LACTATE AS A PREDICTOR FOR FACTORS OF METABOLIC SYNDROME IN MALES AND FEMALES WITH OVERWEIGHT

By

Rebecca Sulpar

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Director of Thesis: Nick Broskey, PhD

Major Department: Kinesiology

ABSTRACT

Background: The prevalence of obesity has dramatically increased by more than 10% over the last twenty years. Risks of obesity include the development of metabolic syndrome (MetSyn) with concomitant development of conditions such as heart disease, stroke, type 2 diabetes, and some cancers. MetSyn is classified as having three of the following: a large waist circumference, hypertension, high blood sugar, high triglycerides, and dyslipidemia. Poor skeletal muscle metabolism is linked to obesity due to a low rate of fatty acid oxidation. Blood lactate is an indicator of oxidative capacity at rest and there is an inverse relationship between them. Elevated fasting plasma lactate levels in the body are present in obesity. Thus, lactate may be a precursor for obesity and metabolic diseases such as MetSyn in both males and females. **Purpose:** The purpose of this study was to determine if there is a relationship between blood lactate levels and its indices in men and women with overweight that has the potential to be used as a precursor for the development of MetSyn. **Methods:** Overweight subjects (n=29) with a body mass index (BMI) of 25.0-29.9 kg/m^2 were screened for plasma lactate concentration. The subjects then

returned for two additional visits. The first visit consisted of a DEXA scan, a 3-D body scan, and a maximal exercise test on a cycle ergometer. The participants returned for a Resting Metabolic Rate measurement. **Results:** Average fasting plasma lactate was 1.05 ± 0.11 mmol/L. Plasma lactate was positively associated with factors of MetSyn such as total cholesterol ($r=0.611$, $p=0.0004$), triglyceride levels ($r=0.49$, $p=0.007$), LDL levels ($r=0.582$, $p=0.0009$), HOMA-IR values ($r=0.626$, $p=0.0003$), insulin levels ($r=0.595$, $p=0.0007$), android/gynoid ratio ($r=0.396$, $p=0.034$), and visceral adipose tissue (VAT) mass ($r=0.672$, $p<0.0001$). Other factors of MetSyn such as BMI, HDL, blood pressure (systolic and diastolic), and waist-to-hip ratio were not statistically significant. **Conclusion:** The results from the current study show that plasma lactate levels have positive relationships with factors of MetSyn. Analyzing plasma lactate values may help clinicians and future researchers predict the development of metabolic diseases such as metabolic syndrome.

Lactate as a Predictor for Factors of Metabolic Syndrome in Males and Females with Overweight

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Rebecca Sulpar

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Director of Thesis: Nick Broskey, PhD

Thesis Committee Members:

Joseph Houmard, PhD

Donghai Zheng, PhD

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Chapter 1: Introduction

Introduction

The prevalence of obesity in the United States has increased drastically from 30.5% to 41.9% over the last twenty years (CDC, 2022). The problem of obesity comes with its risks. These risks include an increased chance of developing obesity-related conditions such as heart disease, stroke, type 2 diabetes, and some cancers (CDC, 2022). Individuals with obesity are estimated to spend $1,861 more per year on medical costs than those without obesity. On a national scale, the United States spent $173 billion dollars in 2019 on obesity-related factors (CDC, 2022). As stated above, having obesity can put one at a higher probability to develop different health problems. Some of these health conditions together can lead to a diagnosis of metabolic syndrome (MetSyn). MetSyn is a group of conditions that together raise one's risk of coronary artery disease, diabetes, or stroke (NIH, 2022). Typically, MetSyn is classified as having three of the following: a large waist circumference, hypertension, high blood sugar, high triglycerides, and dyslipidemia (NIH, 2022). MetSyn is also associated with insulin resistance within skeletal muscle (Stump et al., 2006). The term metabolic inflexibility comes from the inability to efficiently transition between using fat and glucose oxidation as the body's sources of fuel during rest or exercise (Stump et al., 2006). MetSyn is linked to metabolic inflexibility and insulin resistance within skeletal muscle (Stump et al., 2006). It is important to note that skeletal muscle needs energy to create movement and muscle contraction.

There are different ways in which the body produces energy, also known as adenosine triphosphate (ATP). One is the Cori Cycle. The Cori Cycle is the pathway where lactate is produced by glycolysis in the muscles. Lactate is moved to the liver where it is converted into pyruvate and then by using ATP, is then converted to glucose by a process called

gluconeogenesis. From there the glucose is shuttled back to the muscles where it is metabolized back into lactate and the cycle continues (National Library of Medicine, 2021).

The TCA Cycle (tricarboxylic acid cycle) is a multi-step process that provides energy for cells in the form of ATP and is important for cellular respiration (Baldwin & Krebs, 1981). This cycle is an essential part of glucose and fatty acid oxidation that produces NADH and FADH2 for ATP production. After glucose is oxidized to pyruvate, the pyruvate is then oxidized to acetyl-CoA where two molecules enter the TCA cycle. Acetyl-CoA is combined with oxaloacetate to form citrate (National Library of Medicine, 2022). Citrate releases two carbons that produce NADH. The remaining four-carbon molecule makes one ATP and then reduces FAD to FADH2 which feeds into the Electron Transport Chain to produce the ATP via oxidative phosphorylation. No more than 6 ATP can be created due to NADH2 being needed in a different reaction (Baldwin & Krebs, 1981). It is possible for problems to arise within the TCA cycle and consequently may be a marker for obesity or impairment within skeletal muscle metabolism (Broskey et al., 2021).

Lactate drives both processes of the Cori Cycle and the TCA cycle as it is a substrate for both. Due to the observation that those with obesity have an impaired ability to oxidize different substrates, there may be a relationship between lactate production and the function of these cycles. Due to what we know, blood lactate may be a precursor for obesity and metabolic diseases such as MetSyn in both males and females.

Research question

Is there a relationship between plasma lactate levels and factors of metabolic syndrome in men and women with overweight?

Hypothesis

H_a: Having elevated plasma lactate levels is associated with factors of metabolic syndrome such as adiposity and insulin resistance in men and women with overweight.

Purpose

The purpose of this study was to determine if there is a relationship between blood lactate levels as a precursor for the development of metabolic syndrome and its different factors in men and women with overweight.

Chapter 2: Literature Review

Introduction

From 1980 to 2004, the prevalence of obesity in the United States dramatically increased and continues to rise (Ogden et al., 2007). It is estimated that more than 68% of adults in the United States are considered overweight and 35% are considered obese (Wright & Aronne, 2012). Unfortunately, child and adolescent obesity are also on the rise which may increase the prevalence of obesity-related conditions such as MetSyn (Wells et al., 2008). Obesity can increase one's risk of morbidity and mortality (Ogden et al., 2007). Current literature that is available discusses the physical health problems that can occur with obesity and inactivity in adults. This can include MetSyn which is defined as a collection of different interconnected elements and disorders that can increase one's risk for coronary heart disease, cardiovascular disease, and type 2 diabetes (Kassi et al., 2011). The main characteristics of MetSyn are dyslipidemia, hypertension, central obesity, and insulin resistance; other conditions include chronic inflammation, non-alcoholic fatty liver disease, and sleep apnea (Kassi et al., 2011). Metabolic syndrome is associated with insulin resistance within the skeletal muscle as well as an impaired ability to transition between the usage of fat and carbohydrates for energy (Stump et al., 2006). Blood lactate may be a precursor for obesity and metabolic diseases such as MetSyn. The following review of the literature discusses the current research on obesity, MetSyn, skeletal muscle metabolism, and blood lactate.

Obesity

Obesity has been a growing epidemic among adults and children in the United States since 1980 and is generally defined as excess body fat (Ogden et al., 2007). Obesity can be estimated utilizing a formula that combines both height in meters and weight in kilograms. The formula most frequently used is body-mass index [BMI] (Kopelman, 2000). Within the adult population,

a BMI of 30kg/m^2 or greater is considered having obesity while greater than 25kg/m^2 and less than 30kg/m^2 is considered having overweight (Ogden et al., 2007). Obesity is caused by a myriad of factors such as the built environment including our food products, physical activity levels, use of medications, our social networks, and several other individual and communal factors (Wright & Aronne, 2012).

It is known that men and women have different body compositions overall. Research shows that men typically have higher lean mass and women typically have higher fat mass, meaning women are more likely to have obesity (Chooi et al., 2019) & (Geer & Shen, 2010). The distribution of body fat and muscle mass is a bit different between the two genders as well. Men tend to have a central distribution of body fat or visceral obesity, while women have a more peripheral distribution of body fat, also called gynoid obesity; therefore, men tend to be more "apple-shaped", and women appear to be more "pear-shaped" (Geer & Shen, 2010).

Abdominal, or visceral, obesity is a marker of an increased risk for type 2 diabetes and insulin resistance as well as a risk factor for cardiovascular disease (Cornier et al., 2008). There is evidence of high inflammation in those with abdominal obesity due to the infiltration of macrophages in adipose tissue (Després & Lemieux, 2006). It is also noted that those with a positive energy balance from a lack of physical activity and a surplus of high-caloric food in addition to smoking, an adverse genotype, and poor stress responses, may be at higher risk to develop visceral adiposity (Després & Lemieux, 2006). Visceral adiposity is known as dysfunctional adipose tissue due to its altered metabolism of free fatty acids. The extra lipids lead to ectopic fat on the muscle, heart, liver, and other organs that present themselves as clinical criteria for metabolic syndrome (Després & Lemieux, 2006).

Obesity can cause an increased risk for a myriad of diseases such as type 2 diabetes, cardiovascular disease, hypertension, lipid disorders, and different types of cancer. Obesity, specifically visceral obesity, is the driving factor for the increased production of free fatty acids which may interfere with insulin's role in the body (Bosello & Zamboni, 2000). The combination of conditions that are partially caused by obesity is termed metabolic syndrome. MetSyn is comprised of obesity specifically visceral adiposity, dyslipidemia, high triglycerides, hypertension, and insulin resistance (Ogden et al., 2007). Therefore, it is imperative to reduce visceral adiposity to avoid preventable health conditions.

Metabolic Syndrome

Metabolic syndrome is a cluster of factors that have a direct impact on increasing risk for coronary heart disease, cardiovascular disease, and type 2 diabetes (Kassi et al., 2011). MetSyn's main components are elevated triglycerides and low high-density lipoproteins or dyslipidemia, hypertension, and poor glucose homeostasis. Abdominal obesity and insulin resistance are becoming more frequent with MetSyn as well (Kassi et al., 2011). If MetSyn is left untreated, it can lead to changes in multiple important organs due to accumulation of lipids, oxidative stress, and inflammation (Richter-Stretton et al., 2020).

According to NHANES which utilized data from 2003-2012, the prevalence of metabolic syndrome in the United States was 33%, with women having a higher prevalence than men (Aguilar et al., 2015). The study analyzed the self-reported data and determined if an individual had MetSyn if they had three or more of the following: waist circumference greater than 88 cm or 102 cm in women and men respectively, triglyceride level of 150 mg/dL or greater, HDL cholesterol level of <40 mg/dL or <50mg/dL in men and women respectively, systolic/diastolic blood pressure of 130/85 mmHg or greater or taking hypertension medications, fasting glucose level of 100 mg/dL or greater or taking medications for diabetes mellitus (Aguilar et al., 2015). It

is known that prevalence is impacted by sex, age, gender, ethnicity, as well as lifestyle habits and socioeconomic status (Cornier et al., 2008); where Hispanics have a higher prevalence than non-Hispanic and African American individuals (Aguilar et al., 2015); and MetSyn is more prevalent in those of older ages (Cornier et al., 2008). There are certain lifestyle factors that increase the risk for a MetSyn diagnosis which include: lower educational status, smoking tobacco, moderate to heavy carbohydrate intake, physical inactivity, and lower household income (Cornier et al., 2008).

It is imperative to note that physical activity is an important component to prevent cardiovascular disease, and studies show there is an inverse relationship between participating in 150 minutes of moderate-intensity physical activity per week and prevalence of MetSyn (Strasser, 2013). There is also a dose-response relationship between levels of physical activity and risk of developing MetSyn, and higher levels of muscular strength result in a decreased risk of MetSyn (Strasser, 2013). This is because physical activity has a positive impact on all factors related to MetSyn: abdominal obesity, blood lipids, glucose levels, and blood pressure (Strasser, 2013).

Most people with obesity are also insulin resistant (Cornier et al., 2008). Typically, insulin would inhibit adipose tissue lipolysis, but this process is blocked by insulin resistance resulting in more free fatty acids being released into the plasma (Cornier et al., 2008). Data from the NHANES 1999 to 2002, indicated that an increased BMI had a positive relationship with an increased prevalence of hypertension and diabetes, both of which were higher among those who have morbid obesity (Nguyen et al., 2008). The same data concluded that the prevalence of dyslipidemia increases until the BMI exceeded 30 kg/m^2 and then decreases with a BMI greater than 40 kg/m^2 (Nguyen et al., 2008). Consequently, MetSyn is most prevalent in those who have

overweight, or obesity compared to those who are normal weight (Nguyen et al., 2008). Therefore, one of the cardinal ways to prevent MetSyn is to not have overweight or obesity since a healthy weight and enough physical activity are imperative for maintaining normal skeletal muscle metabolism (Stump et al., 2006).

Skeletal Muscle Metabolism

Metabolic syndrome is associated with insulin resistance within the skeletal muscle as well as an impaired ability to transition between the usage of fat and carbohydrates for energy (Stump et al., 2006). Skeletal muscle is a fundamental unit of metabolic health. In skeletal muscle, insulin is responsible for promoting glucose usage and storage by increasing the transport of glucose and glycogen synthesis. If a person has higher insulin levels where it is required to lower glucose then they may be considered insulin resistant (Petersen & Shulman, 2018). Skeletal muscle happens to be the most abundant insulin-sensitive tissue and is responsible for about a quarter of the body's resting oxygen consumption and most insulin-facilitated glucose removal; therefore, changes in skeletal muscle mass, metabolic rate, and hormone responses drastically change overall energy stores and metabolism (Stump et al., 2006). Research suggests that abnormalities within skeletal muscle may come before developing metabolic syndrome and contributes to the etiology of the condition (Richter-Stretton et al., 2020). Skeletal muscle, which is high in mitochondria, converts energy stored to adenosine triphosphate (ATP) via oxidative phosphorylation. The oxidation of glucose or lipids is determined by the functionality and number of mitochondria in the skeletal muscle and low numbers are related to obesity and the development of metabolic syndrome (Richter-Stretton et al., 2020). A reduction in mitochondria number and function has the potential to impact insulin sensitivity and can lead to other conditions such as sarcopenia (Richter-Stretton et al., 2020). Research shows that obesity is

related to an impaired ability to use fat as fuel during post-absorptive conditions and during exercise (Blaak & Saris, 2002).

Metabolic flexibility is an organism's ability to oxidize both carbohydrate and lipid fuels and its capability to transition between them (Storlien et al., 2004). Both glucose and lipids are utilized when moving from rest to exercise in order to support higher energy demands. During times of sleep, skeletal muscle uses high rates of fatty acid oxidation compared to more glucose oxidation after eating. (Goodpaster & Sparks, 2017).

There are several pathways the body can use to utilize different energy substrates during different types of exercise. Duration and intensity of exercise are the two determinants of the energy pathway that will be used by the body (Hargreaves & Spriet, 2018), but other factors such as training status, diet, and sex can also have an impact on substrate use (Alghannam et al., 2021). In short, high-intensity short-duration exercise comes from creatine phosphate degradation and the breakdown of muscle glycogen to lactate that yields the most energy. This is anaerobic (Hargreaves, 2000). During prolonged exercise, glucose, and lipid oxidation supply most of the ATP required where muscle glycogen, blood glucose, and free fatty acids are the fuels for energy (Hargreaves, 2000). The body uses glucose that comes from muscle glycogen and blood glucose that is originated from the liver and the gut via consumed carbohydrates. Fatty acids come from adipose tissue and intramuscular triglyceride breakdown (Hargreaves & Spriet, 2018). It is known that at higher intensities, carbohydrates provide most ATP via oxidation, and at lower intensities fat is oxidized to create ATP (Hargreaves & Spriet, 2020). Skeletal muscle is extremely abundant in the body and has many important functions, two of which are to dispose of glucose and to improve insulin sensitivity in muscle (Marette et al., 2014).

There are several systems that the body utilizes during exercise to generate ATP for energy. During very short and very intense spurts of exercise such as during the 100-400m dash, ATP is made from the breakdown of phosphocreatine (PCr) as well as glycogen to lactate (Hargreaves & Spriet, 2020). Once the exercise bout surpasses approximately 1 minute, ATP is produced by oxidative phosphorylation. In several-minute to hours-long bouts of exercise both fat and carbohydrates are oxidized to provide ATP to the muscles (Hargreaves & Spriet, 2020).

In those who have obesity, fatty acid oxidation in skeletal muscle is impaired where glucose uptake and oxidation are increased (Blaak & Saris, 2002). Research shows that the decreased oxidation of lipids may be a risk factor for weight gain and obesity. Endurance exercise training increases fat oxidation during submaximal exercise, which is the appropriate response (Blaak & Saris, 2002). Free fatty acids are used by the muscle tissue at intensities lower than 65% VO2max as well as during prolonged exercise durations (Ranallo & Rhodes, 1998). There is an inverse relationship between blood lactate levels and fatty acids. Higher blood lactate levels are associated with lower levels of fatty acids during exercise at high workloads. However, lactate is present at a low level during long-duration and low-intensity exercise (Ranallo & Rhodes, 1998). Having poor skeletal muscle metabolism is linked to obesity due to a lower rate of oxidizing fatty acids and glucose in muscle, suggesting that carbohydrates supply a majority of the energy in those who have obesity (Broskey et al., 2020).

Blood Lactate

Blood lactate is known to continuously form under aerobic conditions and is considered a large energy source. (Brooks, 2018). Research shows that elevated plasma lactate levels can be an early biomarker for the prediction of metabolic disease (Broskey et al., 2020) At rest, blood lactate may be an indicator of oxidative capacity. When oxidative capacity is reduced, blood lactate rises (Matsushita et al., 2012). Normal plasma lactate values fall below 2.0 mmol/L (De-

Cleva et al., 2021). According to a study utilizing white and African American men, elevated plasma lactate levels were associated with a higher BMI, larger waist circumference, high triglycerides, insulin resistance, low HDL, and increased prevalence of type 2 diabetes (Crawford et al., 2010). Specifically, this study team saw a very strong, linear relationship between lactate levels and prevalence of type 2 diabetes (Crawford et al., 2010). Increasing blood lactate levels is a biomarker for a discrepancy between the production of lactate and the removal of the metabolite (Brooks, 2018). Research shows that blood lactate is increased in those who have obesity but is decreased after 6 months of endurance training (Broskey et al., 2020). This demonstration of reducing blood lactate concentration through exercise training was also observed in running rats (Brooks, 2018). A research study by Broskey et al. (2020) identified an inverse relationship between the oxidation of fatty acids and increased lactate levels in healthy, lean women. This research team also saw an inverse relationship in the ratio of HDL to LDL cholesterol and plasma lactate, a positive relationship in waist-to-hip ratio and plasma lactate, an inverse relationship in time to exhaustion via VO2max test and lactate, and no relationship between plasma lactate and HOMA-IR values in healthy women. Via the Cori Cycle, lactate is transported between the muscles and the liver to create glucose, and if this process is stressed during rest, fasting blood glucose levels will rise which may lead to a higher risk for glucose intolerance, possible insulin resistance, and other metabolic diseases. Therefore, high lactate levels may be owed to problems in skeletal muscle oxidative capacity (Broskey et al., 2020).

Research has shown that in those with obesity, there is a larger release of lactate seen in the skeletal muscle which implies a problem in the TCA cycle that could potentially be a factor causing metabolic inflexibility (Broskey et al., 2020). Jones et al. (2019) predict that increased lactate levels are likely due to increased production in the muscle while the oxidation of fatty

acids and glucose is decreased in those with obesity. Further, because those with obesity tend to have a problem with the oxidation of pyruvate in the Cori Cycle of the muscle, too much lactate is then transported to the liver which drives gluconeogenesis which can then lead to metabolic diseases. (Broskey et al., 2020). Jones et al. (2019) stated that due to a lesion in the Cori Cycle, glucose production is elevated, and the Cori Cycle is constantly running, even at rest in obese subjects. This can lead to an increase in lactate and metabolic syndrome; this is termed the Vicious Cori Cycle. Therefore, high blood lactate concentration levels in the body are related to obesity and consequently also the development of metabolic diseases such as metabolic syndrome

Chapter 3: Methods

Participants

This study recruited and enrolled a total of 29 individuals with overweight (BMI 25.0-29.9 kg/m^2). The study included both males and females aged 18 to 50 years.

Materials

To assess plasma lactate concentrations, a sterile lancet was used to prick the finger in order to draw the blood droplet sample. A lactate test strip was used to collect the blood droplet and acquire a lactate reading, which was analyzed by a lactate meter (Lactate Plus, Waltham, MA) device. Resting heart rate was recorded using a Polar heart rate monitor (Kempele, Finland), baseline fingertip pulse oximeter (Fabrication Enterprises, Elmsford, NY), and blood pressure was assessed manually using a mobile sphygmomanometer (Welch Allyn, Skaneateles Falls, NY) and a stethoscope (Littmann, USA). For the measurement of REE and the respiratory exchange ratio (VCO_2/VO_2,), Parvo Medic's True 2400 metabolic cart (East Sandy, UT) was used for indirect calorimetry. The metabolic measurement software within the Parvo metabolic cart analyzed the O_2 and CO_2 flow/volume ratio. Dual-energy X-ray absorptiometry (DEXA; Horizon, Danbury, CT) scanner and 3-D Body Scanner (Size Stream, Cary, NC) were used to analyze the subject's body composition. An Excalibur Sports cycle ergometer (Lode, Netherlands) was used for the modality of exercise for VO_2 max test and submaximal exercise. Blood analysis was determined by using a hematology analyzer (Beckman Coulter, USA).

Protocol

<u>Screening:</u>

After utilizing various recruitment strategies such as the ECU Announce List, Pirate 411, Brody Graduate School, Pitt Community College, School of Allied Health, and the School of Nursing at ECU, participants responded 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 Body Mass Index 25.0-29.9 kg/m^2, 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 GI disease, psychiatric disorders, or diabetes. Medications such as those that help with anxiety are also part of the exclusion criteria due to a possible alteration in metabolism. The subjects who met the inclusion criteria then came into the Human Performance Lab at East Carolina University where they signed a consent form, had blood drawn for analysis, and filled out several questionnaires such as an informed consent form, an IRB-approved personal family form, physical activity questionnaire, DEXA radiation consent form, and a menstrual cycle questionnaire when relevant. Height (m) and weight (kg) were acquired to calculate BMI. Heart rate was measured using a finger pulse oximeter and blood pressure was taken manually using a stethoscope and a sphygmomanometer. Blood lactate was the next thing to be measured using the Lactate meter for blood lactate assessment. The third finger on the subjects' non-dominant hand was used for the lactate sample. The finger was wiped with a 70% isopropanol wipe. A one-time use sterile lancet needle was used to puncture the fingertip and blood was gathered with a lactate strip and introduced into the Lactate Plus where it can be measured. Levels of blood lactate were measured twice to make sure that the values were within 0.3 mmol/L and if there was a discrepancy between the two readings greater than 0.3 mmol/L an extra measurement was taken. To measure the other blood metabolites, blood was drawn during the screening visit from the antecubital vein and blood cells were separated by centrifugation. If the subjects still meet the requirements, they were invited back for two additional visits. The first visit consisted of having the subject's body composition measured via DEXA and 3-D scanning. The subject also did a

maximal exercise test on the cycle ergometer. When the subjects returned, we obtained a Resting Metabolic Rate in a quiet area.

Visit 1

Subject: 7 days prior to the visit, the subject will be given a food diary to keep and is instructed to come into the lab in a fasted state.

Visit: For the first visit, there are several measurements that were taken which include body composition and VO2max test. First, resting measurements were taken which include resting heart rate recorded with a pulse oximeter and resting blood pressure that was measured manually with a stethoscope and a sphygmomanometer. To assess body composition, a DEXA (Dual-Energy X-Ray Absorptiometry) scan and a 3-D body scan was performed.

Prior to the scan, the subject removed shoes and anything in their pockets, any jewelry and let the staff know of any metal in their body. The subject was then instructed to lay on the bed with their head on the right side and feet on the left. The research staff then ensured the correct alignment of the body and limbs and used a Velcro strap to secure the subjects' toes together. Once the subject was in the correct position, they were instructed to lay still for the duration of the scan (approximately two and a half minutes). Once the scan was complete, the subject may relax, and the results were gathered. The 3-D body scan included the subjects removing their clothes in an enclosed space, following the instructions given to stand properly in the 3-D scanner for approximately one minute then they were able to get dressed and move on to the maximal exercise test.

Next, the subject 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 used to collect the data 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, a 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 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.

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

Visit 2

When the subject arrived, they were directed into the REE room where they will be in a reclined position. They were asked to come in fasted, which meant they had restrained from

exercise for 72 hours, no food, stimulants (including caffeine, nicotine, and medication that are stimulants), and alcohol for at least 10-12 hours. The testing room was kept at approximately 70 degrees F (21 degrees C) with dimmed lighting for a relaxed setting. The subject was instructed to stay awake and as still as possible for the duration of the test. After a resting period of approximately 10-15 minutes, an open circuit canopy hood that is equipped for REE measurement using indirect calorimetry was placed on the subject for a 20-minute measurement. The research staff adjusted CO2 levels to stabilize them to about 1.0% for the duration of the test. The patient may be left alone for several minutes at a time to ensure an adequate resting state. After 20 minutes of good data, the test concluded, the hood was removed from the subject, and test results were acquired.

Data Processing

BMI was calculated by dividing the subject's weight by height squared (kg/m^2). Subjects' exercise energy expenditure and both resting and exercise RER from indirect calorimetry were collected from the metabolic cart. The DEXA scanner provides body composition which consisted of Fat mass (FM) and Fat-Free Mass (FFM). Plasma and serum were sent to Labcorp for analysis of glucose, lactate, insulin, and lipids. HOMA-IR was calculated as (glucose*insulin)/405. The normal value was <2 and hyperinsulinemia was classified as ≥12 µIU/ml.

Statistical Analysis

To determine the relationship between lactate and factors of metabolic syndrome, Pearson product-moment correlation was used to measure the strength of the association between the variables of plasma lactate and metabolic factors. The statistical significance was set *a priori* at a probability level of $p < 0.05$. Statistical analyses was performed utilizing JMP®, Version 14 (SAS Institute Inc.).

Chapter 4: Results

Participants (n=29) were both men and women (n=16, n=13), respectively, ages averaged 29 ± 7 years. Most subjects were of overweight BMI (27.3 ± 1.9 kg/m^2). The average fasting plasma lactate was 1.05 ± 0.6 mmol/L. Fasting glucose levels were elevated at 91.2 ± 1.4 mg/dL and insulin was 9.4 ± 0.9 μIU/mL. The subjects had mild insulin resistance as classified by HOMA-IR (2.1 ± 0.2). Total cholesterol and triglyceride levels were in the normal range at 184.9 ± 8.3 mg/dL and 103.1 ± 16.1 mg/dL, respectively. HDL levels were below the desirable range (50.2 ± 2.1 mg/dL) and LDL was slightly above the normal range (115 ± 7.4 mg/dL).

Using a bivariate fit model between factors of metabolic syndrome and lactate, the results are as follows. Total cholesterol ($r= 0.611$, $p= 0.0004$), triglyceride levels ($r=0.489$, $p=0.007$), and LDL levels ($r=0.582$, $p=0.0009$) were directly and positively associated with lactate (Figure 1, Panels a, b, and c). Insulin levels ($r=0.595$, $p= 0.0007$) and HOMA-IR values ($r=0.626$, $p=0.0003$) were positively correlated with lactate values. (Figure 2, Panels a and b). Lactate was positively correlated with Android/Gynoid Ratio ($r=0.396$, $p= 0.034$) and VAT Mass ($r=0.672$, $p<0.0001$) (Figure 3 Panels a and b). Many of the variables provided no statistically significant correlations with lactate (Table 1). Lactate was regressed in bivariate associations with factors of blood metabolites including total cholesterol, LDL, triglycerides, insulin, and HOMA-IR. These relationships were significant ($p<0.05$). However, when adjusted for statistically detected outliers, total cholesterol ($p=0.972$), LDL ($p=0.972$), and android/gynoid ratio ($p=0.449$) became statistically nonsignificant (see Figure 4). In addition, diastolic blood pressure ($p=0.012$), systolic blood pressure ($p=0.017$), and HDL ($p=0.014$) became statistically significant when adjusting for statistically detected outliers (see Figure 5).

Table 1: Subject Characteristics (n=29)

	Mean	Range
Sex (M/F)	16/13	
Age (years)	29 +/- 7	48-20
BMI (kg/m²)	27.3 +/- 1.9	30.5-23.4
Systolic Blood Pressure (mmHg)	118 +/- 13	137-90
Diastolic Blood Pressure (mmHg)	73 +/- 9	87-52
Visceral Adipose Tissue (g)	421.1 +/- 136.7	739-237
Percent Body Fat (%)	30.6 +/- 8.0	41.7-19.5
Waist Circumference (cm)	38.5 +/- 3.2	44.7-33.2
Hip Circumference (cm)	43.5 +/- 2.2	47-39.6
Waist-to-Hip Ratio	0.88 +/- 0.05	0.97-0.75
Android/Gynoid Ratio	0.97 +/- 0.15	1.37-0.71
Resting Metabolic Rate (kcal/day)	1752 +/- 332	2425-974
VO2Max (mL/kg/min)	32.4 +/- 8.3	49.8-17.8

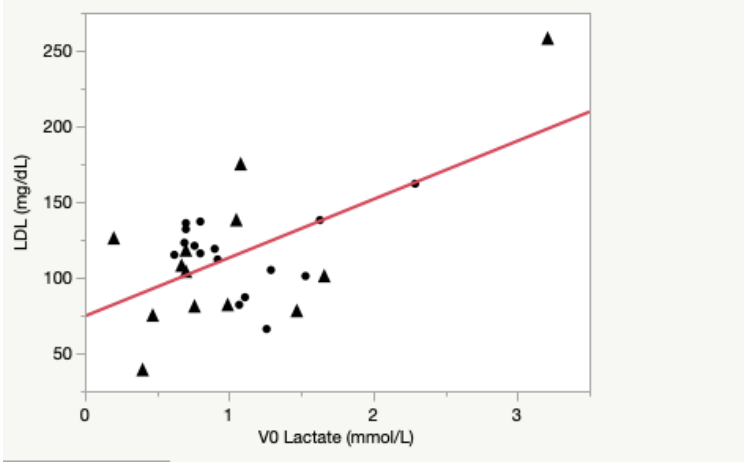
Mean +/- SD

Table 2: Blood Metabolite Averages Across Participants

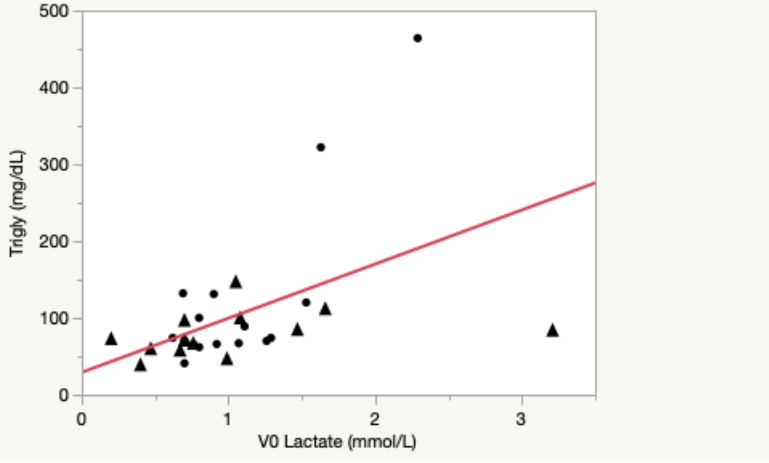
	Mean	Range
Lactate (mmol/L)	1.0 +/- 0.11	3.2-0.2
Glucose (mg/dL)	91.2 +/- 1.4	108-76
Insulin (uIU/mL)	9.4 +/- 0.9	22.8-2.9
HOMA-IR	2.1 +/- 0.2	6.1-0.6
Total Cholesterol (mg/dL)	184.9 +/- 8.3	330-129
LDL (mg/dL)	115 +/- 7.4	258-39
HDL (mg/dL)	50.2 +/- 2.1	87-30
Triglycerides (mg/dL)	103.1 +/- 16.1	464-39

Mean +/- SEM

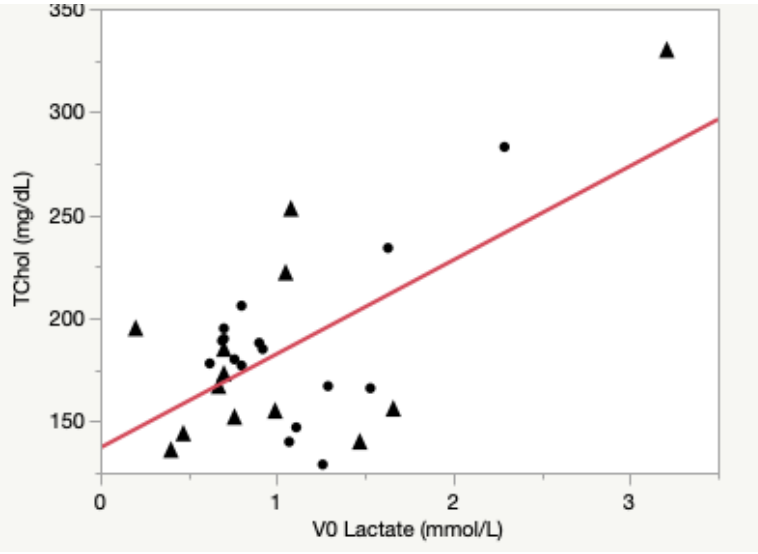
FIGURE 1: Correlations between cholesterol-related factors associated with metabolic syndrome and fasting plasma lactate.



p=0.001, r=0.582

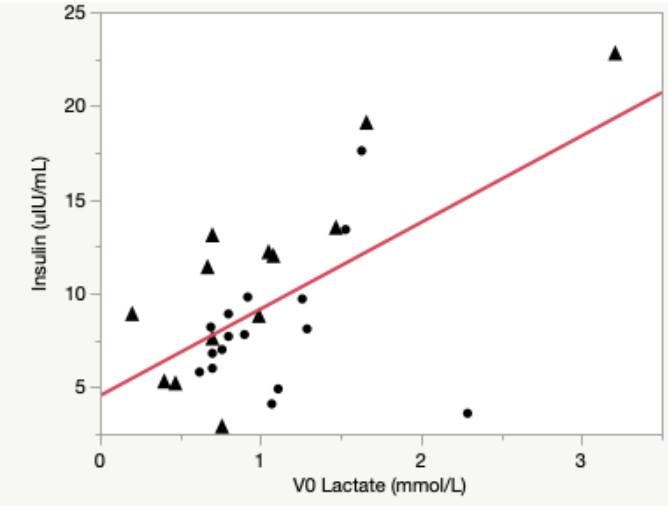


p=0.01, r=0.489

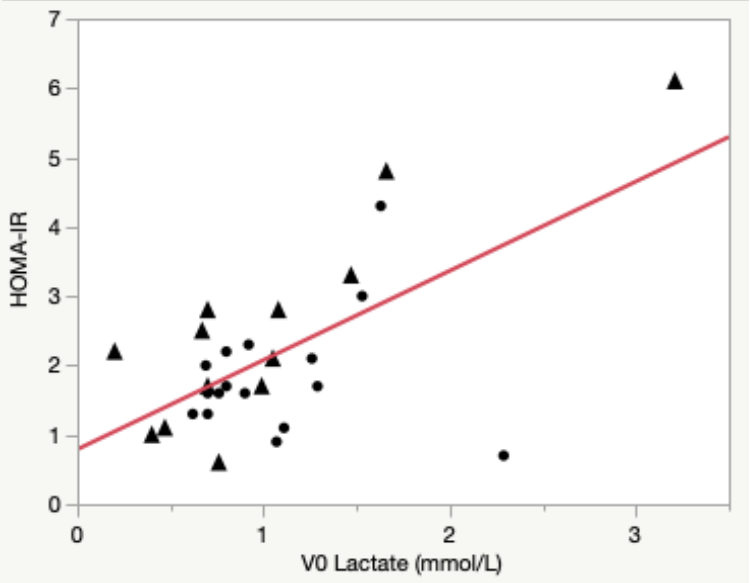


p=0.0004, r=0.611

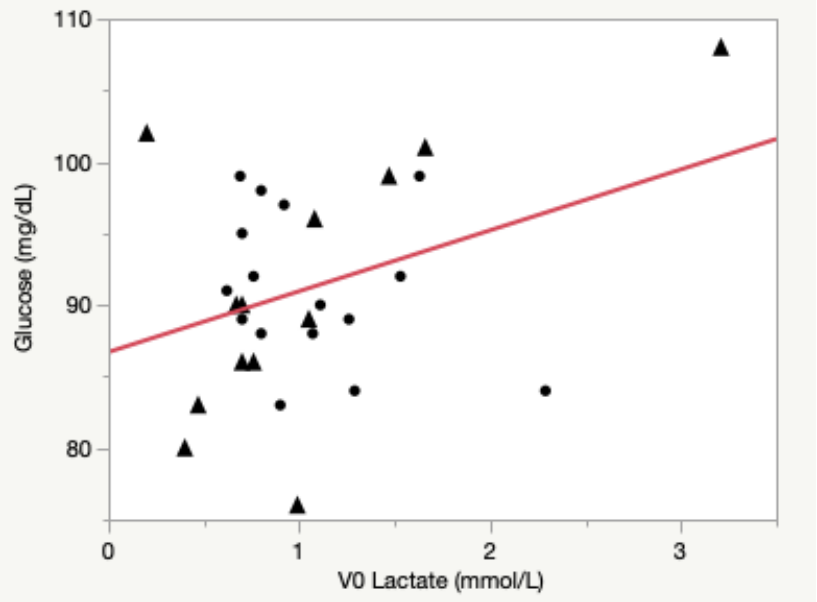
FIGURE 2: Correlations between insulin-related factors associated with metabolic syndrome and fasting plasma lactate.



p=0.001, r=0.582

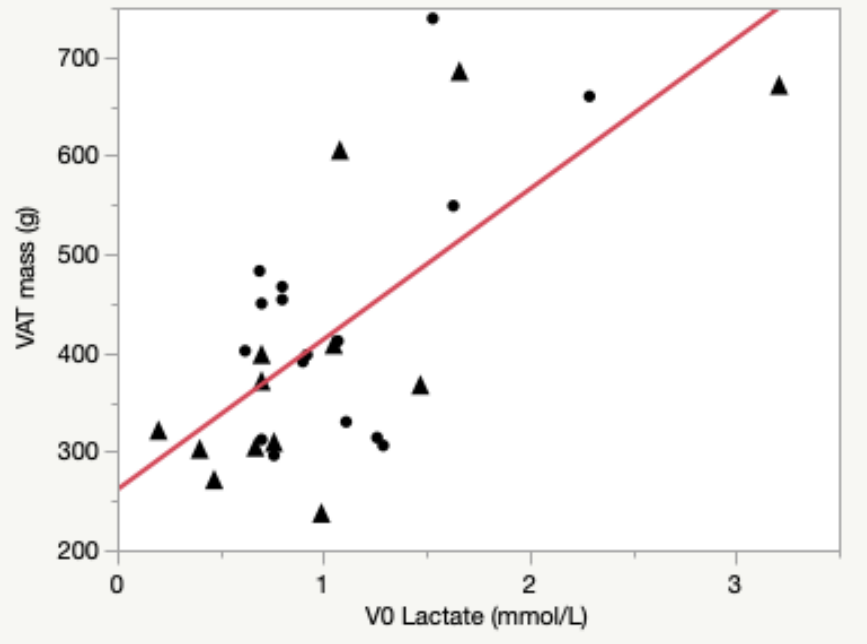


p=0.0003, r=0.62

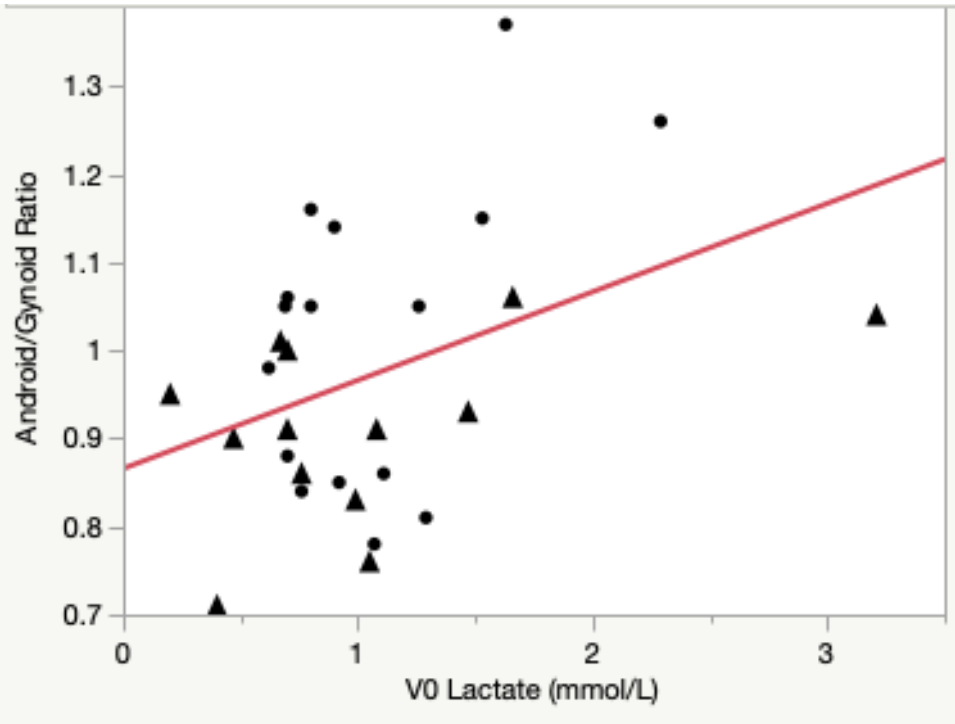


p=0.06, r=0.353

FIGURE 3: Correlations between visceral fat-related factors associated with metabolic syndrome and fasting plasma lactate.

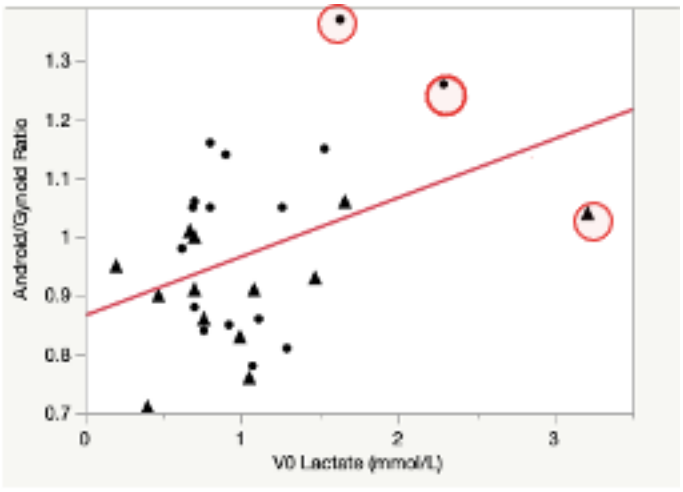


$p<0.0001$, $r=0.672$

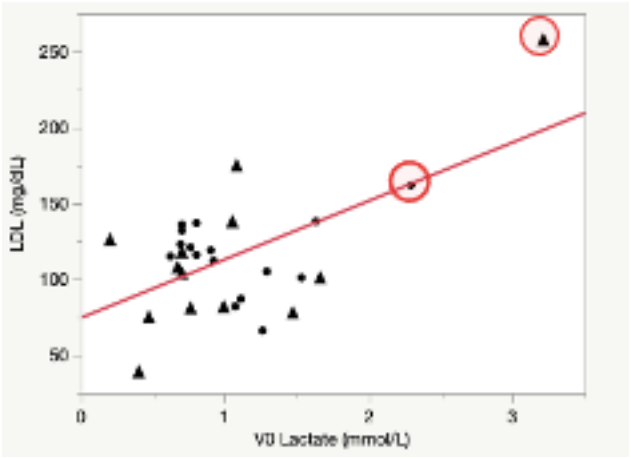


$p=0.034$, $r=0.3$

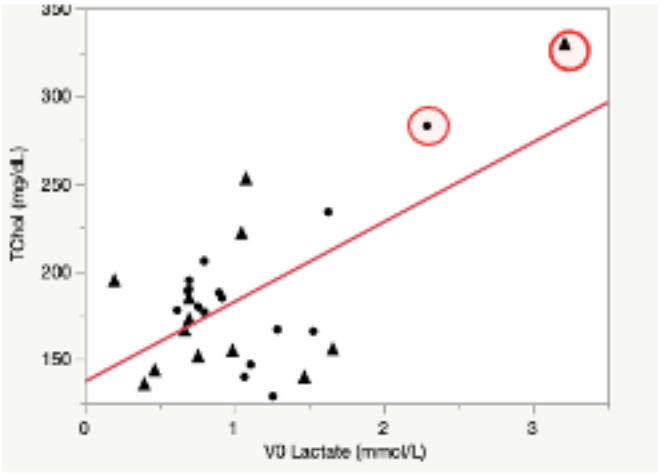
FIGURE 4: Correlations that turned non-significant when controlling for statistically determined outliers.



p=0.034, r=0.396

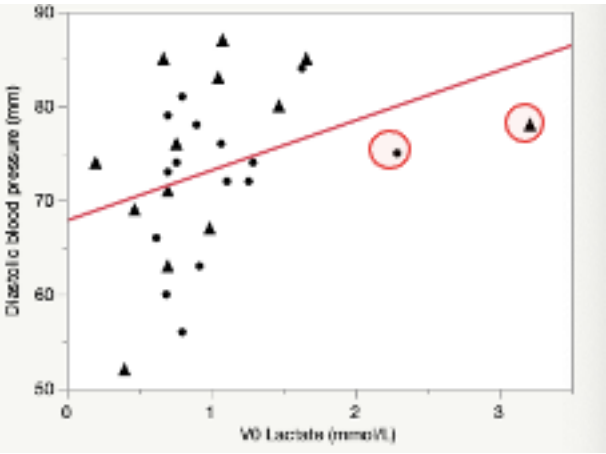


p=0.001, r=0.582

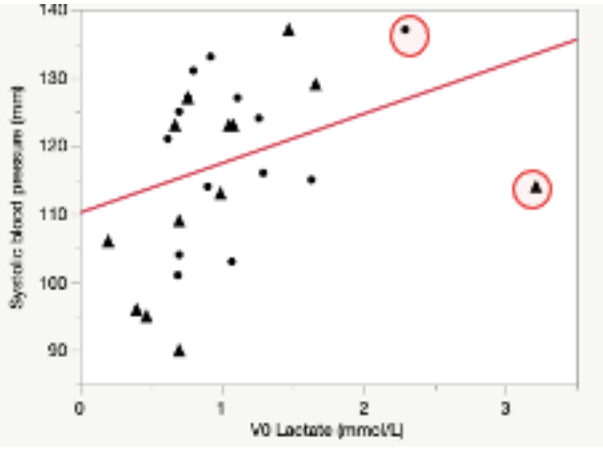


p=0.0004, r=0.611

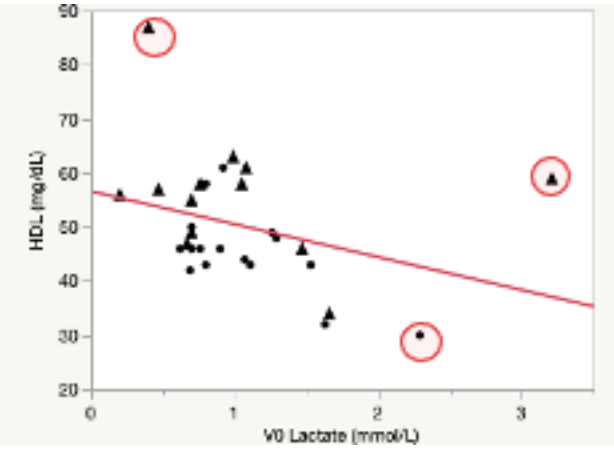
FIGURE 5: Correlations that turned statistically significant when controlling for statistically determined outliers.



p=0.06, r=0.363



p=0.08, r=0.3371



p=0.08, r=-0.3289

Table 3: Correlation and Significance of Metabolic Syndrome Factors with V0 Lactate Levels (Non-Statistically Significant)

Factor	r	p
Age	0.084	0.665
BMI	0.235	0.2182
HDL*	-0.329	0.0814
HDL: LDL	-0.351	0.0613
Glucose	0.353	0.0606
Systolic Blood Pressure*	0.337	0.0794
Diastolic Blood Pressure*	0.363	0.0576
Waist-to-Hip Ratio	0.011	0.9546
Time to Exhaustion	-0.069	0.7197
VO2max (mL/kg/min)	-0.111	0.5797
Resting Metabolic Rate	0.005	0.9771

Statistically significant at $p<0.05$

Chapter 5: Discussion

Metabolic syndrome, defined by a collection of different elements and disorders including dyslipidemia, hypertension, central obesity, and insulin resistance, has the potential to increase a person's risk for conditions such as coronary heart disease, cardiovascular disease, and type 2 diabetes (Kassi et al., 2011). Previous research shows that elevated plasma lactate levels can be an early biomarker for predicting metabolic diseases (Broskey et al., 2020). This study aimed to answer whether a relationship exists between plasma lactate levels and factors of MetSyn in men (n=16) and women (n=13) with overweight. The study demonstrated a positive relationship between lactate levels and metabolic syndrome factors, including total cholesterol, triglycerides, LDL levels, insulin, HOMA-IR, Android/Gynoid ratio, and VAT mass when outliers are not controlled for. Surprisingly, some factors that could be elevated and contribute to the metabolic syndrome such as BMI, glucose levels, and waist-to-hip ratios did not have a significant relationship with plasma lactate. Systolic and diastolic blood pressures were originally non-significant but became statistically significant when outliers were controlled for.

Prior research has established that basal lactate values typically increase, corresponding to an increase in BMI as demonstrated by a study using lean and obese subjects with normal glucose tolerance (Lovejoy, et al., 1990). However, the current study did not illustrate that same relationship. Another study looking at the effect of type 2 diabetes and plasma lactate levels found that lactate was highest in obese subjects with type 2 diabetes compared to non-obese and obese individuals with normal glucose tolerance (Chen et al., 1993). However, a different study described similar results to ours and did not report an association between lactate changes and BMI changes following rapid weight loss (Crawford, et al., 2008). This may be because the current study examined those with overweight yet are otherwise healthy, while the previous research focused on obese individuals versus lean individuals and those with type 2 diabetes.

Another reason why BMI may not be related to plasma lactate in the current study could be due to the fact that BMI does not take into consideration lean mass vs fat mass and there could be a lean individual in our population with high muscle mass, but their BMI indicates an overweight calculation (Rothman, K., 2008)

Measuring blood lactate is an easy way to measure oxidative capacity where there is an inverse relationship between the two variables and it is hypothesized that decreased oxidative capacity may lead to obesity-related hypertension (Crawford et al., 2008). This study by Crawford et al., examined if blood lactate is associated with high blood pressure and if reducing weight via a very low-calorie diet would change both blood pressure readings and blood lactate levels. The researchers studied adults with obesity –with and without metabolic syndrome, and lean controls. Following rapid weight loss, the subjects with obesity and - metabolic syndrome had a significant reduction in lactate levels as well as both SBP and DBP where these variables were significantly higher than their lean counterparts at baseline. However, once adjusted for baseline lactate, BMI change, and demographic factors, a 10% change in lactate after rapid weight loss was associated with a 1 mmHg change in DBP but not SBP. Nevertheless, this was still significant (Crawford et al., 2008).

Another study by Jones et al. (2019) had comparable results regarding glucose levels as the current study. Individuals with obesity underwent gastric bypass surgery and factors such as lactate production, glucose, and insulin sensitivity were measured pre-and post-surgery. In a separate group, metabolically impaired subjects participated in a 6-month exercise training program and had the same values measured pre-and post-intervention. Results indicated that after weight loss, lactate production decreased while there was no change in insulin sensitivity and therefore glucose, even though lactate and glucose are often synchronously elevated in

metabolic disease (Jones et al., 2019). Chondronikola et al. (2018) found that lactate concentration and glucose production are correlated in participants who are obese before and after weight loss via a low-calorie diet, behavior education sessions, and dietary counseling sessions. The participants in this study also showed a decrease in fasting lactate independent of the amount of weight lost. These results show that a 6-month exercise program and weight loss by gastric bypass surgery are both successful at significantly reducing fasting plasma lactate values, but not glucose levels. Thus, it can be concluded that weight loss via diet, exercise, or gastric bypass surgery has the potential to reduce lactate levels.

The current study did not find an association between lactate levels and waist-to-hip ratios in the subjects with overweight. Although a high waist-to-hip ratio is a factor for metabolic syndrome, the relationship was not significant. The only other study found that directly measured plasma lactate and waist-to-hip ratio was Broskey et al., (2021) where they discovered a positive correlation between lactate levels and waist-to-hip ratio in young, lean women. This difference may be due to the differences in population between Broskey et al. and the current study. In addition, the Broskey et al. study examined women only, and with the current study's population of men and women, it is known that men and women carry fat and weight differently. Men carry more visceral fat and women carry more peripheral fat which could lead to the insignificant relationship between plasma lactate and waist-to-hip ratio (Geer & Shen, 2010).

Insulin resistance (IR) is illustrated by weakened insulin action and therefore a reduction in the uptake of glucose by insulin-sensitive tissues (Barbosa et al., 2022). Research with healthy, young, and lean women saw no relationship between lactate levels and HOMA-IR values (Broskey et al., 2020) while the current study saw a significant relationship with the addition of males as well. This could mean that insulin resistance is associated with elevated

lactate and that there is dysfunction in both sexes. To support this finding, Digirolamo et al. (1992) saw an inverse relationship between plasma lactate levels and insulin sensitivity, translating to a direct relationship between insulin resistance and plasma lactate. However, it is unknown whether insulin resistance precedes lactate overproduction or vice versa (Digiorolamo et al, 1992). Previous research utilizing prepubescent children ages 5 to 9 years saw higher lactate levels in those with higher insulin resistance (Barbosa et al., 2022). This means that the findings of the current study may go back to childhood. Mechanisms by which insulin resistance would cause lactate accumulation are still unknown. In a study that assessed the utilization of HOMA-IR to establish insulin resistance, in women diagnosed with polycystic ovary syndrome (PCOS), the researchers determined that HOMA-IR performed better than using hyperinsulinemia to identify insulin resistance (Majid et al., 2017). Low HOMA-IR values indicate being more sensitive to insulin. Elevated lactate that is related to obesity may be accountable for insulin resistance (Crawford et al., 2010). However, this relationship between lactate and its role in insulin resistance should be studied further.

It is known that those with elevated triglyceride concentrations are more insulin resistant than those with healthy triglyceride levels (Laws & Reaven, 1992). Previous research has shown that dyslipidemia is related to not only obesity but also to elevated plasma lactate and metabolic dysfunction (Jones et al., 2019). Dyslipidemia is characterized by high triglycerides, low HDL cholesterol, high LDL cholesterol, and/or high total cholesterol readings. Research also shows that triglyceride levels increased as plasma lactate levels increased as well (Crawford et al., 2010). The current study illustrated a positive association between plasma lactate and total cholesterol levels, LDL cholesterol, and triglycerides while not controlling for outliers. When outliers were accounted for, total cholesterol and LDL became insignificant while HDL levels

became significant. This is on par with previous research especially since the current study also saw a positive association between triglycerides and plasma lactate. Cholesterol levels can increase via several different factors such as genetics, diet, stress, and sedentary lifestyles, and can often be combatted with a healthy diet, exercise, stress management, and sometimes medications such as statins (Huff et al., 2022).

The results show that both the android/gynoid ratio and visceral adipose tissue (VAT) mass have a positive association with plasma lactate levels when outliers were not controlled for. Android/gynoid ratio was insignificant when controlling for outliers while VAT mass was still significant. Something to take away from this study is that with high levels of visceral obesity comes more health risks that could lead to metabolic syndrome (Després & Lemieux, 2006). Visceral adiposity is a driving factor for the increased production of free fatty acids and an inability to metabolize them which may lead to insulin resistance (Després & Lemieux, 2006). Additionally, visceral adiposity coupled with dysfunctional adipose tissue can lead to fatty depots in and around the muscle, heart, and liver which can therefore cause an altered metabolic profile that can lead to insulin resistance and other factors of metabolic syndrome (Després & Lemieux, 2006). Due to the nature of visceral adipose tissue, one does not have to be within the overweight or obesity category via BMI to have the cardiovascular risk factor of central obesity (Bosello & Zamboni, 2000). However, another study noted a direct and significant linear relationship between BMI and lactate levels in healthy lean, and obese subjects (Digirolamo et al., 1992). The current study may not have seen the same relationship due to using subjects with overweight. It is important to note that the android/gynoid ratio is another way to determine visceral fat due to the ratio of abdominal fat and hip and thigh fat. The current study is the first study to directly correlate VAT mass and plasma lactate levels.

There are limitations to this study, however. One limitation would be the sample size as there were only 29 subjects, which may have also contributed to the differences when controlling for outliers. A confounding variable may be that we did not time the data collection with the female menstrual cycle. We also only analyzed those with overweight BMI so for a future study it may be worthwhile to look at individuals with healthy weight and obesity in the same study to see the differences between all three groups.

To conclude, there is evidence that plasma lactate values may be able to predict the development of metabolic conditions as a biomarker. With high cholesterol, triglycerides, and central obesity indicators it is imperative to be active and keep a balanced diet to lower plasma lactate levels and improve these other metabolic factors.

Chapter 6: Conclusion

The research aimed to determine if there is a relationship between plasma lactate levels and factors of MetSyn in overweight men and women. By analyzing participants who completed measurements of MetSyn factors and others, it can be concluded that there is a statistically significant relationship between plasma lactate levels and some, but not all, factors of MetSyn. While the small sample size acquired limits the generalizability of the results, this research provides new insight into plasma lactate and how it may be a precursor and biomarker for metabolic diseases such as MetSyn. Future research may want to utilize a larger sample size with more variation in BMI to be able to illustrate those potential differences. While more research needs to be done to help determine if elevated plasma lactate levels precede elevated metabolic factors or vice versa, practitioners and clinicians may be able to utilize this data to emphasize the importance of measuring resting plasma lactate values.

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APPENDIX: IRB APPROVAL LETTER

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board
4N-64 Brody Medical Sciences 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: 2/27/2023
Re: CR00010015
UMCIRB 19-000914
Metabolic inflexibility is related to elevated muscle anaerobic glycolysis

I am pleased to inform you that at the convened meeting on 2/22/2023 at 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 2/22/2023 to 2/21/2024.

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
APPHoumard-Dohm_NIH_R01_12 (1).pdf(0.01)	Study Protocol or Grant Application
Flyer and email(0.07)	Recruitment Documents/Scripts
Flyer Canvas distribution(0.01)	Recruitment Documents/Scripts
Group 1 and 2 Information Sheet V.1.docx(0.02)	Recruitment Documents/Scripts
Group 3 Information Sheet V.3 1.20.22(0.03)	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.7 07.13.22-Clean.doc(0.16)	Consent Forms
MetFlex Main ICF v.8 07.08.22-Clean.doc(0.14)	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.5.0 07.26.22.docx(0.01)	Study Protocol or Grant Application
MetFlex Screening ICF v.7-0 1.25.22.docx(0.13)	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:

None

The following UMCIRB members with a potential Conflict of Interest did not attend this IRB meeting: M. L. Pories