**Abstract**

**Blood Lactate Predicts Resting Energy Expenditure in Non-Obese Caucasian Females**

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**Background:** Obesity and Type 2 Diabetes are both global epidemics that continue to skyrocket. High basal plasma lactate levels, reduced oxidative capacity, and insulin resistance are co-morbidities with these metabolic disorders. However, it is unknown if those co-morbid traits are also displayed in the non-obese who may be predisposed to obesity. A higher plasma lactate concentration is the product of the shift to anaerobic glycolysis due to the reduction of oxidative capacity. With aerobic respiration being limited, we believed it would reduce the resting energy expenditure (REE), and potentially propel an individual toward obesity and related metabolic disease(s). **Purpose:** The purpose of the study was to investigate if plasma lactate can be used to predict resting energy expenditure in non-obese humans. **Methods:** Non-obese subjects (n=40) with body mass index (BMI) between 18.5-26.0 $kg/m^2$ were screened for plasma lactate concentration. Subjects with the highest (n=10) and lowest (n=11) plasma lactate were selected and grouped into a High Lactate (High) and a Low Lactate (Low) Group. Both groups had their body composition, REE, resting respiratory exchange ratio (RER), and aerobic capacity ($VO_2$ peak) assessed on the first testing day. Blood measurements, and oxidative capacity were assessed using near infrared spectroscopy (NIRS), and submaximal exercise RER

(low intensity at 50% $VO_2$ peak) were assessed on the second testing day. **Results:** Lactate and REE were negatively correlated (R=-0.6093) in the High Lactate Group. This correlation was stronger when REE was adjusted for fat free mass (FFM) (R=-0.6823, P<0.05). Aerobic capacity adjusted for FFM was significantly lower in the High Lactate Group (P<0.05). Lactate and aerobic capacity adjusted for FFM also revealed a strong relationship (R=-0.7232, P<0.001). Lactate and oxidative capacity demonstrated a negative relationship (R=-0.364) in the High Lactate Group. Lactate and substrate change (ΔRER) from resting to submaximal exercise were strongly correlated (R=0.6393, P<0.05). Laboratory blood analysis showed no difference in plasma lactate, insulin or glucose. **Conclusion:** Lactate is associated with resting energy expenditure in non-obese Caucasian females. The inability for higher lactate individuals to match more fat substrates utilized as the lower lactate subjects may be an indication of a lesser mitochondrial and capillary density. The implication of early signs of elevated lactate concentrations may be an indicator of a compromised energy transduction capacity, which may be a contributor to obesity. Further research is needed to look at the plasma lactate concentration, and oxidative capacity affecting energy expenditure.

# Blood Lactate Predicts Resting Energy Expenditure in Non-Obese Caucasian Females

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## Chapter I: Introduction

Obesity is one of the largest health epidemics in North America [1]. The excessive weight gain among the U.S. population is one of the main contributors to metabolic diseases and negative health related risk factors [1]. These diseases include Type 2 Diabetes, cardiovascular disease (CVD), hypertension, and dyslipidemia [2]. Body mass index (BMI) is a widely used tool to categorize bodyweight as a risk factor for obesity. BMI is a fast and simple way to predict global health risks and it has also been shown to be highly associated with all-cause mortality. As a person deviates further above the normal BMI range (18.5-24.9 $kg/m^2$), mortality and morbidity increases [3,4]. Obesity is defined with a BMI above 30.0 $kg/m^2$, and obesity related risk factors such as cardiovascular disease, hypertension, Type 2 Diabetes, are magnified even further when accompanied with a sedentary lifestyle [5]. Moreover, the cost associated with obesity continues to rise. It is estimated that Americans spend between 147 to 210 billion dollars annually on obesity related health issues [6]. The annual cost for treating an obese woman is around $4,879 and $2,646 for an obese man. According to the Center for Disease Control Prevention, close to 40% of the U.S population is obese [2]. If this epidemic continues, the cost is projected to reach up to $580 billion by year of 2030 [7].

Metabolic diseases become more prevalent the more obese an individual becomes [8]. To understand more on the topic of obesity and obesity related diseases, we were interested in looking at the biomarker plasma lactate in the non-obese to see if it can be served as a predictor of resting energy expenditure. In the basal/resting state, plasma lactate concentrations of 0.5-1.0 mmol/L has been suggested to be indicative of a lower risk for developing metabolic diseases, while levels above 2.5 mmol/L have a significantly higher association [4,9–11]. In the normal healthy state, low plasma lactate levels are expected. Plasma lactate serves as a good indicator of

an individual's substrate oxidative capacity. The problem arises when there is a larger concentration of lactate within the blood. This is noted in the presence of a low mitochondrial oxidative capacity, which has been associated with dysfunction of the pathway involved the Tricarboxylic Acid Cycle (TCA) observed in the obese state [12–14]. Evidence suggests that the pathway for the TCA Cycle has been mechanistically attenuated, which results in a shift of substrate utilization away from lipids/fatty acids (and carbohydrate) toward anaerobic metabolism of carbohydrate [15,16]. It is known that high lactate concentration is associated with both obesity and Type 2 Diabetes [11,17]. However, the characteristics of individuals with elevated lactate above the normal level has not been thoroughly investigated in the non-obese state with no known diagnosis of metabolic or cardiovascular diseases. We believed that high lactate concentration is an indication for lower oxidative capacity, which would reflect a lower resting energy expenditure (REE), and resting higher RER.

The purpose of the study was to focus in the direction towards plasma lactate prediction of resting energy expenditure in non-obese humans. The hypothesis entertained currently is that a reduced energy substrate (carbohydrates or fats) oxidative capacity leads to heightened anaerobic metabolism of carbohydrates due to an inability to shift between carbohydrate and fat substrates for oxidation [18,19]. As such, we hypothesized that resting lactate levels in the blood would be predictive of an individual's REE.

In relation to REE, it is imperative to address the characteristics of skeletal muscle bioenergetics in terms of fuel metabolism. Possessing high levels of fat mass and low levels of physical activity is associated with a low muscle mass, which can lead to low whole body oxidative capacity. Skeletal muscle obtains energy through competing substrates between glucose and free fatty acids [15]. The cell utilizes both glucose (via aerobic and anaerobic

glycolysis) [20], and fatty acids for fuel ( via β-oxidation)[21] in response to energy demand. However, reduced lipid oxidation has been observed in the obese. Due to this decrease in lipid oxidation, it's speculated that REE would be reduced. While skeletal muscle oxidative capacity was not measured *in vitro* within the present study other measurements such as $VO_2$ max, resting and exercise respiratory exchange ratio, and near infrared spectroscopy was assessed to indirectly describe whole body substrate oxidation.

Substrate utilization was another component of interest with the present study. Substrate utilization is commonly measured by the RER through indirect calorimetry. Indirect calorimetry assesses the amount of carbon dioxide produced by oxidative metabolism, which requires oxygen consumption to drive ATP synthesis. The procedure of indirect calorimetry is expressed mathematically as the volume of carbon dioxide ($VCO_2$) produced divided by the volume of oxygen ($VO_2$) consumed required for mitochondrial respiration and ATP synthesis [22]. The concept was first developed by Antoine Laurent Lavoisier [23], and his work made it possible to assess metabolic inflexibility toward substrate switching between the predominant energy supply (carbohydrate or lipid) in overweight or the obese population [11]. Studies have shown that metabolic inflexibility is prominent in the obese and those who are insulin resistant, but the mechanism is still under investigation [12,18]. It is unclear if metabolic inflexibility was developed after obesity or before. It is unknown if metabolic inflexibility contributes to obesity and/or insulin resistance. This is relevant in to the present study since both are associated with plasma lactate. Thus, the study of RER and its association with blood-lactate might aid identifying individuals who are predisposed or currently afflicted with metabolic disease notably in the non-obese. The literature suggests the obese population has a different muscle metabolic profile than the lean population [14,24]. As a person gradually becomes obese, the decline in muscle oxidative

capacity follows [25]. In this regard, a reduction in slow twitch oxidative, or Type I muscle fibers, was suggested to be one mitigating factor for reduced energy production from oxidative metabolism [19,26].

When the body becomes less adaptive in using fat substrates, the reaction of glycolysis becomes more predominant in the anaerobic condition and proceeds to a greater pathway end product of lactate production [27,28]. This altered energy production (shift from oxidative metabolism of carbohydrate as pyruvate) was commonly observed in individuals with significant differences in fat mass and fat free mass. In context of the obese population, REE adjusted to body weight, was significantly lower compared to their lean counter parts [29,30] . Lower REE was associated with lower levels of respiration of energy substrates (carbohydrates and lipids) leading to "forced" anaerobic metabolism as mentioned previously. The rise in lactic acid production was the concomitant result for anaerobic respiration. Thus, lactate may serve as a surrogate marker for reduced oxidative potential (via reduction in mitochondrial respiration) and a predisposition towards reduced aerobic energy production.

Collectively, metabolic diseases become more apparent with obesity [3,27]. It was then reasonable to speculate that REE may be an indicator of a propensity or overt presence of the inability to generate sufficient energy aerobically. The result accompanied would be an elevation in plasma lactate production as the end product of anaerobic metabolism. As an outcome, it was predicted that the non-obese, predisposed to metabolic disease would also display a lower REE respective to elevated lactate concentration. This is a fundamental dependent variable (REE) to be assessed in the present project and forms a basis for the proposed hypothesis. Therefore, if our hypothesis was correct, blood lactate concentrations would be reflective of metabolic

dysfunction of the aerobic capacity in individuals leading to an earlier phenotype manifested as obesity and Type 2 Diabetes.

**Preliminary Data**

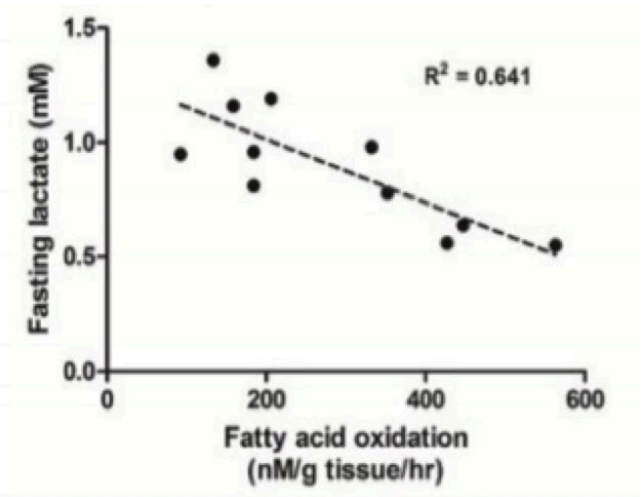



**Figure 1.** Correlation between fasting lactate and fatty acid oxidation in muscle homogenates of non-obese subjects.

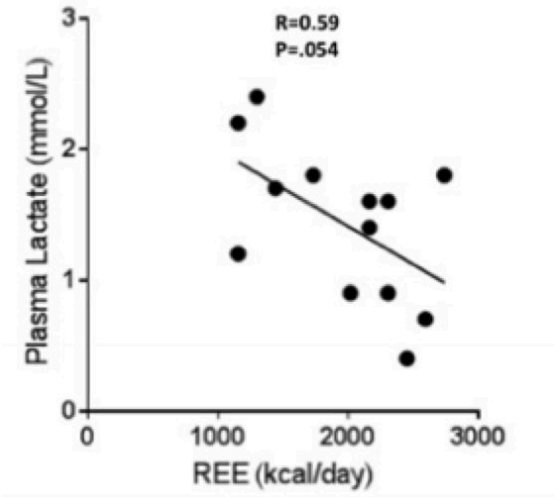



***Figure 2.** Resting energy expenditure REE is high in subjects who have low plasma lactate.

*Provided by Charles Tanner and his ECU students who collected the data

Our preliminary data (Fig. 1) showed a negative association between fatty acid oxidation and fasting lactate. We also showed a negative association between the resting blood plasma lactate and resting energy expenditure (Fig. 2). The current understanding of elevated lactate is due to characteristics that were related to reductions in aerobic metabolism of fuel substrate (carbohydrate and fatty acids) in obese individuals [31,17]. An insulin resistance increase and lower aerobic capacity are both associated with lactate, and are prevalent in the obese population [10,32]. These traits have also been noted recently in the non-obese [11,33].

As supported from our preliminary data, there is a negative correlation between blood lactate and REE. In relation, plasma lactate is also negatively correlated with fatty acid oxidation. We believed that the non-obese who demonstrated elevated blood lactate levels (2.0 mmol/L) were exhibiting lower energy expenditure before reaching the obese state. Therefore, plasma lactate and REE was studied to help us further understand energy production in these "pre-disposed" non-obese individuals.

**Research Question:**

Is there an association between blood lactate level and resting energy expenditure, and by extension aerobic capacity in young healthy non-obese females?

**Hypothesis:**

$H_0$ : There is no association with plasma lactate and resting energy expenditure in non-obese individuals.

$H_a$: Resting blood levels is associated in reduction of energy expenditure in the non-obese. To expand, higher plasma lactate level is associated with many metabolic diseases; REE would potentially be lowered due to a shift in energy substrate towards anaerobic respiration.

**Clinical Significance:**

Since a higher plasma lactate level is likely associated with many metabolic diseases such as obesity and Type 2 Diabetes, measurement of blood lactate levels may be used as an early predictor of a predisposal toward metabolic disease. The findings from this research project allowed for an economical diagnostic tool for clinicians to assess and/or predict metabolic disease and thus serve as an early treatment recommendation, thus allowing physicians to treat patients prior to the development of metabolic diseases after the fact.

**Purpose:**

The purpose of this study is to correlate blood plasma lactate level with resting energy expenditure (REE).

**Experimental Design**

A total of 40 non-obese subjects were screened for the blood lactate. The lower (n=11) and higher (n=10) end of blood lactate spread were selected for further testing. Participants performed 20 min of indirect calorimetry to determine their REE. In addition, we assessed the metabolic response to a standardized aerobic exercise test to assess possible "inflexibility" in "substrate shifting" toward lipid versus carbohydrate. Furthermore, we obtained adjunct measurements of *in vivo* mitochondrial oxidation of substrate by near infrared spectroscopy (NIRS) to further correlate our data in plasma lactate and REE. We focused this investigation on non-obese subjects as preliminary data from our laboratories suggested that a dichotomy in resting energy expenditure exists even among non-obese individuals suggesting a "pre-disposition" toward metabolic disease in non-obese individuals (suggested by BMI and body composition).

**Statistical Design**

Pearson product-moment correlation was used to measure the strength of the linear relationship between depend variables (resting energy expenditure, REE adjusted to FFM, BMI, body fat percentage, resting and exercise RER, absolute and adjusted to FFM $VO_2$, oxygen utilization) and the predictor variable (lactate). Regression analysis provided the strength of association and predictive power ($r^2$) of plasma lactate concentrations and each dependent variable. Two sample unpaired t-test was used to compare independent variables between the

High and Low Lactate groups. Two-way ANOVA was used to compare resting/exercise RER between groups and throughout time.

**Limitations:**

1. Error of the indirect calorimetry method to determine resting energy expenditure
2. Age of the population
3. Sample population from Greenville and surrounding area
4. The degree of insulin resistance and metabolic flexibility
5. Recruitment of lean subjects

**Delimitation**

1. Females
2. Caucasian
3. Random selection of subjects with a BMI between 18.5-26 $kg/m^2$
4. The duration of exercise indirect calorimetry
5. Selected adult volunteers
6. Level of daily physical activity among subjects as sedentary

**Operational Definition**

1. Body mass index (BMI): $kg/m^2$ an index that classify an individual's health risk. Normal: 18.5-24.9, overweight: 25.0-29.9 $kg/m^2$, obese class I: 30-34.9 $kg/m^2$, obese class I: 35.0-39.9 $kg/m^2$, obese class III: >40.0 $kg/m^2$.
2. Obesity: excessive body fat that is beyond overweight, having BMI ranging from 30-34.9 $kg/m^2$ is grade I obesity, 35-39.9 $kg/m^2$ is grade II and over 40 $kg/m^2$ is grade III

3. Metabolic flexibility: the ability to switch between two substrates, fat and carbohydrates, and its response to a change in availability
4. Lactate: a product that yield from pyruvate by a redox reaction via enzyme lactate dehydrogenase (LDH)
5. Insulin resistance: The blunted response of cells to insulin
6. Type 2 Diabetes (Mellitus): chronic disease, which the cell becomes resistance to insulin and it, blunts the movement of glucose into the cells.
7. Respiratory exchange ratio (RER): the ratio between the amount of carbon dioxide ($CO_2$) produced in metabolism and oxygen ($O_2$) used. The ratio is determined by comparing exhaled gases to room air
8. Substrate: the reactant, which catalyze by the enzyme. In relevant of this, our substrate is our macronutrients (glucose and fat)
9. Glycolysis: the metabolic pathway of breaking down glucose
10. Adenosine Triphosphate (ATP): a complex organic compound that participates in many processes. Also known as the unit of currency of intracellular energy transfer.
11. Indirect Calorimetry: technique that measures inspired and expired gas flow volumes and concentration of oxygen and carbon dioxide.
12. Resting Energy Expenditure (REE): the amount of total energy that the body requires in a resting state
13. Krebs Cycle (TCA: Tricarboxylic acid cycle): a series of chemical reaction used by all aerobic organisms to release stored energy through the oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins into carbon dioxide and chemical energy in the form of ATP.

# Chapter II: Literature review

## Introduction

The purpose of the study was to investigate the potential association between resting energy expenditure (REE) and blood plasma lactate. It is important to note that our focus was not to measure metabolic inflexibility or insulin resistance. The following literature review contains the current knowledge of The Epidemic of Obesity, Lactate, Insulin Resistance/Type 2 Diabetes, and Skeletal Muscle, Metabolic Flexibility and Resting Energy Expenditure.

## The Epidemic of Obesity

Obesity is recognized by health professionals as one of biggest health epidemics in North America, USA [2]. The estimated expenses that Americans spend on obesity treatment was between 147 to 210 million dollars annually [2]. To be specific, an average annual cost of treatment for an obese woman is around $4,879 and $2,646 for an obese man. If the epidemic continues, the cost for the U.S. alone could reach up to $580 billion dollars by year of 2030[7].

Obesity leads to a higher chance of developing other diseases, such as Type 2 Diabetes, hypertension, dyslipidemia, and cardiovascular disease. The risk for the development of these diseases is increased even more with a sedentary lifestyle [3]. The consequences of obesity are severe as it's strongly associated with diseases such as cardiovascular disease and cancer [34]. A meta-analysis investigated the all-cause mortality rate across Australia, Asia, Europe, and North America. The study collectively analyzed BMI over 3.65 million individuals with no known chronic diseases with a 5 years follow up. The data showed the potential association between all-cause mortality rate and BMI through the use of hazard ratio analysis, which consisted of participant/death of participants. This meta-analysis demonstrated the positive association of all-cause mortality with overweight and obese populations [35].

It's commonly reported that obesity is negatively associated with aerobic capacity. Increased physical activity and exercise may help with weight management and improve aerobic capacity. Aerobic capacity is one of the strongest predictors of all-cause mortality and health related risk factors in men and women [36]. Aerobic capacity is measured by oxidative capacity or volume of oxygen a person consumes for respiration ($VO_2$). A Lancet study assessed the relationship of low oxidative capacity and mortality in normal, overweight, and obese men [35]. The study observed over 25,000 men with a mortality follow up. The baseline of cardiovascular disease (CVD), Type 2 Diabetes, dyslipidemia, smoking, and low aerobic capacity was examined prior to the follow up. In the course of this study, a total of 1025 subjects were deceased, and 439 deaths were due to CVD. Men who were either overweight or obese essentially had a greater risk of CVD and Type 2 Diabetes. This study highlighted that the obese group had the highest risk with 2.6 times higher risk for CVD and 1.9 times higher risk for all-cause mortality. The study concluded low aerobic capacity as a strong independent predictor of all-cause mortality, Type 2 Diabetes, and other relative risk factors of CVD[5].

Losing weight and sustaining a lower BMI by exercising can ultimately lower the risk of mortality [5,26]. However, reducing energy intake and increase energy expenditure may be more difficult in people who are obese or overweight. Obesity has an inverse relationship with oxidative capacity [37], which is determined by aerobic respiration. The reduced content of oxidative, type I fibers precedes a dependence on anaerobic respiration for energy demand, resulting in an increase of blood lactate [12,38]. The elevation of blood lactate is a distinct feature in obesity. Insulin resistance and metabolic inflexibility are also characteristics that have been observed in obesity [18,19,27]. This suggests there might an alteration in energy production from metabolic diseases.

We understand that obesity and other metabolic diseases carries negative physiological traits, and reduce the quality of life. The population of interest in our study was the non-obese, since the aerobic capacity of the non-obese has not been thoroughly studied along with lactate. Our question was that do people develop these metabolic alterations and then become obese or do they become obese and then developed disease traits? With obesity rate rising, studying the non-obese could potentially help in understanding the predisposition of obesity.

**Lactate (Adipose tissue and Lactate)**

Elevated plasma lactate level has been associated with many diseases such as obesity, insulin resistance, diabetes, hypertension, cardiovascular disease, and dyslipidemia [10,11,30,39,40]. The etiology of lactate is mainly from metabolizing glucose substrate via glycolysis. The end product of glycolysis yields 2 moles of pyruvate, 2 NADH, and net of 2 ATP. In the aerobic condition, pyruvate is translocated into the mitochondria by pyruvate translocase, and then converted to acetyl-CoA by a series of reactions to initiate the Krebs (TCA) Cycle[38]. However, in the anaerobic condition, pyruvate interacts with lactate dehydrogenase to yield lactate and $NAD^+$ [24]. Lactate is then transported to the liver to make glucose (via gluconeogenesis) to supply the nervous system and the red blood cells with energy substrate. In contrast, the surplus generated glucose can be converted and stored as glycogen via the process known as glycogenesis, but this does not occur in the catabolic condition such as during an overnight fast.

Lactate in the blood can be produced by skeletal muscle, perivenous liver cells, red blood cells, adipose cells, and skin cells. Lactate that was produced would essentially get metabolized by various organs, with liver being the main contributor for disposing lactate. The liver accounts for 70% of lactate clearance in the blood, and the rest is being metabolized by the renal system, skeletal muscle, and cardiac myocytes[24]. Under normal conditions basal lactate level is 0.5-1.0

mmol/L[39] [24], depending on the studies noted in the literature. Lactate concentration above 1.0 mmol/L deviates from normal levels. However, elevated or high plasma lactate is not universally determined. Most studies have elevated lactate concentration marked as 2.0 to 2.5 mmol/L [39], and labeled >4.0mmol/L as "high". In the terms of glucose metabolism, people who have a lower basal blood lactate level are able to aerobically metabolize glucose, which would decrease the net basal lactate concentration. In most cases, elevated lactate concentration is an indication of a greater dependence on glucose for anaerobic metabolism due to attenuation of its oxidation of glucose.

It was believed that adipose tissue contributes to a greater lactate concentration. Hagstrom et al. were the first to examine the role of adipose tissue toward lactate production *in vivo*. The study selected non-obese subjects and performed the microdialysis sampling technique within the subcutaneous adipose tissue after glucose injection. The dialysate determined there was an elevated level of pyruvate and lactate production that was independent from muscle [41]. Previously, lactate production was believed to account for 1-3% of glucose uptake within the adipose tissue [31], but was later reconsidered to be more. It is estimated that lactate production is between 10-15% in lean individuals, and 15% to >30% in the obese [31]. Adipose tissue in the obese or diabetic state is able to uptake 50-70% of glucose, which has been shown in humans and mice[31]. This shows that extra adipose tissue in the obese is able to uptake more glucose in the blood and proceed to a greater lactate production. This may be the result in part from the reduction in muscle to aerobically utilize glucose, and subsequent to a greater conversion of glucose to lactate. Accordingly, adiposity has been associated with higher lactate production, and elevated lactate has shown to be more prominent with increasing BMI [10]. The non-obese subjects that were recruited in the present study had relatively lower body fat percentage compared to

their obese counterparts. This ruled out the potential lactate production from adipose tissue, as described from Hagstrom and colleagues. Poor aerobic capacity in the obese has been well documented [16,42]. Decreased oxidative capacity would then be a better association factor for higher lactate production than adiposity in the present study.

**Skeletal Muscle in the Obese**

Aside from extra adipose tissues, the obese also possess different muscle composition as one of the characteristics of reduced aerobic respiration. Skeletal muscle performs many biological processes, which metabolically requires high-energy demand. Skeletal muscle consists of two main phenotypes, Type I and II[26]. Type I are slow twitch fibers and are known for their high oxidative properties that require aerobic glycolysis to produce ATP. The energy system for slow oxidative fibers involve the mitochondria [38,43]. Within the mitochondria, the process known as the Krebs Cycle or Tricarboxylic Cycle (TCA) is linked by mitochondrial derived pyruvate (from glucose) and beta-oxidation for lipid metabolism [33]. The common molecule from glycolysis and/or β-oxidation is acetyl-CoA. Type II fibers are fast twitch and are more diverse with several subtypes (IIa, IIb, and IIx). The predominate energy pathway for type II fibers is anaerobic glycolysis to which the end product of is lactic acid which lactate is derived from. The aerobic Type I fibers produces acetyl-CoA derived from both glucose/pyruvate (instead of lactic acid) and fatty acids from mitochondrial β-oxidation. The amount of Type I fibers is a determining factor (although not exclusive; e.g., oxygenated blood deliver and $O_2$ extraction) of an individual's aerobic capacity [14,44].

Muscle phenotype is strongly associated with obesity. A greater fiber ratio favoring Type I muscle fibers has been shown to be reduced in the obese population [26]. Tanner et al. examined body mass index (BMI) and muscle phenotype between lean and obese individuals. The lean

group had a greater composition of Type I oxidative muscle fiber than the obese group. Conversely, the obese group had a higher composition of Type II anaerobic muscle fiber, and a lower Type I fiber content. The study suggested that there is a positive relationship between BMI and Type II muscle fibers, but inversely with oxidative capacity at the whole body level. [26] Other studies have concluded adiposity has much to do with the amount of slow oxidative Type I fibers[45,46] as described briefly above. The increasing amount of Type II fibers leads to elevated blood lactate during respiration[10]. This is due to the end product of glycolysis, pyruvate, which is unable to enter the mitochondria without the presence of oxygen. Therefore, compromising aerobic glycolysis, pyruvate is oxidized by lactate dehydrogenase (LDH) and converted to lactate. Lactate then gets transported to the liver to engage in gluconeogenesis to make glucose as mentioned above. At the whole body and cellular level, the ability to utilize oxygen is to a large extent dependent on the amount of Type I fiber an individual possess[26,45,46]; this is metabolically related to greater content of mitochondria [43].

**Insulin Resistance/Type 2 Diabetes and Lactate**

Elevation of lactate in obesity and insulin resistance has been suggested in previous studies[10,47]. The inverse relationship was earlier established between insulin sensitivity and lactate production [10]. Skeletal muscle and adipose tissues are both receptive to insulin, and high lactate concentrations have been observed in diabetics. Accordingly, in order to utilize glucose for fuel, insulin is required in tissues such as skeletal muscle (expanded upon below). Insulin's function is to initiate aerobic glycolysis via stimulation of glucose uptake from the blood, followed by other regulatory proteins within the insulin signaling cascade [48]. After glucose entry, cells begin to perform glycolysis for catalyzing glucose molecules for production of energy. In the aerobic condition, pyruvate, as stated above, enters the mitochondria to initiate the Krebs

Cycle [38], also known as aerobic glycolysis. To expand, with the presence of oxygen, pyruvate bypasses lactate dehydrogenase (LDH), and enters the Krebs cycle to avoid lactate production. In the anaerobic condition, pyruvate undergoes an oxidation-reduction reaction resulting in the conversion to lactic acid [49].

Insulin is essential for the entry of glucose into the cell. Normally, insulin responds to the elevation of glucose, and amino acids[10]. Mechanistically, when glucose is present in the blood stream, it gets transported into pancreatic β-cells by the insulin independent GLUT 2 transporter protein. The transported glucose then gets phosphorylated and oxidized to generate ATP within the pancreas. The accumulation of ATP cause potassium channels to close and the β-cells to depolarize. The depolarization ensues via by an accumulation of intracellular calcium levels by the opening of the voltage sensitive calcium channels. The content of the insulin storing vesicles are then released to the blood stream via stimulated exocytosis following the increased level of intracellular calcium. Once the insulin is released into the blood stream, it binds to the cell surface receptors on sensitive tissues such as skeletal muscle and adipocytes. Thereafter, an insulin signaling cascade is stimulated to uptake glucose into the cell by facilitated diffusion. Glucose then would either be metabolized to ATP via glycolysis or stored as glycogen in the muscle[50]. In contrast, the hepatocytes (liver cell) are insulin independent with regards to glucose uptake[51]. When there is a positive energy balance (energy surplus), the liver can become resistant to insulin at the intracellular level, notably in the inability to restrict gluconeogenesis (production of new glucose) [52]. The clinical outcome is an exacerbated blood glucose content due to liver's increased glucose production from uncontrolled gluconeogenesis, resulting in a hyperglycemic state in the blood (primary outcome in the diabetic state). In response, the pancreatic β-cells secretes more insulin to mediate the elevated blood glucose. If the pancreas

become over-taxed to maintain normal blood glucose level, the body will reduce the ability of insulin to evoke the signaling cascade responsible for glucose uptake over. The prolonged excitation of pancreatic β-cells will sequence into hyperinsulinemia, the hallmark of the insulin resistant state and leading to an exacerbation of the Type 2 Diabetic condition (concomitant hyperglycemia and hyperinsulinemia)[18,53].

Insulin not only regulates blood glucose, but it also activates different kinases (JNK, PKC,mTOR, S6K, IKK, and ERK), and other enzyme cascades (endothelin-1, free fatty acids, cytokines, amino acids, and cellular stress) that would phosphorylate the insulin receptor and its substrates[48]. These kinases and other signaling cascades specifically target insulin receptor substrate-1 (IRS-1), which is associated with insulin resistance (i.e., insulin receptor tyrosine kinase activity and downstream signaling). With phosphorylation of specific serine residues on IRS-1, the body reduces the ability of insulin to evoke the signaling cascade responsible for glucose uptake. If not treated, insulin resistance would be induced to leading eventually to Type 2 Diabetes (hyperglycemia and "forced" hyperinsulinemia) [11].

As noted, hyperinsulinemia contributes to insulin resistant and potentially leads to Type 2 Diabetes. Fasting insulin level increase with obesity [54]. Evidence suggests the accumulation of intramuscular lipid may be part of the reason for provoked insulin resistance [53,55–57]. In addition, studies have shown an inverse relationship between plasma FFA and insulin sensitivity. Accordingly, an accumulation of intracellular FFA (so-called bioactive FFA such as diacylerol glycerol and ceramides) are associated with an inhibition of IRS-1[58]. These observations direct an alteration of insulin-activated glucose transport and subsequently phosphorylation of IRS-1 commonly found in the obese and individuals with Type 2 Diabetes [48]. For this reason, we were interested in looking at the blood analytes of insulin and glucose concentration in our non-obese

subjects to draw association with lactate. We investigated insulin and lactate concentration to potentially explain the association of lactate and REE.

To summarize, the pancreas accommodates the reduction in insulin stimulated glucose uptake by secreting more insulin to the blood, subject to the induced hyperglycemia. Over time, the pancreas becomes "worn out" and stops secreting appropriate amounts of insulin. The result is clinically presented as the development of Type 2 Diabetes [59]. In this regard, skeletal muscle is the largest insulin dependent tissue in the body by weight and hence responsible to clear ~85% of blood glucose following a meal[60]The unifying theory is that Type 2 Diabetes exhibits higher-levels of blood lactate[11]. We were interested in observing the glucose and insulin levels that correspond to the recruited subject's lactate level. Investigating REE (see immediately below) and its relations with lactate would better help to understand glucose/insulin action in subjects who may be predispose to metabolic diseases (clinically for the early detection of metabolic diseases such as obesity and Type 2 Diabetes).

**Resting Energy Expenditure**

Knowing the alteration of metabolism with obesity, it is essential to understand energy expenditure. As first described by the French chemist Antoine Laurent Lavoisier, the metabolic energy transformation involving oxygen consumption and carbon dioxide production can be used to determine energetic requirements. The muscle constantly synthesizes ATP, and the energy requirement at rest accounts for a great amount of the daily energy demand. The demand for ATP at rest is referred as the resting energy expenditure (REE), which is commonly measured by indirect calorimetry. Accumulated evidence suggests the existing lean mass is a major determining factor accounting for daily energy requirements.

Studies on energy production vary across obese populations [49,61]. There are conflicting data between REE and obesity. The obese was shown to have either a lower, or no difference in REE when adjusted to their fat free mass (FFM)[62]. The studies that showed no association between the two did not consider metabolic disease as a factor. Other studies however, do suggest a negative correlation between obesity and REE[61,63]. The difference of a lower REE could be an early sign of the development of metabolic diseases. Obesity is associated with an alteration in metabolic processes that correlate with the inability to oxidize fat in skeletal muscle, precipitating insulin resistance, and impaired metabolic flexibility [18,19,27,64], which can be explained in part by a lower REE. Obese individuals who showed no difference in lowered REE, may have yet to develop metabolic diseases, which demonstrated no variance in REE from disrupted energy production. Concurrent with these studies, blood lactate has become an interest that corresponds with metabolic dysregulation specifically reflective of necessitated enhancement of anaerobic respiration due to reduction in oxidative capacity of all substrates [10,11,46,61]. Blood lactate has been studied in conjunction with obesity and obesity related health conditions. However, REE and lactate have not been thoroughly investigated. The main purpose of this study is to test for the potential relationship between REE and blood lactate.

As stated, skeletal muscle tissue is a major determining factor in energy expenditure[37], due to the fact that it has a high turnover rate of ATP. Measuring resting energy expenditure (REE) is a useful assessment for weight control. As alluded to above, the REE contributes to 50-75% of total energy expenditure[65]. As stated before, indirect calorimetry is reputably accurate and it is an non-invasive method in retrieve REE and RER[66 67 68].

In some studies, REE has been shown to have a positive correlation with BMI between obese and non-obese[62]. While this duly noted, it's important to recognize that BMI does not

account for both fat mass (FM) and fat free mass (FFM) independently. According to Kleiber, basal energy expenditure corresponds to three quarters of the body weight[69]. Kleiber proposed that with an increase of the total adipose tissue, muscle mass would subsequently increase due to the energy demand for the excess fat. Therefore, REE would increase, displaying a positive correlation between the two. This could explain why in some studies REE has a positive association when adjusted to FFM[62]. In contrast, other studies have suggested that the increase in muscle mass to positive REE has its limitation[70,71], and that there was no association in adjusted REE in obese and non-obese. The reasoning is that the mass of excess adipose would eventually dilute the contributions from the lean mass. Both sets of data were conflicting. However, they were not specific among subject samples examined. Throughout these studies, metabolic activity of the FFM, such as oxidative capacity, was not considered. The conclusion from these studies is that strongly associates between BMI, and tissue composition may result in the human metabolic variation across obesity.

In the present study, we hypothesized that the REE would decrease as fasting lactate increases. Although lean tissue is the determinant factor in energy expenditure, it may vary depending on the type of lean tissue. FFM consists of bones, skeletal muscles, and smooth muscles. Each are individualized in their contributions to metabolic activity, with skeletal muscle being the greatest contributing tissue. Even within the skeletal muscle however, oxidative fibers affect energy production as discussed [37,72].

To expand, metabolism systematically varies across the levels of obesity. For example, Heymsfield et al. examined adjusted REE and FFM differently from previous studies. In their findings, magnetic resonance imaging (MRI) was used to supplement adjusted body composition obtained from DEXA[63]. MRI served to measure the thermogenesis within various fat free mass

(FFM) components. Four types of FFM were obtained in this study: 1) low-metabolic-rate fat-free adipose tissue (18.8 kJ/kg), 2) skeletal muscle (54.4 kJ/kg), 3) bone (9.6 kJ/kg), and 4) residual mass (225.9 kJ/kg). The thermogenesis value for the FFM was then combined with body composition to correlate with REE. The reported result of REE from this study was negatively correlated with adjusted FFM. This was a better representation of computing REE and FFM than the studies that were mentioned in the beginning. They accounted for metabolic activity in different regions of the FFM, which previous studies ignored.

In addition, other metabolic alterations may also affect REE. People who are exposed to insulin resistance and metabolic inflexibility present a lower REE. Adamska-Patruno et al. investigated the effects of postprandial REE and substrate utilization in normal and obese subjects [61]. When given high carbohydrate meals, glucose utilization corresponded accordingly to the amount of available substrate in both groups. Glucose utilization was higher when given high carbohydrate meals compared to normal carbohydrate meals. However, the normal group represented a higher level of glucose utilization and at a faster rate than the overweight/obese group. In contrast, fat oxidation level was respectively opposed to glucose oxidation. When the subjects were introduced to high fat meals, both groups showed an increase of fat oxidation, but the rate of fat oxidation were slower and lower in overweight/obese group than the normal group. With respect to REE in these studies, both groups showed an increase in postprandial REE, but it was notably lower in the obese/overweight than the normal group. The overweight/obese group had a lower fasting and postprandial REE than the normal group. The outcome of this study demonstrated that overweight/obese group had a slower time adapting to the available substrates, and a lower fasting and postprandial REE. The author described the obese/weight group as metabolically inflexible. Lower aerobic capacity may be an underlying factor for lower REE.

Thus, in the obese population, a lower REE is observed, perhaps related to the presence of metabolic disease. This may cause metabolic alterations in the cell which affect mitochondrial function. This can result in secondary effect to conversion of Type II muscle fiber. As stated, Type II muscle fibers are lactate producers due to the reliance on glycolytic anaerobic metabolism. We contend that a lower REE would be reflective of a higher lactic acid generation potential and a lower oxidative capacity [45].

**Metabolic Flexibility and Energy Expenditure**

Although not directly measured in the present research design, it is important for the reader to have an underlying concept of substrate switching in the normal physiological state. Metabolic flexibility is defined as the capacity by which the body can adapt fuel oxidation to match fuel availability. The two primary fuels that the body utilizes are carbohydrates (glucose) and fats (fatty acids). Cells metabolize both glucose and fatty acids for energy; flexibility becomes impaired when the body cannot shift between the two available substrates [16,18,73]. Research has shown that metabolic inflexibility is displayed in people who cannot adapt to the available substrates whether a predominance of carbohydrate or lipids [73]. These individuals also have an accelerated rate of hepatic gluconeogenesis to compensate for a lack of fat utilization [31]. This metabolic alteration is associated with elevated lactate compare to someone who can switch substrate utilization, or metabolically flexible. The etiology of metabolic inflexibility is still unknown; [74]however, obesity and insulin resistance are associated [18,73,75].To measure substrate ratio utilization of carbohydrate and lipid fuel sources, indirect calorimetry is considered to be one of the best methods at the whole body level to assess the cells ability to accommodate substrate flux due to its non-invasiveness and precise estimation of substrate oxidation [66,67]. The indirect calorimetry procedure (measurement of $VCO_2/VO_2$ at the mouth using carbon dioxide

and oxygen measurements with a metabolic cart) would compute the respiratory exchange rate (RER). RER indicates the amount of carbon dioxide being expired as the result of substrate oxidation in the mitochondria, divided by the amount of oxygen being consumed required for oxidative metabolism by the electron transport chain ($VCO_2/VO_2$)[19]. The RER serves as an indirect measurement on cellular substrate utilization.

There are many studies that implicate metabolic inflexibility in fuel oxidation associated with obesity and insulin resistance [8,15,76,77]. The origin and underlying mechanism of metabolic inflexibility is still speculative. It is unclear if one develops the condition before or after they've become obese. It is believed that most would ultimately degrade to some degree of metabolic inflexibility without intervention. One study found RER and oxidative capacity to be positively associated with fat mass in sedentary premenopausal women[78]. The subjects that were included in the study were overweight to Class 1 obesity. This suggested the mitochondria within those women were aerobically functional, and responded accordingly to the amount of fat they possessed despite being overweight or obese. It is important for us to study lactate as we rationalized elevated lactate is the result of lower oxidative capacity. The reduction in glucose uptake by the cell reduced oxidative phosphorylation is associated with metabolic inflexibility, which would eventually exacerbate the development obesity and diabetes. With regards to the present study, we indirectly emphasized another suspected mitigating factor leading to metabolic diseases; the reduction in glucose and lipid oxidation due to compromised mitochondrial capacity. The end result is that the cell is being forced to generate energy solely by anaerobic-glycolytic metabolism of carbohydrate, as manifested by plasma accumulation of lactate.

**Summary**

The purpose of this study is to determine if plasma lactate can be a predictive tool in measuring resting energy expenditure (REE). Due to many aspects of obesity leading to metabolic disease, it is speculated that plasma lactate may be indicative of a predisposition toward an inability to generate energy aerobically. The implied concomitant result is an elevation in lactate production as the end product of anaerobic metabolism that leads to elevations in plasma lactate concentrations. The hypothesis entertained currently is a reduced oxidative capacity leading to heightened anaerobic metabolism of carbohydrate due to the inability to oxidize carbohydrate or fat substrates.

# Chapter III: Methods

## Subjects

A total of 40 subjects were recruited from East Carolina University and Greenville area. All subjects were Caucasian non-obese females. Subjects' age ranged from 18 to 26, BMI ranged from 18.17- 25.9, and none were diagnosed with metabolic or heart disease. From the 41 subjects, the top (n=10) and bottom (n=11) of the 40 subjects pre-screened for resting blood lactate levels was selected for further testing. Each of the subjects were non-smokers, showed no physical impairment to exercise, non-pregnant, no known cardiovascular or metabolic diseases, and were not currently enrolled in a diet or supervised exercise program.

## Instrumentation

To screen for 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 ration. Dual-energy X-ray absorptiometry (DEXA; Horizon, Danbury, CT) scanner was used to analyze the subject's body composition. An Excalibur Sports cycle ergometer (Lode, Netherlands) was used for the modality of exercise and $VO_2$ max test. An OxiplexTS (ISS, Champaign, IL) Near Infrared Spectroscopy (NIRS)

instrument was used to determine the recovery rate of muscle $VO_2$ ($mVO_2$) saturation as an indirect measurement of oxidative capacity. Blood analysis was determined by using a hematology analyzer (Beckman Coulter, USA).

**Measurements Protocol**

Screening:

After subjects responded to posted advertisements, prescreening was conducted through the phone or email to determine if all the inclusion criteria were met. The qualified participants were then scheduled to come into the Human Performance Lab/FITT building at East Carolina University. Subjects were asked to arrive in a fasted state, which consisted of refraining from any exercise for three days, food, stimulants (caffeine, nicotine, stimulant medication, and etc.), and alcohol for at least 10-12 hours. Upon arrival, informed consent was obtained by IRB approved study staff (verbally and in writing) and signed by each subject prior to the experiment. Participants were also asked to complete the IRB approved personal family history form, a physical activity questionnaire, DEXA radiation consent form, and menstrual cycle questionnaire prior to REE measurement and $VO_2$ max test.

Upon subject's arrival, weight (kg) and height (m) was first obtained for BMI. Resting heart rate was recorded using a finger pulse oximeter, and blood pressure was assessed manually using a mobile sphygmomanometer and a stethoscope. Lactate was then obtained by using the Lactate meter for blood lactate assessment. Lactate Plus has been shown to provide reliable and accurate readings of blood lactate when compared to laboratory-based analyzers [79]. When ready, the third finger or the "ring" finger of the non-dominant hand was selected for the lactate sampling. The finger was rendered aseptic with a 70% isopropanol wipe and a sterile onetime

use lancet needle was used to penetrate the skin of the fingertip for venous blood exposure. Blood was retrieved with a lactate strip and inserted into the Lactate Plus for measurement. Blood lactate was analyzed twice to ensure lactate values were within 0.3 mmol/L. If there was a difference between the two readings that is greater than 0.3 mmol/L, an extra lactate measurement was obtained with a value within 0.3 mmol/L of the last measurement. In the case of lactate meter error readings, measurement was obtained again. Sterile gauze was used to wipe off any excessive blood on the finger, and a bandage was applied to cover up the wound upon the completion of lactate analysis.

First Visiting Day

*For the subject:* A copy of food diary was sent to the subject 7 days in advance to be completed prior in coming in on the scheduled day. Prior to arrival, subjects were instructed to remain in the fasted state for 10-12 hours. Subjects were encouraged to ceased eating after 10:00 PM the night before.

*During the visit:* On this day, subjects' body composition, REE, blood draw, and their $VO_2$ max was assessed. Upon arrival, subjects' resting heart rate was recorded using a Baseline Fingertip pulse oximeter, and blood pressure was measured manually using a sphygmomanometer and a stethoscope. Dual-energy X-ray absorptiometry (Horizon, Danbury, CT) or DEXA scan was performed first to assess subject's body composition.

Body composition was assessed using the DEXA scanner. In preparation for the scan, the study staff reviewed for safety precautions such as no metallic jewelry, and with minimal chance of pregnancy. The subjects were then correctly aligned on the bed of the DEXA scanner. The subjects were aligned to lay with their head on the right end of the bed, and their feet on the left

end of the bed. A Velcro strap was used to wrap the subjects' feet together. The limbs of the subject were checked to ensure they were within the marked parameter of the bed. The scan started after confirmation of the subjects' correct positioning; the subjects were instructed to stay still and minimize any movement. The scan lasted approximately two minutes and 30 seconds for all participants. After the scan was completed, the results were acquired.

Upon completion of the DEXA scan, a REE test was performed. During the REE assessment, subjects were seated for 20-30 minutes in a quiet room where the assessment took place. The environment of the testing room was temperature-controlled at around 70 °F (21 °C). A desk lamp was dimmed for the room to provide a relaxed setting to help the subject to adjust to the resting state. The subjects were reminded to stay awake and minimize movement before initiating the REE assessment. Once the subject rested for 20-30 minutes, an open circuit canopy hood was equipped for REE measurement via indirect calorimetry for a 20-minute measurement. $CO_2$ levels were adjusted in the canopy and stabilized as close to 1.0% as possible. Throughout the REE measurement, the subject was left alone in the testing room, and was periodically monitored every 5 minutes through an open door. $CO_2$ levels through the canopy were also monitored closely and adjusted if it necessary through that 5-minute check-up. The test was terminated after 20 minutes of successful measurement. Equipment was detached from the subject, and the REE test results were acquired from the metabolic cart.

Upon completing the REE assessment, a $VO_2$ max test was performed via a cycle graded exercise protocol. Indirect calorimetry technique was used to obtain aerobic capacity and exercise RER. Before the $VO_2$ max test, procedures were explained to the participants, and the seat was adjusted for subjects' comfort. A mouthpiece and a headgear were fitted to subject's comfort. A Polar Electro heart monitor was equipped to the subject for recording of their heart

rate, and was manually recorded by hand. Subjects resting heart rate and blood pressure were also measured. Before the start of the test, subjects were encouraged to exercise as long as they can. At the start of the test, a baseline of gas exchange was established on the metabolic cart. The workload on the bike was preprogrammed to automatically enter each stage. The participant was informed to pedal at a comfortable frequency, or at a frequency between 50-70 revolutions per minute. A 10 second warning was given to the participant before entering a new stage. At the end of each stage, RPE and heart rate was recorded. Blood pressure was recorded at the end of the first stage and then every other stage. Subjects progressed through each stage until maximum effort was achieved, or subjects fell below 40 revolutions per minute on the cycle ergometer. All subjects reached a max status by meeting at least 2 of the following criteria: 1) RER ratio of 1.10, 2) RPE above 17 on the Borg scale, and 3) peak heart rate within +/- 5% of max heart rate (using age predicted formula, 220-Age). Upon successful completion of the test, heart rate and blood pressure was recorded immediately, as the subject entered the active recovery phase. During active recovery, the load of the cycle was set to zero watts, and was adjusted to the comfort of the subject if needed. Heart rate was recorded every minute, and blood pressure was recorded every 2 minutes to monitor normal hemodynamics response. The test was terminated when a normal hemodynamic response was observed, and subject's heart rate and blood is close to the resting state.

LODE Bike $VO_2$ Max Test Protocol (Leg Cycle Ergometer)

| **Minutes** | **Stages** | **Work Load (Watts)** |
|---|---|---|
| 1 | 1 | 30 |
| 2 | | 30 |
| 3 | 2 | 60 |
| 4 | | 60 |
| 5 | 3 | 90 |
| 6 | | 90 |
| 7 | 4 | 120 |
| 8 | | 120 |
| 9 | 5 | 150 |
| 10 | | 150 |
| 11 | 6 | 180 |
| 12 | | 180 |
| 13 | 7 | 210 |
| 14 | | 210 |
| 15 | 8 | 240 |
| 16 | | 240 |
| 17 | 9 | 270 |
| 18 | | 270 |
| 19 | 10 | 300 |
| 20 | | 300 |

Second Visiting Day

*For the experimenter:* A metabolic cart was turned on, and calibrated for at least 30 minutes prior to subject's arrival. The computer and the OxiplexTS were turned on for at least 20 minutes before taking any measurements. During this time, the 2 independent data acquisition channels (probe) equipped with the OxiplexTS was calibrated. The 15-gallon air compressor (Model D55168, Dewalt, Baltimore, MD) was set to 30 psi to control the blood pressure cuff. Polar Electro heart rate sensor/watch, baseline fingertip pulse oximeter, sphygmomanometer, and a stethoscope were gathered in place for heart rate, and blood pressure measurements.

*For the subjects:* All test subjects were reminded to be in a fasted state for at least 10-12 hours, and refrain from any physical activity for at least 48 hours. Subjects were also reminded to eliminate stimulants for 12 hours, and alcohol for 24 hours before the second visiting day. The subject was directed to wear comfortable athletic wear.

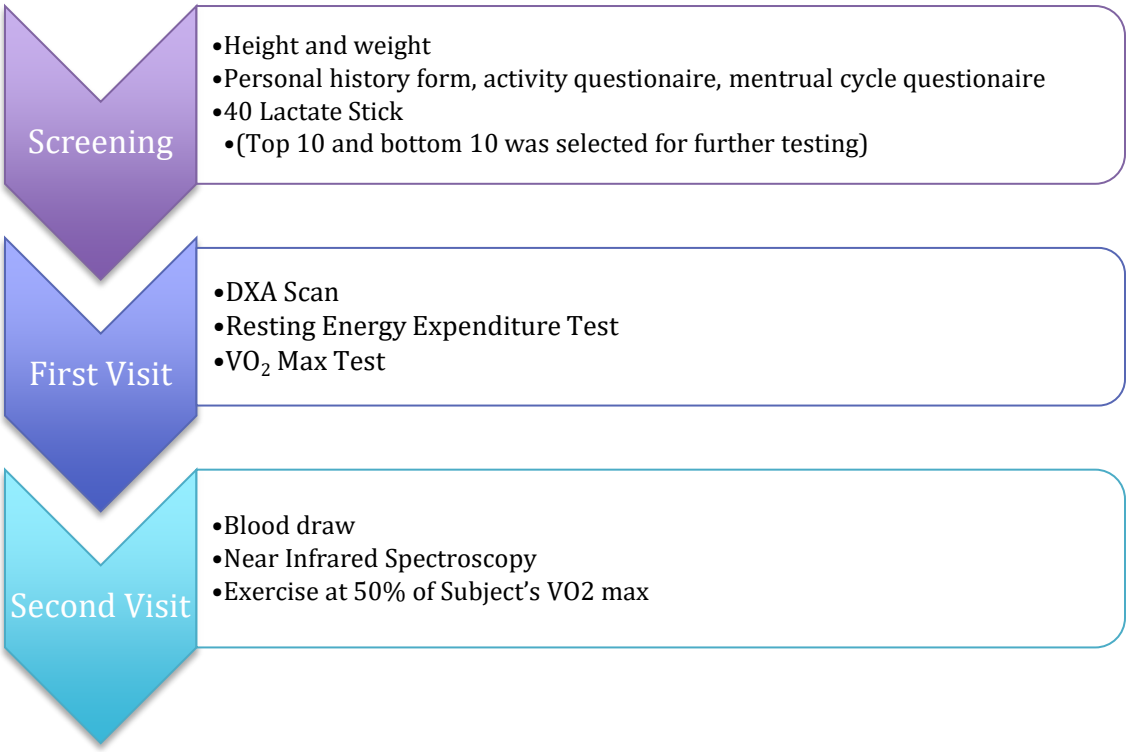
*During the visit:* On day two of testing day, blood draw, near infrared spectroscopy (NIRS) measurement and a submaximal exercise session were conducted. A total of three 5 mL (Mel or approximately 3 teaspoons) of blood sample were collected via a venipuncture and sterile butterfly by one of the research staff. To prevent cross contamination, 5 mL of the "red top" evacuated collection tube was attached to draw the blood first. Then, a 5 mL blood sample of "purple top" evacuated collection tube, and a 5 mL blood sample of "purple top" with DPPIV inhibitors evacuated collection tube was collected. After each tube is filled with blood, the samples were immediately spun down in a centrifuge, and each was aliquoted into two cryovials. The purple top without the DPPIV inhibitor blood sample was analyzed using a hematology analyzer (Beckman-Coulter, USA) for fasting glucose, insulin, and lactate. The red top and purple top with DPPIV inhibitor was stored appropriately in an -80° C freezer for future analysis.

The NIRS measurement followed the blood draw. The NIRS protocol is a non-invasive method of assessing mitochondrial respiratory capacity at the whole body level. The NIRS probes consisted of 8 infrared diode lasers (four emitting at 691 nm and four at 830 nm) and a detector within each (emitter-detector distances 2.0-4.0 cm) was secured to the vastus lateralis belly of the dominant leg using a double-sided adhesive tape and Velcro straps. To prep for the NIRS assessment, a skinfold caliper (Lange, Beta Technology, Santa Cruz, CA) was used to measure subcutaneous adipose tissue thickness at the probe site (~10 cm above the patella). This was used to determine which probe to use as each probe is set to measure $mVO_2$ dependent on the subcutaneous fat. Probe B was used for individuals with skinfold of 30 mm or less, and probe A was used for individuals with skinfold greater than 30 mm. For each NIRS measurement, participants were positioned supine on a medical examination table with both legs fully extended. After the correct probe was selected, blood occlusion was then initiated. A blood pressure cuff was placed as high as anatomically possible, and ensured the NIRS probe is outside contact of the cuff after inflation. A 15-gallon air compressor was set to 30 psi and was used to control the blood pressure cuff. Upon securing the probe, three 5-second practice occlusions were done to ensure subjects were familiar with the pressure around their leg, and making sure there was no pinching on the participants' leg. The first arterial occlusion was the baseline measurement, where participants completed a 60 second duration of occlusion. Subjects were rested for 2-3 minutes before the second occlusion. After resting, isometric contraction of the quadriceps was performed for 15-20 seconds. Following isocontraction, the cuff was inflated for the second arterial occlusion (300 mmHg) for 5 minutes to determine the total deoxygenated hemoglobin (deoxy Hb). The collected data was then processed, and the rate of deoxy Hb was determined using the oxygen kinetics obtained from the one-minute occlusion divided by the

total deoxy Hb oxygen kinetics obtained from the 5-minute. Percent-deoxy Hb was then calculated by taking rate of deoxy Hb dividing the total deoxy Hb (umol/minute).

The submaximal exercise portion of the experiment was followed upon completion of NIRS measurement. The submaximal exercise test was conducted on the Excalibur Sport cycle ergometer for 20 minutes. The intensity of the exercise was set to 50% of their $VO_2$ max obtained from the 1st visit, and exercise RER was collected via indirect calorimetry. In preparing for the submaximal exercise test, subjects were equipped with Polar Electro heart rate sensor and Polar Electro watch to assess their heart rate. The cycle ergometer was adjusted to the participants' comfort with headgear and the mouthpiece secured. The metabolic cart assessed for $VO_2$ baseline, while the subject was instructed to cycle at a workload that matched their 50% of their $VO_2$ peak. Adjusting workload for the participants took approximately 2-3 minutes. Subjects were instructed to pedal at a comfortable frequency, and that was 50-70 RPM range to maintain consistency. Subjects cycled for 20-minute in duration while maintaining 50% of their $VO_2$ max. Exercise heart rate was recorded at the end of every minute. A baseline fingertip pulse oximeter was used as backup for assessing heart rate. Throughout the exercise, subject was monitored to ensure the participant is exercising at the given rate. Upon completion of the test, the load of the cycle was lowered to the comfort of the participant. Hemodynamics was monitored to ensure subject's safety. After the submaximal exercise test, data was acquired from the metabolic cart for subject records and stored in a locked file cabinet located in a secure room for HIPPA compliance.

Experimental Overview




**Data Processing**

BMI was calculated by dividing the subject's weight over height squared ($kg.m^2$). Out of the 40 subjects of the screening process, only the top (n=10) and bottom (n=11) quarter from the lactate spread was selected for further testing. Subjects' exercise energy expenditure, and both resting and exercise RER from indirect calorimetry was collected from the metabolic cart. The DEXA scanner provide body composition which consisted of Fat mass (FM) and Fat Free Mass (FFM). FFM from the DEXA was used to adjust participant's REE in kcal/kg of FFM/day. Blood samples were collected in the order of red top, purple top, and purple top with DPPIV inhibitors. Beckman-Coulter hematology analyzer was used to determine the concentration of fasting insulin, glucose, and lactate then processed Blood samples. The oxygen utilization was obtained from the NIRS analysis and was processed manually using Microsoft Excel.

**Statistical Analysis**

To determine the relationship between lactate and REE (adjusted to their lean mass), Pearson product-moment correlation was used to measure the strength of the association between the variables of plasma lactate and REE in High and Low Lactate group. Furthermore, BMI, REE (adjusted to lean mass), resting RER, exercise RER, $mVO_2$ data was analyzed individually with plasma lactate for strength of association using the Pearson Product-moment correlation. In addition, an unpaired two sample T-test was used to analyze the high and low plasma lactate group for REE (adjusted to lean mass), resting RER, exercise RER, $mVO_2$, fasting insulin and fasting glucose to determine the level of statistical significance. The statistical significance was set *a priori* at a probability level of $P < 0.05$.

# Chapter IV: Results

## Subjects

All subjects (n=40) recruited were non-obese, with no known cardiovascular or metabolic diseases. Subjects with higher (n=10) and lower (n=11) blood lactate concentration were categorized as High and Low Lactate groups respectively, and were further studied. Both High and Low Lactate groups were similar in age (22.5±0.68, 21.21±0.58 year), height (1.656±0.02, 1.82±0.02 m), weight (60.05±2.06, 64.37±1.30 kg), BMI (21.91±0.69, 22.82±0.62 kg/$m^2$), total fat mass (19.65±1.54, 21.25±0.99 kg), and total fat free mass (41.13±1.15, 43.69±1.203 kg) respectively. However, relative body fat percentage was significantly higher in the Low Lactate Group ($P<0.05$). Laboratory blood characteristics of lactate (0.8578±0.0721, 0.7391±0.1478 mmol/L), insulin (4.271±0.5162, 5.167±0.5605 uIU/mL) glucose (90.77±1.351, 88.26±1.335), and HOMA-IR (0.9505±0.1094, 1.135±0.1293) were also respectively similar between High and Low Lactate groups.

**Table 1. Participant's Characteristics**

| | Low Lactate Group (n=11) | High Lactate Group (n=10) | P-value |
|---|---|---|---|
| **Age (year)** | 21.21 ± 0.58 | 22.5 ± 0.68 | |
| **Height (m)** | 1.682 ± 0.02 | 1.656 ± 0.02 | |
| **Weight (kg)** | 64.37 ± 1.30 | 60.05 ± 2.06 | |
| **BMI (kg/m$^2$)** | 22.82 ± 0.62 | 21.91 ± 0.69 | |
| **Lactate Conc. (mmol/L)** | 0.52 ±0.02 | 1.20 ±0.11 | |
| **Body Fat (%)** | 32.72±1.27 | 28.92±1.22 | *0.0442 [a] |
| **Total Fat Mass (kg)** | 21.25±0.99 | 19.65±1.54 | 0.2512 [a] |
| **Total Fat Free Mass (kg)** | 43.69±1.203 | 41.13±1.15 | 0.1971 [a] |
| **REE (kcal)** | 1294±52.52 | 1290±28.47 | 0.9725 [a] |
| **Adjusted REE (kcal/lean mass)** | 29.66±1.013 | 31.49±0.80 | 0.2512 [a]<br>*0.0429 [c] |
| **Resting RER ($VO_2/VCO_2$)** | 0.8182±0.02 | 0.831±0.18 | 0.6181 [a]<br>0.0888 [b]<br>0.0511 [c] |
| **Exercise RER ($VO_2/VCO_2$)** | 0.8003±0.02 | 0.8317±0.02 | 0.2041 [a]<br>*<0.0001 [b] |
| **Absolute $VO_2$ (L/min)** | 1.99± 0.09 | 1.844 ± 0.10 | 0.2899 [a]<br>0.1961 [c] |
| **Relative $VO_2$ max (mL/kg/min)** | 30.83±1.23 | 27.7±1.75 | 0.1540 [a]<br>0.1169 [c] |
| **Adjusted Relative $VO_2$ max (mL/kg lean mass/min)** | 67.55± 3.35 | 44.76± 2.02 | *<0.0001 [a]<br>* 0.0002 [d] |
| **NIRS Aerobic Capacity (%)** | 35.38 ±4.20 | 27.69±3.53 | 0.1815 [a]<br>0.3891 [c] |
| **ΔRER** | | | *0.0466 [c] |

Data are expressed as mean ± SEM for Low Lactate Group (n=11), High Lactate Group (n=10); age, height, weight, BMI (Body Mass Index), lactate concentration, body fat percentage, total fat mass, total lean mass, resting energy expenditure (REE), adjusted REE, resting respiratory

exchange ratio (RER), Absolute $VO_2$ max, relative $VO_2$ max (mL/kg/min), adjusted Relative $VO_2$ max (mL/kg lean mass/min). * Significance $P<0.05$.

[a] Unpaired t-test between groups

[b] 2-way ANOVA

[c] regression for the High Lactate group

[d] regression for both Group

**Table 2. Blood Characteristics**

| | Low Group | High Group | P-value |
|---|---|---|---|
| **Lactate (mmol/L)** | 0.7391±0.1478 | 0.8578±0.0721 | 0.1628 [a] |
| **Insulin (uIU/mL)** | 5.167±0.5605 | 4.271±0.5162 | 0.2637 [a] |
| **Glucose mg/dL** | 88.26±1.335 | 90.77±1.351 | 0.2072 [a] |
| **HOMA-IR** | 1.135±0.1293 | 0.9505±0.1094 | 0.3021 [a] |

Data are express as mean ±SEM for Low (n=11) and High (n=9) Lactate Group for Lactate, insulin, glucose, and HOMA-IR.

[a] Unpaired two sample t-test

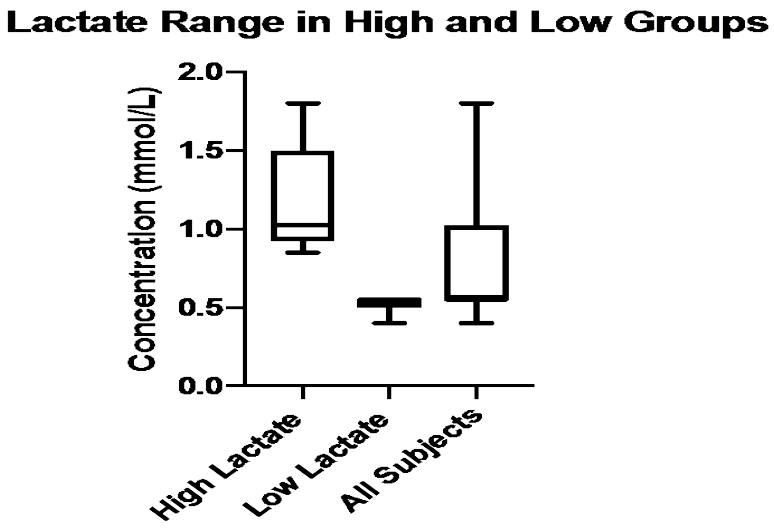



**Figure 1. Lactate Concentration (mmol/L).** Data is presented with number of participants, and a 5 number summary of each group as minimum, Q1, median, Q3, and maximum for: High (n=10, min=0.85, Q1=0.925, med=1.25, Q3=1.5, max=1.8, Spread=0.95 mmol/L), Low (n=11, min=0.40, Q1=0.50, med=0.55, Q3=0.55, max=0.55, Spread=0.15 mmol/L), Overall (n=40, min=0.40, Q1=0.55, med=0.55, Q3=1.025, max=1.80, spread=0.95 mmol/L)

**Lactate and Body Composition**

Lactate and BMI was compared between the High and Low Lactate group (Fig. 1). The correlation coefficient was reported to be r = 0.01035 for the High Lactate Group ($r^2$=0.0001), and r =-0.2288 for the Low Lactate Group ($r^2$=0.05237).

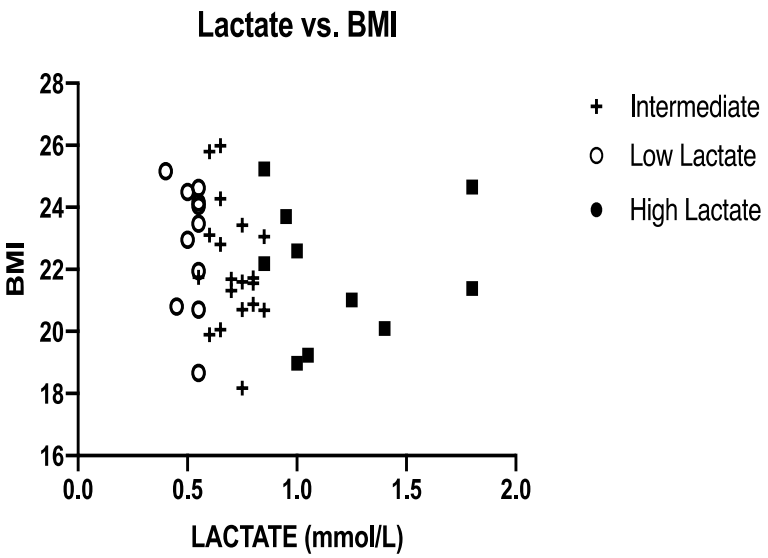



**Figure 2. Lactate and Body Mass Index (BMI).**

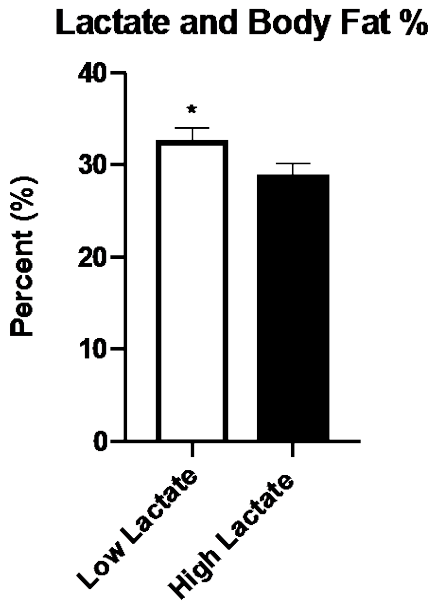



**Figure 3. Body fat percentage (BF%)**
* Significance P<0.05

Body composition was analyzed in both High and Low Lactate Group by using the DEXA scanner (Fig. 3). BF% was compared between groups by using a two sample unpaired t-

test was performed. There was a significant difference between High and Low Lactate Group (p=0.0442) with alpha set at 0.05.

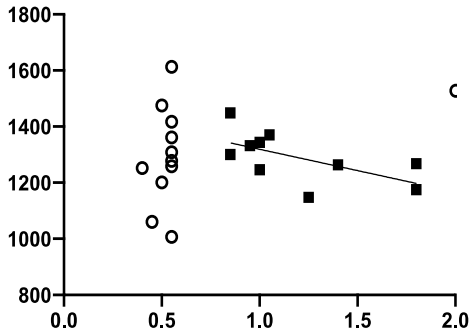



**Figure 4. Lactate and REE.**

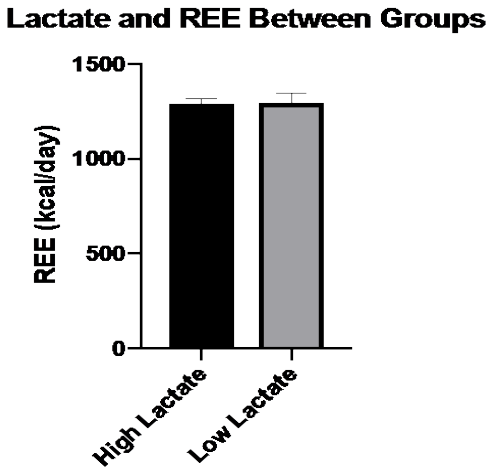



**Figure 5. Lactate and REE.**

**Resting Energy Expenditure and Lactate**

REE of High (n-10) and Low (n=11) Lactate group were measured for 20 minutes over a ventilated canopy (Fig. 4). The data $VO_2$ was obtained with less than 10% coefficient of variance

in all participants in computing REE. REE was then compared to lactate concentration using Pearson Product Moment Correlational Analyses (Fig. 4). The subjects from the High Lactate Group (□=9) were shown to have R=-0.6093, and $r^2$=0.3712. The Low Lactate Group (n=11) with R=0.2822, and $r^2$=0.07963. Linear regression line was fitted in High Lactate Groups. No significance was found in Low (P=0.4005), High (P=0.06015) Lactate groups. No significant difference was found when REE was compared using unpaired two sample t-test.

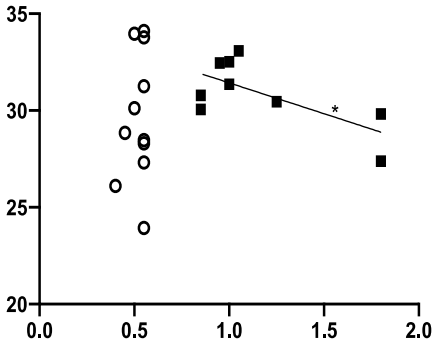



**Figure 6. Lactate and Adjusted REE.** REE was adjusted to the subject's lean body mass represented as mL/kg of FFM/min.

* Significance P<0.05

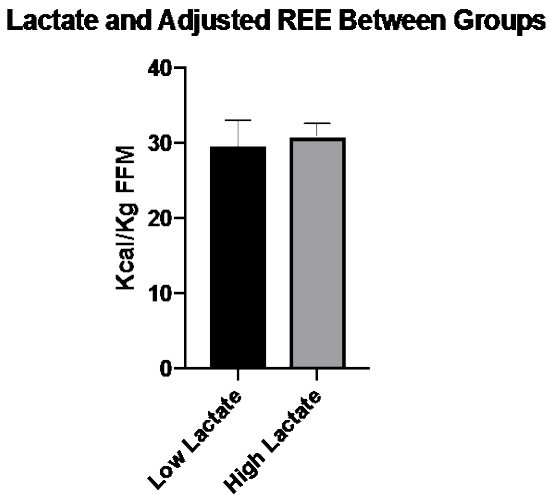



**Figure 7. Lactate and REE.**

REE of High (n=9) and Low (n=11) Lactate group were adjusted to the participant's FFM (Fig.6). One outlier was detected 2 standard deviations above the mean in the High Lactate Group, and was excluded from the analysis. Pearson Product Moment Analysis, and a line of regression were used to determine the association and significance between lactate and adjusted REE. REE adjusted for FFM demonstrated a strong association in High ($r^2$=0.4656) Lactate group, with significance (P=0.0429). No significant difference found in adjusted REE between groups using unpaired two sample-unpaired t-test (Fig. 7).

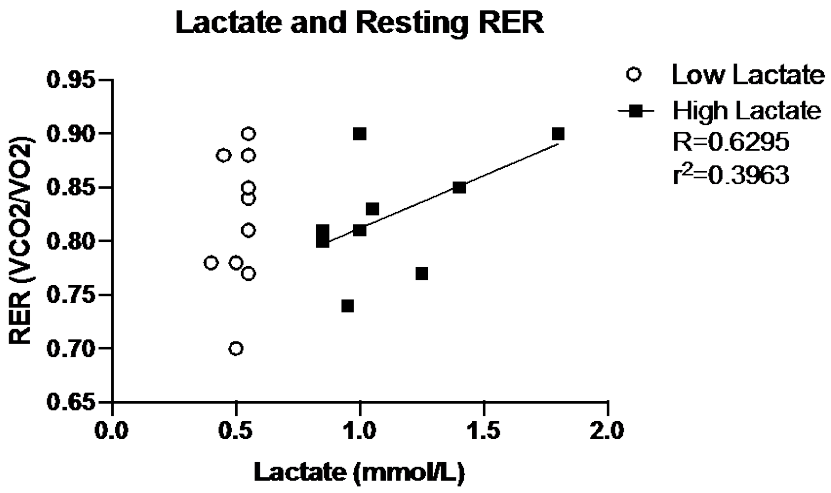



Figure 8. Lactate and Resting Respiratory Exchange Ratio (RER)

**Resting Respiratory Exchange Ratio (RER)**

Resting RER was measured concurrently with the REE assessment (Fig. 8). Using Pearson Product and regression analysis, High Lactate Group demonstrated a strong correlation coefficient of correlation with R=0.6295, and $r^2$=0.3963 (P=0.05).

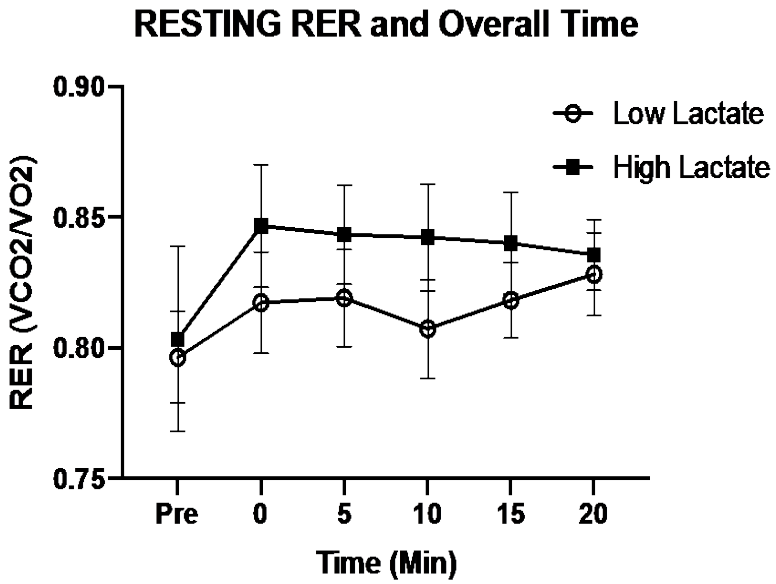



**Figure 9. Resting RER and Overall time.** Time points are represented as mean ± SEM within each time points

**Respiratory Exchange Ratio Overall Time**

Resting RER of High (n=9) and Low (n=11) Lactate group was ploted across time as represented (Figure 9). Resting RER is the same data set from acquired from resting REE. Measurements of resting RER for one subject were lost in the High Lactate Group. Resting RER data collection depicts a PRE phase, minute 0, 5, 10, 15 and 20. The PRE stage depicts subjects being acclimated before the 20 minute measurement. Resting RER at minute 0 to 20 represents the average RER of the given group after reaching steady. A two-way ANOVA was used to compare the potentia for significant differences of RER between groups at each time point, RER of both groups at each time point, and RER within the group at each time point. Resting RER showed no signifigant difference between groups, or between time.

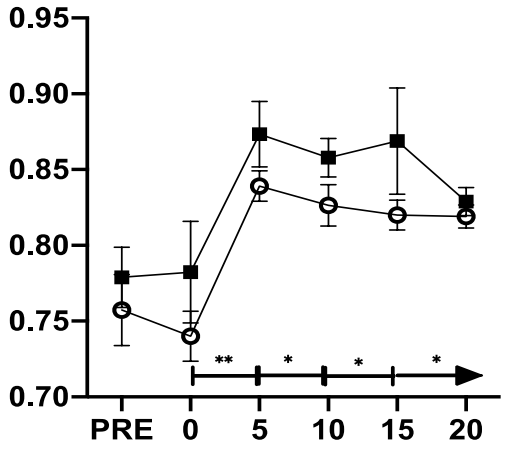



**Figure 10. Exercise RER and Overall time.** Time points are represented as mean ± SEM within each time points. * Significance P<0.05

Exercise RER of High (n=10) and Low (n=11) Lactate group was ploted across time as represented in Figure. 10. All subjects' exercise RER was determined by 20 minute low intensity (50% of $VO_2$ max) on the cycle ergometer. For exercise RER, minute 0 to 20 represents to the average RER of the given group after reaching 50% of $VO_2$ max for submaximal exercise to the end of minute 20. A two-way ANOVA was used to compare the potentia for significant differences of RER between groups at each time point, RER of both groups at each time point, and RER within the group at each time point. There was no significant difference in exercise RER between High and Low group factor, or the group by time factor. Exercise RER for the Low Lactate Group was significantly higher from beginning of measurement at minute 0 to 5 ($p=<0.0049$), minute 0 to 10 ($p=<0.0257$), minute 0 to 15 ($p=<0.0190$), and minute 0 to 20 minute($p=<0.0137$).

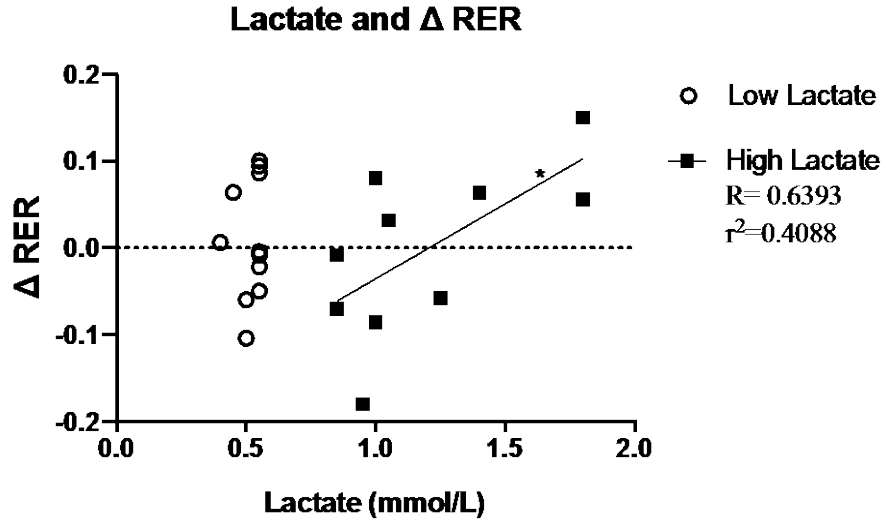



**Figure 11. Lactate and Change of RER.** RER was compared from resting to submaximal exercise

* Significance P<0.05

The change of RER from resting to submaximal exercise ΔRER of High (n=10) and Low(n=11) Lactate group was analyzed (Fig. 11). Pearson Product showed a correlation of R=0.6393, and $r^2$=0.4088 (P=0.0466) in the High lactate Group.

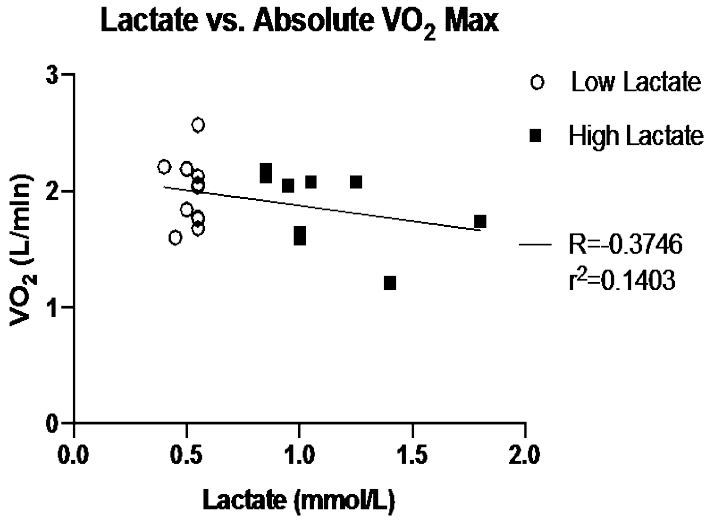



**Figure 12. Lactate and Absolute $VO_2$ Max**

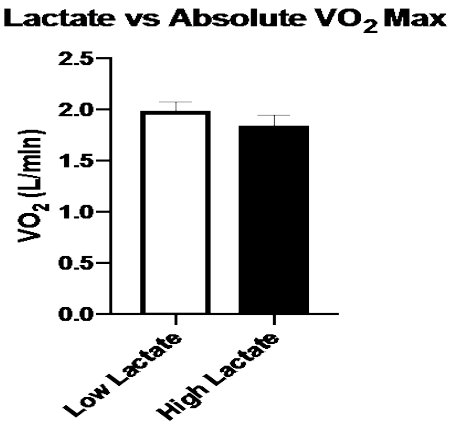



**Figure 13. Absolute $VO_2$ Max Comparison Between Groups (mean ± SEM)**

**Lactate and $VO_2$ Max**

Absolute $VO_2$ max of High (n=10) and Low (n=11) Lactate groups were determined using a Lode Excalibur bike via indirect calorimetry (Fig. 12). Pearson Product-Moment correlation coefficient was reported be to R=-0.3746 (Fig. 9) with both groups. No significance was found when a linear regression line was plotted. No significant difference was found in $VO_2$ max between groups using a unpaired two sample unpaired t-test (Fig. 13).

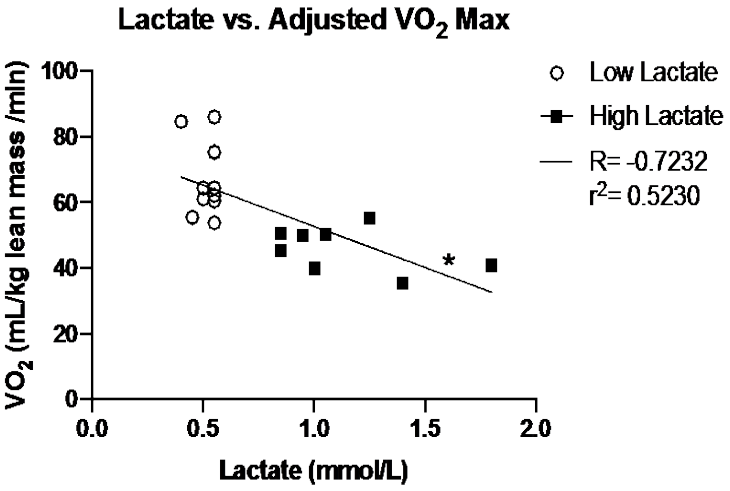



**Figure 14. Lactate and Adjusted Relative $VO_2$ peak** * Significance P<0.05

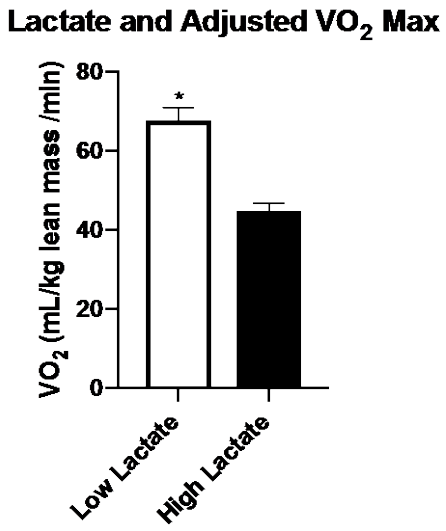



**Figure 15. Adjusted Relative $VO_2$ Max Between Groups (mean ± SEM).** * Significance P<0.05

$VO_2$ max adjusted for FFM of High (n=10) and Low (n=11) Lactate groups of was plotted (Fig. 14). Pearson Product-Moment correlation coefficient was reported to be R=-0.7232, $r^2$=0.523, linear regression line was plotted (p=0.0002) (Fig. 14). Low Lactate Group showed a significant high $VO_2$ max adjusted for FFM using two sample unpaired t-test (p=0.0001) (Fig. 15).

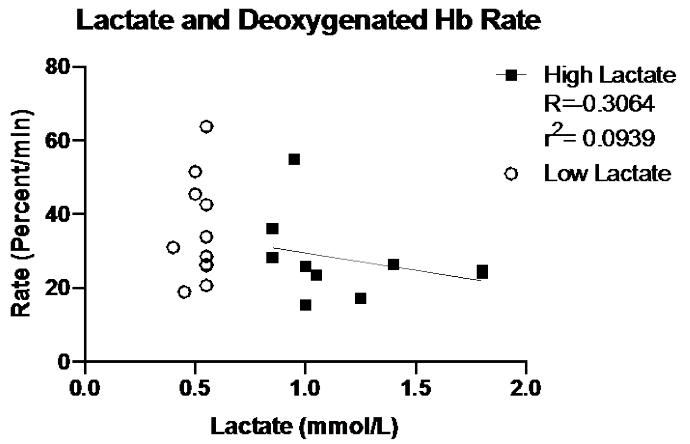



**Figure 16. Lactate and Deoxygenated Hemoglobin Rate**

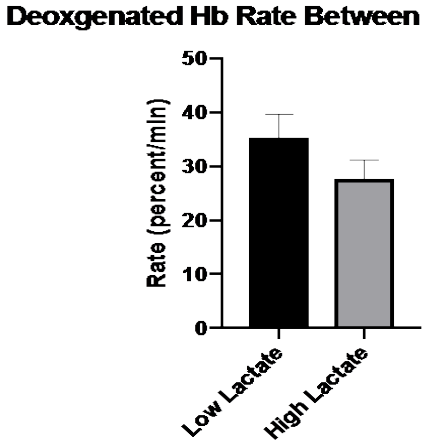



**Figure 17. Deoxygenated Hemoglobin Rate**

**Comparison Between Groups (mean ± SEM)**

**Lactate and Deoxygenated Hemoglobin Rate**

Deoxygenated hemoglobin percent (deoxy Hb %) of High (n=10) and Low (n=11) Lactate group was analyzed using near infrared spectroscopy (NIRS) instrument (Fig. 16). The coefficient of correlation for the Low Lactate Group was determined to be R=0.1366 with $r^2$= 0.0187, and the High Lactate Group reported R= 0.3064, with $r^2$=0.0939. No significant difference was found using a two-sample unpaired t-test (P=0.1815).

## Chapter V: Discussion

In our study, lactate was shown to have some association with REE in non-obese Caucasian females in the High Lactate Group. While not significant, subjects with high basal plasma lactate level showed a negative correlation with REE. This relationship became stronger when REE was adjusted FFM. Similarly, lactate and $VO_2$ max adjusted for FFM was strongly associated. Subjects in the High Lactate Group displayed a positive relationship in ΔRER from resting to submaximal exercise. The inability for higher lactate individuals to match the same substrate as the lower lactate is an indicative of lower oxidative capacity.

There is no universally accepted value for basal lactate, but most literature reports levels as “normal” to be in the 0.5-1.0 mmol/L range. These individuals with "normal" lactate concentration present with the least morbidity for all diseases [9,11,80]. People with obesity and/or Type 2 Diabetes display a wide range of basal lactate levels ranging from 4.0-10 mmol/L [11]. While those diseases are multifactorial, we believe as one deviates further from the “normal” range for blood lactate, the lower daily REE would become, thus possibly contributing to obesity and/or Type 2 Diabetes. The first finding in the present study suggests overall there was no association with lactate and REE. When looking at the Low and High Lactate groups individually, the Low Lactate Group showed no correlation between lactate concentration and REE. The cut off for lactate concentration was a predetermined limitation to our design in the Low Lactate Group. It was unexpected that the lower spread was as narrow as 0.40 to 0.55 mmol/L, along with a high variability of REE. As a result, the data range was too small to be included for an overall meaningful analysis. Conversely, the High Lactate Group had a wider lactate range from 0.85-1.80 mmol/L, which allowed for a correlation to be plotted. The High Lactate Group showed some correlation with REE (R= - 0.6093) (Fig. 4). When a regression line

was plotted for the High lactate Group alone, no statistical significance was obtained (P=0.06015). In this context, it is important to note the characteristics of subjects were similar in terms BMI, physical activity level, along with no known cardiac or metabolic diseases. Despite the similarities, we were still able to see some association with lactate and REE.

In the obese, absolute REE is commonly reported to be higher than the leans [62]. In our study, we were also interested to see if energy expenditure was different when REE is adjusted for FFM. Body composition was first analyzed between the two groups; no significance was found in either fat mass or fat free mass. We hypothesized the High Lactate Group would have a higher body fat percentage if there was going to be a difference in body composition. Interestingly, our study showed the Low Lactate Group had a significantly higher body fat percentage than the High Lactate Group (P=0.0442). Even though, we found significant difference in body fat percentage between groups, our subjects were non-obese and not different by a larger margin. We speculate that lifestyle factors such as diet and level of physical activity may have played a role in different body fat percentage between two groups. Upon analysis of REE adjusted for FFM, one outlier was detected, and no significant difference was found in adjusted REE between groups. When the correlation was plotted, adjusted REE for the High Lactate Group showed a negative correlation coefficient of R= - 0.6823 (Fig. 6) with statistical significance ($r^2$=0.4656, P=0.0429). REE adjusted for FFM had a stronger association with lactate than comparing just REE alone.

While relationships were shown between blood lactate and REE within the High Lactate Group, it's important to take note of the REE within the Low Lactate Group. Given the Low Lactate Group had a smaller range of lactate concentration; REE was highly variable (Fig. 4 and Fig. 6). This indicates that some individuals with lower lactate can also have a lower REE, and

blood lactate may not be a good predictor of resting expenditure. Within our results, High Lactate suggests that measurements of blood lactate could potentially used to predict lactate, while correlation of Low lactate groups is indicating that lactate is not associated with REE. Based on these data, it's suggested that blood lactate measurement is less sensitive in predicting REE in the Low Lactate Group, and becomes more reliable as lactate increases, as shown in the High Lactate Group.

We believe that testing all 40 subjects would have help us draw a better conclusion between lactate and REE. Due to the time restraint and our limited budget, were unable to test all 40 subjects. Instead, we thought selecting the higher and the lower lactate subjects would provide us with distinct difference in REE, but we know this is not the case within healthy non-obese subjects. In retrospect, we believe by including the middle 19 untested subjects for further testing would help us clarify if blood lactate could be a good overall predictor of REE. We acknowledged that excluding the Low Lactate Group in part of our analysis couldn't fully justify the overall association of blood lactate and REE. However, the inverse relationship between blood lactate and REE within the High Lactate Group should also be acknowledged.

In previous studies, association was diminished when REE was adjusted to FFM. These studies implied energy expended adjusted to FFM was equally matched between both the leans and the obese. Even though our finding of REE adjusted for FFM is different in comparison, our result does not necessarily conflict with previous studies [81–83]. One factor that may have been previously overlooked within those studies was not considering aerobic capacity when examining REE. Aerobic capacity is directly associated with cellular oxidative capacity, which may impact REE, as shown in the High Lactate Group. Thus, we also investigated aerobic capacity along with REE which may explained the dissimilarities. In our result, aerobic capacity

adjusted to FFM was significantly higher in the Low Lactate Group (P=0.0001). In addition, lactate was shown to have an inverse relationship with aerobic capacity and adjusted REE (Fig. 6 and 14). As plasma lactate level elevates, REE was lowered as a consequence of lower aerobic capacity. In support of our findings, Elbert et al. investigated energy expenditure and physical activity patterns between normal/overweight groups and different categories of obese groups, which was consistent with our data [84]. In their experiment, adjusted activity thermogenesis and adjusted REE was observed to be significantly lowered as obesity progressed. Accordingly, we believe lactate levels are indication reflecting aerobic capacity, which possibility could, contributes to a lower rate of REE, at least in the High Lactate group.

Aerobic capacity is highly associated with all-cause mortality and morbidity [5,36], and it has been used as a one of the factors for explaining metabolic inflexibility. In determining aerobic capacity, $VO_2$ peak was assessed in all of our participants. In our data, we found a negative correlation (R= -0.3746) between absolute $VO_2$ peak and plasma lactate (Fig. 12). Our data therefore suggests that there was little to no relationship between the plasma lactate groups and absolute $VO_2$ peak alone.

Furthermore, we were also interested in examining aerobic capacity adjusted for FFM to analyze if aerobic capacity was equally matched per kilogram of lean mass. The adjusted $VO_2$ peak relationship with lactate became distinctively stronger (R= -0.7232, P=0.0002) compared to absolute $VO_2$ peak alone. The Low Lactate Group showed a significantly higher $VO_2$ adjusted to FFM (P<0.0001). Aerobic capacity has been shown to be a direct reflection of mitochondrial oxidative capacity and $O_2$ delivery (capillary density) [43]. The Low Lactate Group demonstrated a notably higher aerobic capacity, which is an indication of a higher mitochondrial and capillary density.

In an attempt to further "unmask" potential relationships between plasma lactate concentrations and aerobic capacity; NIRS was used to study mitochondrial respiratory capacity between the lactate groups at the whole body level. Ryan et al. found measuring mitochondrial aerobic capacity with NIRS to be strongly correlated with measuring *in situ* for mitochondria respiration [85]. In our experiment, NIRS showed no significant difference between High and Low Lactate groups. It is important to note that NIRS technology is highly variable with adipose tissue differences among subjects, which could explain absence of a significant difference between groups. The NIRS measurement was taken on participant's vastus lateralis muscle of the left leg. Since females hold most of their adipose tissue in the lower body, it was challenging to get an accurate representation of deoxygenated hemoglobin, which was used to analyze the rate of oxygen utilization in determining mitochondria aerobic capacity. The fat distribution of the left leg of our participants ranged from 29.7-47.8%. Thus we detected a high variability between lactate concentrations and rate of oxygen kinetics (R=0.3064, Fig. 14). While NIRS measuring for mitochondrial aerobic capacity didn't show a significant difference or a strong correlation as originally projected, the data is still agreeable with $VO_2$ peak assessment when adjusted for FFM.

Our study showed relationship with increased lactate and reduced aerobic capacity. Plasma lactate level is a distinctive marker for aerobic capacity (Fig.14 and 15). The reflection of an inverse relationship between lactate and REE adjusted to FFM (Fig. 6) further suggest the non-obese with higher lactate may be experiencing energy alteration within the cell. Lactate detection might then potentially be served as a clinical biomarker in determining aerobic capacity and REE, as it may be a useful for disease prevention. This speculation requires further studies with a larger number of subjects.

Our data showed that plasma lactate reflects on aerobic capacity. We were also curious to determine potential relationships between lactate levels and the RER in the non-obese in determining their substrate utilization. High resting RER has been extensively studied in the obese Type 2 Diabetes individuals [19,45,46]. RER reflects the carbohydrate and fat substrates utilized, and we were interested to see if plasma lactate concentration would be a factor to show distinct differences between the two groups. The resting RER was obtained when performing REE measurements. We hypothesized that the High Lactate Group would yield a higher resting RER concomitant with a higher plasma lactate production. We found no significant difference when resting RER was compared between two groups. However, a positive correlation ($R=0.6295$, $r^2=0.3963$, $P=0.05$) was reported within the High Lactate Group (Fig. 8). The increased lactate concentration corresponded to the increase resting RER, which suggests individuals with high lactate levels were utilizing more glucose at rest. Furthermore, resting RER was analyzed during 5-minute time points within the 20-minute measurement between High Lactate and Low Lactate Groups (Fig. 9). No statistical difference in resting RER was found between groups within each time points, or at each time point within each group. Although there was no significance, the High Lactate Group elicited a higher average resting RER than Low Lactate Group throughout the 20-minute measurement. This was still important to take notice of when accounting for the similar characteristics of our subjects. A possible explanation may be that the High Lactate Group has a lower composition of type I oxidative muscle fibers. Type I muscle fibers have higher mitochondrial and capillary density [86], which could reduce the production of lactate. Our results disprove our hypothesis, as RER did not show a distinct difference in groups. However, correlation and lactate still shared some relationship. We

postulate that High Lactate Group may have not yet completely reached the characteristics of obesity or Type 2 Diabetes.

Submaximal exercise RER was also measured for 20 minutes with the introduction of low intensity exercise (Fig. 10). The workload was set as close to 50% of subject's $VO_2$ peak (ranged 50-60% of subjects' $VO_2$ peak) on the cycle ergometer. The acclimation took 2-3 minutes before the 20-minute measurement. We were interested in the response of substrates switching during the protocol. No significant difference was found between groups throughout the 20 minutes. The exercise RER for both groups increased simultaneously when exercise was induced. However, RER response in the Low Lactate Group increased significantly from the start at minute 0 to minute 5, minute 0 to 10, minute 0 to 15, and minute 0 to 20 (Fig. 9). No significant difference was found in the High Lactate Group throughout the time of exercise. The Low Lactate Group was able to noticeably switch to more carbohydrate utilization from the start of the exercise and sustain their exercise RER throughout the rest of exercise. The High Lactate Group also shifted to more carbohydrate substrate, but showed no significant difference over any given time point during the exercise. Higher exercise RER was more prominent in High Lactate Group, and it was consistent from the start of the measurement to the end. It was not until the $20^{th}$ minute that exercises RER for the High Lactate Group dropped closer to the Low Lactate Group. Given sufficient time, we observed that the High Lactate Group could adapt to more fat substrate utilization similar level to the Low Lactate Group between minute 15 and 20.

The inability for the High Lactate Group to match a lower RER in the beginning of exercise could be indicative of metabolic inflexibility. Accordingly, lactate and ΔRER was shown to be positively correlated with statistical significance ($R=0.6393$, $P<0.05$). This illustrated the increasing lactate level corresponds to more glucose substrates being utilized given

by the low intensity. However, longer measurements of exercise RER data would be needed to determine if the High Lactate Group was experiencing an adaptation in substrate shifting. To expand, the term metabolic inflexibility has been coined by Kelly and colleagues referring that the ability for substrate transition of carbohydrate when fat is limited [15,76,42]. While the mechanisms for metabolic inflexibility is presently open to debate, it is commonly observed in people who are obese, have Type 2 Diabetes, and/or are sedentary [18,19,45]. While our exercise RER data showed no significant difference between groups (Fig. 10), higher lactate might potentially be an indicative factor limiting the ability to adapt to substrate utilization.

The trend of higher RER in the High Lactate Group was consistent at both resting or during exercise (Fig. 8,9,10, and 11). We believe that this was one of the outcomes subsequent from elevated lactate that is independent from body composition within the non-obese. Previously in a study by Goodpastor et al., it was reported that a higher body fat percentage is associated with a higher RER [87]. The study showed that free fatty acid oxidation is related to a subject's body fat proportion during moderate exercise. In our results, body fat percentage was found to be significantly higher in the Low Lactate Group. This observation leads to the suspicion that a higher fat oxidation may be more related to a higher amount of body fat, instead of a higher aerobic capacity. While this conjecture is sensible, it's important to note that the comparison made in the previous study was comparing leans to obese individuals across a large range of BMIs, opposed to our matched model examining individuals with resting lactate levels that represent the lowest and highest lactate. Additionally, the exercise intensities studied in the past were higher compared to the present study. Therefore, the past literature examining fat oxidation relative to body fat may be misrepresenting the present findings due to different subject characteristics, and exercise intensity that was conducted. In contrast, Geerling et al.

suggest that body fat is not proportional to fatty acid oxidation [88]. The intensity of the exercise (45% $VO_2$ max), and their subject's characteristics (non-obese) were more appropriately matched to our model. The Low Lactate Group showed a lower exercise RER response at each time point, perhaps due to a higher aerobic capacity, which is independent from higher body fat percentage.

**Summary**

Increasing lactate above the normal range demonstrated some association with decreasing REE in the High Lactate Group ($r^2$=0.3712, P=0.0615). The inverse relationship became stronger when REE was adjusted to subject's body weight ($r^2$=0.4656, P=0.0429). However, resting and exercise RER were not different between groups over exercise time. While not significant, it is important to mention lactate and resting RER are positively associated within the High Lactate Group ($r^2$=0.3963, P=0.0511). Additionally, ΔRER from resting to submaximal exercise was strongly associated ($r^2$=0.4088, P=0.0466). No statistical significance was noted in the absolute $VO_2$ peak when compared to High and Low Lactate groups. Surprisingly, aerobic capacity adjusted to FFM was significant higher in Low Lactate Group (P<0.0001). Lactate and adjusted $VO_2$ peak showed to have a strong association ($r^2$=0.5230, P=0.0002).

**Conclusion**

It was unclear if people developed traits of obesity and Type 2 Diabetes before or after they've developed the disease. Decreasing energy expenditure was one of the traits that we thought was prominent in the obese and people who have Type 2 Diabetes. We believed that REE would be distinguish between individuals with low versus high plasma lactate and that stronger difference would become evident as resting lactate increases. Our experimental findings demonstrated that REE decreased when lactate levels increased above the normal range (0.5-1.0

mmol/L), which was displayed in our High Lactate Group. This implies that the early signs of elevated lactate may be an indication of energy alteration within the body as a whole. Concurrently, resting RER also increased with lactate. In the resting state, subjects with a higher lactate level utilized more carbohydrates than fat. Lactate and the adaptability of substrate utilization (ΔRER) shared a positive relationship. The inability for higher lactate individuals to match the same substrate utilized as the lower lactate may be indicative of a lesser mitochondrial and capillary density. Not only lactate and aerobic capacity were inversely associated, $VO_2$ peak adjusted to FFM was significantly higher in the Low Lactate Group. It is important to note that we didn't control for physical activity level as a limitation of our study. The participants were all non-regular exercisers, but their physical activity level may vary from person to person, which is something to consider when looking at the results of our study. Overall, measurement of blood lactate concentrations may be a reflection of aerobic capacity, and substrate utilization during resting and exercise. Thus, further studies are needed with larger subject recruitment to determine the potential clinical adoption of lactate levels as a biomarker for the predisposal toward metabolic disease.

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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** ·
**www.ecu.edu/ORIC/irb**

## Notification of Amendment Approval

From: Biomedical IRB
To: Zhen Yang
CC: Ronald Cortright
Date: 3/5/2019
Re: Ame1_UMCIRB 18-001524
UMCIRB 18-001524
Lactate Predicts Resting Energy Expenditure In Non-Obese Human Subjects

Your Amendment has been reviewed and approved using expedited review for the period of 3/4/2019 to 12/18/2019. It was the determination of the UMCIRB Chairperson (or designee) that this revision does not impact the overall risk/benefit ratio of the study and is appropriate for the population and procedures proposed.

Please note that any further changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. A continuing or final review must be submitted to the UMCIRB prior to the date of study expiration.The investigator must adhere to all reporting requirements for this study.

Approved consent documents with the IRB approval date stamped on the document should be used to consent participants (consent documents with the IRB approval date stamp are found under the Documents tab in the study workspace).

The approval includes the following items:

Description
Changes to Study Team/Personnel

The Chairperson (or designee) does not have a potential for conflict of interest on this study.