grant Application
APPHoumard-Dohm_NIH_R01_12 (1).pdf(0.01)	Study Protocol or Grant Application
Flyer and email(0.08)	Recruitment Documents/Scripts

Document	Description
Flyer Canvas distribution(0.02)	Recruitment Documents/Scripts
Group 1 and 2 Information Sheet V.2 06.05.24.docx(0.03)	Recruitment Documents/Scripts
Group 3 Information Sheet V.3 1.20.22(0.04)	Recruitment Documents/Scripts
Health history 08.13.20.docx(0.02)	Surveys and Questionnaires
Met Flex ppt Script.docx(0.01)	Recruitment Documents/Scripts
MetFlex Bariatric Surgery ICF v.9 06.05.24-Clean.doc(0.19)	Consent Forms
MetFlex Main ICF v.10 06.05.24-Clean.doc(0.16)	Consent Forms
MetFlex Participant website.docx(0.01)	Recruitment Documents/Scripts
MetFlex Protocol v.4.0 02.17.22.docx(0.06)	Study Protocol or Grant Application
MetFlex Protocol v.6.0 06.05.24.docx(0.02)	Study Protocol or Grant Application
MetFlex Screening ICF v.8.0 05.23.23-Clean.docx(0.14)	Consent Forms
PARQPlus2019ImageVersion2.pdf(0.01)	Surveys and Questionnaires
Participant questionnaire 01.13.20.docx(0.01)	Surveys and Questionnaires
Phone screen final 08.13.20.docx(0.02)	Recruitment Documents/Scripts

For research studies where a waiver or alteration of HIPAA Authorization has been approved, the IRB states that each of the waiver criteria in 45 CFR 164.512(i)(1)(i)(A) and (2)(i) through (v) have been met. Additionally, the elements of PHI to be collected as described in items 1 and 2 of the Application for Waiver of Authorization have been determined to be the minimal necessary for the specified research.

The following UMCIRB members were recused for reasons of potential for Conflict of Interest on this research study: M. Pories

The following UMCIRB members with a potential Conflict of Interest did not attend this IRB meeting: N. Broskey

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IRB00000705 East Carolina U IRB #1 (Biomedical) IORG0000418
IRB00003781 East Carolina U IRB #2 (Behavioral/SS) IORG0000418