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Blood Glucose as a Predictor of Resting Lactate, Hrpr, and Hra as Measured by Method Cra

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BLOOD GLUCOSE AS A PREDICTOR OF RESTING LACTATE, HR_{PR}, AND HR_A AS MEASURED BY METHOD CRA

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35 Pages

Objective: The purpose of this study was to examine fasted blood glucose as a predictor of lactate levels measured by Method CRA.

Method: Participants and data for the present study were collected by employees of an orthopedic rehabilitation center in Bloomington, Illinois. Participants recruited for the study followed the Method CRA upright cycle testing protocol. Fasted blood glucose, resting lactate, and heart rate measurements at Prime (2 mmol/L) and Anaerobic (4 mmol/L) were recorded. Blood was sampled every three minutes during exercise testing. The total sample size of 35 was examined as well as two subgroups, BMI > 30 group and the SD group. Data was analyzed using regression models.

Results: A total of 35 participants were included. Participant's average age was 52.8 years, average fasted blood glucose was 120.4mg/dL, average resting lactate was 1.19mmol/L, average Prime of 107.17bpm, and average Anaerobic of 128.2bpm. The study found that blood glucose was a significant predictor of resting lactate in the BMI >30 group ($n = 26$, $p = 0.03$) and a significant predictor of Prime HR in the SD group ($n = 3$, $p = 0.02$).

Conclusion: Blood glucose was found to significantly predict resting lactate in a subgroup with individuals having a BMI > 30. This indicates that in obese individuals, elevated blood glucose could be indicative of an elevated resting lactate. Blood glucose was also found to

be a significant predictor of Prime HR in individuals who met the criteria for significant deconditioning.

KEYWORDS: Prime Heart Rate (HR_{PR}); Anaerobic Heart Rate (HR_{A}); fasted blood glucose; resting lactate; Method CRA

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Introduction

Obesity is an epidemic that seems to be ever increasing in its occurrence. In 2017-2018, the obesity prevalence in the United States was 42.4% for adults. This number is up by nearly three percent from the 2015-2016 obesity rate of 39.6% (Hales et al., 2020). Obesity does not come without complications. It can frequently lead to further, serious metabolic problems. Some examples of these metabolic problems include insulin resistance, hyperglycemia, type II diabetes mellitus, and atherogenic dyslipidemia. The threat of these complications creates a need for increased endogenous fat oxidation amongst obese individuals to reduce body fat and hopefully prevent their onset.

Currently, the way to maximize fat oxidation involves training at a low-moderate intensity between 45 and 75% maximal oxygen consumption (VO_{2max}) (Purdom, 2018). Lactate testing is another method of assessing maximal fat oxidation (MFO) and has led to different intensity recommendations from the previous methods of assessment. In a study by Bircher and Knechtle (2004) where both lactate threshold and VO_{2max} were studied in relation to substrate oxidation, is was observed that trained men and women show MFO around 75% VO_{2max} while obese individuals show MFO around 65% VO_{2max}. While these percentages both fall into the aforementioned range, they are still considerably different percentages. The difference between the trained individuals' MFO and the obese individuals' MFO illustrates an earlier shift from fat oxidation to carbohydrate oxidation in the obese population. The lactate thresholds reached were also significantly higher in the trained population than the obese population, as would be expected (Bircher & Knechtle, 2004). Keeping in mind the relationship between observed lactate

threshold and presence of obesity along with the effect it had on MFO, lactate testing should be further examined as potentially more individualized assessment of MFO.

In past research, it has been shown that an obese individual's resting blood lactate is elevated compared to someone of healthy weight (Crawford et al., 2010). It has also been shown that resting blood lactate increases along with resting blood glucose (Crawford et al., 2010). While this has been observed in the past, studies such as that by Crawford et al. (2010) are few and far between, and most of the research is done with critical care patients and sparsely so with obese individuals. The relationship between fasted blood glucose and resting lactate has not been thoroughly analyzed in an obese population with the use of a tool such as Method Cellular Respiration Analytics (Method CRA), which gives more than just lactate threshold as an output. In short, while it is not yet validated, Method CRA is an incrementally increasing exercise protocol conducted on either an upright bike or a recumbent stepper to assess metabolic fitness. Lactate is sampled at predetermined intervals along with continuous heart rate monitoring. Lactate measures of 2.0mmol/L, 4.0mmol/L and 6.0mmol/L and the corresponding heart rates provide estimates for the following information: maximal fat oxidation, anaerobic threshold, and peak anaerobic capacity.

The purpose of this study was to examine fasted blood glucose as a predictor of lactate levels measured by Method CRA. The research questions leading this study are: Is fasted blood glucose a predictor of resting lactate measured by Method CRA? Is fasted blood glucose a predictor of prime/anaerobic measures? Is fasted blood glucose a predictor of optimal fat oxidation? Knowing the answers to these questions could prove useful for providing a quick and simple indicator of predicted fat oxidation in some populations.

Methodology

Recruitment

Patients of McLean County Orthopedics (MCO) in Bloomington, Illinois enrolled in physical therapy or the body composition program were given the option to participate in this study. Those individuals enrolled in physical therapy typically had a physician's referral, while patients from the body composition program consisted of both internal and external referrals who opt out of therapy but are interested in the program. The patients were given informed consent forms as well as a physical readiness questionnaire by MCO staff in order to participate. New patients are enrolled into the study on a rolling basis. IRB approval was obtained before any data was collected in the current study.

Inclusion criteria. Only adults 18 years or older were allowed to participate in this study. Completion of the lipid and glucose test, as well as completion of the Method CRA testing up to the anaerobic point were required to be included in the present study. Patients were excluded if they did not have blood glucose data and if they did not give consent.

Data Collection

All data was collected by one of 50 trained staff at MCO. Before the method CRA testing, a therapist obtained verbal consent for lipid and glucose test (CardioChek), Method CRA, heart rate monitor needs, and offered an optional DEXA. The CardioChek was strongly suggested to be performed before the CRA testing and could be scheduled no more than three days before the date of CRA testing.

CardioChek. Patients were asked if they are fasted, and their responses were recorded. Blood samples were obtained, and a therapist was notified of all abnormal values. Total

cholesterol, LDL, HDL, triacylglycerols (TAGS), and blood glucose were recorded to a deidentified excel sheet. At this time, CRA appointments were confirmed with the patients.

Method CRA. General anthropometric data was collected prior to Method CRA testing including height, weight, blood pressure (BP), O_2 saturation, BMI, blood lactate, resting heart rate (HR), and waist circumference. Height and weight were measured using a Health O meter professional weigh scale, BP was measured with a standard sphygmomanometer and stethoscope while sitting, O_2 saturation was measured with pulse oximetry, blood lactate was sampled from the ear with the blood sample analyzed using a Nova Biomedical Lactate Plus Meter, resting HR was taken with patient supine for two minutes and measured with pulse oximetry, and waist circumference was measured in centimeters.

In patients meeting the criteria of significant deconditioning (SD) or in the presence of orthopedic co-morbidities, a NuStep model T4r or Scifit Model RST7000 recumbent stepper was used for either the lower or upper body. The SD criteria required ≥2 of the following: resting blood lactate over 1.5mmol/L, resting HR greater than 90bpm, BMI >40, and/or presence of cardiovascular disease (CVD) or diabetes. Those individuals that fell into this category began at a workload of 5-watts while those who do not fit the SD criteria began at a workload of 15-watts.

For those individuals who did not fall in the SD group, they completed CRA testing using a Matrix 3x upright cycle, model HUre-3x-O2-C. Standard protocol participants were fit on an upright cycle so that a 15-20-degree bend in the knee is allowed on the down stroke and SD participants were fit on an upright cycle or recumbent stepper to ensure comfort. A Polar brand H7 or H10 chest strap heart rate monitor was worn around the sternum at the xiphoid process for monitoring heart rate. As the test began at the previously established workloads for standard and SD individuals, workload was increased at a rate of 15-watts every three minutes for the standard

protocol and a rate of 5-watts every three minutes for the SD protocol until 15-watts are achieved at which point 10-watt increments will be applied every three minutes. At the three-minute increments, lactate samples from the ear lobe were taken as well as corresponding HR values and recorded.

The Method CRA application recorded HR values associated with blood lactate values around 2.0mmol/L (HR_{PR}), 4.0mmol/L (HR_{A}), and 6.0mmol/L (HR_{PK}). HR_{PR} is the heart rate that corresponds to a blood lactate concentration of around 2mmol/L and is associated with maximal aerobic fat oxidation. HR_{A} is also known as the lactate threshold and is the point at which significant lactate has accumulated. HR_{PK} is measured at a blood lactate concentration of 6mmol/L and is defined as the point at which exercise cannot be maintained much longer and could result in slight muscular tissue damage due to lactate buildup (Method, n.d.). The tests were terminated after 6.0mmol blood lactate was achieved, if the participant requested the test to stop, or if lactate failed to rise in more than two consecutive samples. All heart rates corresponding to HR_{PR}, HR_{A}, and HR_{PK}, were recorded to the excel spreadsheet containing all other patient information.

Statistical Analysis

The sample was analyzed as a total sample (n=35) and two subgroups from within the sample: individuals with blood glucose data and a BMI>30 (n=26), and individuals with blood glucose data who meet the SD criteria (n=3). A BMI of >30 was used as a cut off for the first subgroup as that is where obesity is defined. Due to the very small sample size in the SD group, this group was examined with much skepticism and viewed mainly in an exploratory capacity. Conclusions cannot reliably be drawn from this group. Linear regression analyses via Microsoft

Excel was utilized for all groups to assess the relationship between the dependent variables of resting lactate, HR_{PR} and HR_A and the independent variable of blood glucose.

Results

This study investigated the relationship between the independent variable of blood glucose, and the dependent variables of resting lactate, HR_{PR} , and HR_A. A total of 35 participants were included in the study. Of the 35 total participants, 17 were male (49%) and 18 were female (51%). The age of participants ranged from 24 to 83 years old. Participants were mainly white (n=30) with one Asian, one Hispanic, and three participants who did not answer. Additional demographic and Method CRA data can be found in Table 1.

In addition to assessing the total sample of 35 participants, two subgroups were also assessed using the same variables. Those subgroups were: all participants with a BMI >30 (n=26) and all participants who met the SD criteria as previously described (n=3). All three groups were subject to the same statistical analysis. Regression analyses via Microsoft Excel was used to analyze for significance (with an alpha value set at 0.05) to assess the ability of blood glucose to predict resting lactate, HR_{PR}, and HR_A.

Total Sample. No significant predictability was found between the independent variable and dependent variables in the full sample size of 35. Blood glucose was not a statistically significant predictor of resting lactate with a p value of 0.19 and an R squared value of 0.05. Blood glucose was not a statistically significant predictor of HR_{PR} with a p value of 0.45 and an R squared value of 0.02. Blood glucose was also not a statistically significant predictor of HR_A with a p value of 0.42 and an R squared value of 0.02. Figures 1-3 contain visualizations for these regression models.

BMI >30 Group. The first subgroup of 26 participants with BMI>30 contained one statistically significant relationship. Blood glucose was found to be a significant predictor of resting lactate with a p value of 0.03 and R squared value of 0.18 suggesting that while the relationship is significant, the graph representing the regression had high variability. Blood glucose was not a statistically significant predictor of HR_{PR} with a p value of 0.13 and R squared value of 0.09. Blood glucose was also not a statistically significant predictor of HR_A with a p value of 0.17 and R squared value of 0.08. Figures 4-6 contain visualizations for these regression models.

SD Group. The second subgroup of 3 participants who met the SD criteria contained one statistically significant relationship. Blood glucose was not a statistically significant predictor of resting lactate with a p value of 0.61 and an R squared value of 0.33. Blood glucose was a statistically significant predictor of HR_{PR} with a p value of 0.02 and an R squared value of 0.99 suggesting a model of which 99% of plotted data fits. Blood glucose was also not a statistically significant predictor of HR_A with a p value 0.37 and an R squared value of 0.70. Figures 7-9 contain visuals for these regression models.

Discussion

The purpose of this study was to examine fasted blood glucose as a predictor of lactate levels measured by Method CRA. Specifically, the ability of blood glucose to predict resting lactate, $HR_{PR,}$ and HR_A was analyzed and assessed with regression models in Microsoft Excel. The first significant finding of this study was blood glucose as a significant predictor of blood glucose on resting lactate in the BMI >30 group ($p<0.05$). The second significant finding of this study was a significant prediction of blood glucose on HR_{PR} in the SD group. Additional findings

of the present study were that in each group, blood glucose was predictive of resting lactate; however, this relationship was only significant in the BMI >30 group.

The first finding could suggest that higher fasted blood glucose is associated with increases in one's resting lactate which is partially supported by some of the mechanisms provided in the literature. Most studies focus on the effects of Diabetes on resting lactate and lactate threshold, but some of the results could be applied here given the elevated BMI of participants which could be similar to that of diabetic individuals. For example, in a study by Crawford et al. (2010), researchers looked to further understand the relationship between type two diabetes and blood lactate. Researchers found that there was a linear, graded relationship between lactate level and the odds of an individual having type two diabetes. The measured lactate was separated into increasing quartiles and when compared to the first quartile, the fourth had a 3.25-fold increase in the odds of an individual from the group to have diabetes. The average BMI of the fourth quartile group was over 30. Researchers in this study also found that in their non-diabetic participants, lactate levels were lowest in individuals with normal, non-elevated fasting glucose levels. They also found that intermediate levels of lactate were present in participants with fasting blood glucose of 100-125mg/dL and the highest lactate levels were observed in subjects diagnosed with type two diabetes. These findings are consistent with the current study.

The second significant finding, similarly to the first, would suggest that optimal fat burning is hard to achieve in deconditioned individuals since they easily surpass it and shift to lactate production via anaerobic metabolism. This finding is difficult to support with previous research due to the specifics of the SD criteria. Similarities can be drawn with other studies that account for individual aspects of the SD criteria, however, such as obesity. Studies such as one

by Chen et al. (2017) found that in obese rats, for example, lactate dehydrogenase expression is blunted resulting in increased blood lactate concentrations that occur more rapidly.

The first total sample group (n=35) was the only group to present no significant relationships. This group had an average BMI of 37.4. This could suggest the significance found in the two smaller subgroups were more likely attributed to chance given their much-decreased size. It is also possible that the increased average BMI of the BMI >30 group resulted in a stronger positive correlation between blood glucose and resting lactate. This group, (n=26) contained only individuals from the original sample that had a BMI > 30. The average BMI for this subgroup was 40.8. In this group, blood glucose was a significant predictor of resting lactate which coincides with some of the literature, such as that by Chen et al. (2017), Zak-Golab et al. (2010), and Metz et al. (2007).

In the SD group (n=3), individuals who met the SD criteria were examined. The SD criteria required ≥2 of the following: resting blood lactate over 1.5mmol/L, resting HR greater than 90bpm, BMI >40, and/or presence of cardiovascular disease (CVD) or diabetes. Within this subgroup, there was a significant negative correlation between blood glucose and HR_{PR} ($p<0.05$). This suggests that as one's fasted blood glucose increases, the HR at which maximal fat oxidation is achieved is lowered. Interestingly, this result can be supported anecdotally by the staff of MCO who stated some individuals they test are at HR_{PR} according to Method CRA far before expected. This would suggest a very disordered metabolism in the individuals meeting the SD criteria. It would suggest that lactate production is much higher at much lower heart rates than in individuals would do not meet the criteria. This idea is supported by the literature as far as in obese individuals. In a study by Chen et al. (2017), researchers found that buildup of blood lactate occurs more rapidly in obese individuals at least partially due to changes in the lactate-

utilizing proteins in the muscle and liver. While studies similar to this do not include criteria such as that used here for significant deconditioning, similar results and changes to metabolism could be observed. It is also noteworthy that athletic conditioning level was not accounted for in this study, which is another variable that has effects on lactic acid onset (Zakynthinaki, 2015).

Another study conducted by Shen & Wen (2019) used a similar graded exercise test to assess lactate threshold onset and its association with HR. This study different from the current one in that there were many more participants (n=84), only untrained individuals participated, and the exercise test was stopped at lactate threshold. The regression models from this study showed that 47%-65% of variance found could be explained with the eight models created. Each model used a different percentage of max HR and the lactate threshold speed. While not 100% effective, the authors concluded that their models could be appropriate for use on the general population for lactate threshold prediction. This article had very similar study design to the current and had similar results.

Limitations

Multiple limitations were present in the current study. The first limitation was the limited sample size. Recruitment for the study as a whole was high but the amount of individuals who had blood glucose data was very limited (n=35). With blood glucose being the independent variable being investigated, this notably affected the sample size being used. Another limitation was the significantly smaller number of individuals who met the SD criteria. One of the main goals of this study was to investigate that subgroup in specific, and with a sample size of only three, thorough and reliable analyses of the subgroup could not be done. Additionally, had sample sizes been larger, more analyses could have been conducted with more subgroups such as

age, BMI subcategories, or similar analysis as conducted here with individuals of specific disease states such as diabetes.

Data for the study was not collected by the researcher, it was collected by MCO staff. This is an additional limitation because it reduced the amount of collectable information for the researcher and the ability for the researcher to make sure that important variables, such as blood glucose, were being more stringently obtained. It also affected the ability for the researcher to confirm strict adherence to the protocols integral to the study.

It was notable that the blood glucose data of many participants was very elevated. This would suggest that either the majority of the participants were diabetic, or that they had not properly fasted. While MCO staff did ask whether or not individuals were fasted, it is not clear how "fasted" was defined to the participants. Having a strict fasting protocol that is applied to all participants is important to make sure data gathered in the study is as reliable as possible. Additionally, blood glucose being recorded three days prior to Method CRA testing reduces the relevancy of the data. Another limitation related to the data collection was the intervals of lactate sampling during exercise. While three-minute interval sampling is common amongst testing such as this, more frequent measures could have proven useful in assessing such precise measures as that included in the present study.

Future Studies

There are many ways that future studies can expand on information and direction from the current study. This study had many limitations secondary to the fact that data collection was not conducted by the researchers. These are limitations easily corrected in future studies. In addition to data collection, future studies need to have much larger sample sizes with more diverse demographics. The present study primarily contained only one ethnicity in addition to

being relatively small. A larger sample size could help provide clarity on significance found in the present study.

In future studies, researchers should break down their samples by age groups to see how, if at all, that additional variable affects metabolite usage. If a large enough sample size is available in the future, BMI subgroups could be a useful addition to the study. Since the literature suggests obesity promotes onset of HR_A, it may prove helpful to assess the specific BMI range associated with this observation. Additionally, the presence of specific disease states could be analyzed separately to see how they impact these variables. By doing this, there is the opportunity to find more clinically significant findings. In previous studies such as one by Gaster et al. (2004), it was found that diabetic patients displayed blunted capacities to oxidize fats. Incorporation of more diabetic individuals in future studies could contribute a lot to the body of knowledge surrounding metabolic changes in diabetic participants.

Future studies could also incorporate an exercise intervention and follow-up to assess effectiveness of physical activity on the variables presented and their correlation to blood glucose in the presence of exercise and without. While some studies that utilize weight loss programs had findings inconclusive regarding correction of disordered metabolism by weight loss alone, it is possible that by not only losing weight, but also adding muscle mass, more significant results could be found.

As far as raw data collection, future researchers should look at branching out from resting data. Information such as resting lactate and fasted blood glucose might not be as useful or practical as more functional measures. Measures such as VO2 that more accurately assess fitness level should be examined and implemented in future studies.

Conclusion

This study was unique in its assessment of blood glucose associated with two different measures of lactate: 2 mmol/L (HR_{PR}) and 4 mmol (HR_A) on a graded exercise test as well as resting lactate. One of the only related studies found was by Shen & Wen (2019) in which a similar, graded exercise test was performed but only lactate threshold and heart rate were examined. The aim of this study was to examine fasted blood glucose as an aspect of metabolic functionality defined by appropriate onset of MFO (HR_{PR}) measured by Method CRA. Specifically, the relationship between blood glucose, resting lactate, $HR_{PR,}$ and HR_A were analyzed via regression analysis. Two significant findings were present in the current study. The first was that in the BMI >30 group, a significant positive relationship was found between fasted blood glucose and resting lactate levels. This finding is in agreeance with the literature that obese individuals with higher fasted blood glucose levels will have higher resting lactate levels. The second statistically significant finding was that although the sample size was very limited, the SD group, which included only individuals meeting the SD criteria, displayed a negative relationship between HR_{PR} and fasted blood glucose. This finding would imply that as one's fasted blood glucose increases, the point at which maximal fat oxidation, in terms of HR, occurs becomes lower.

Table 1

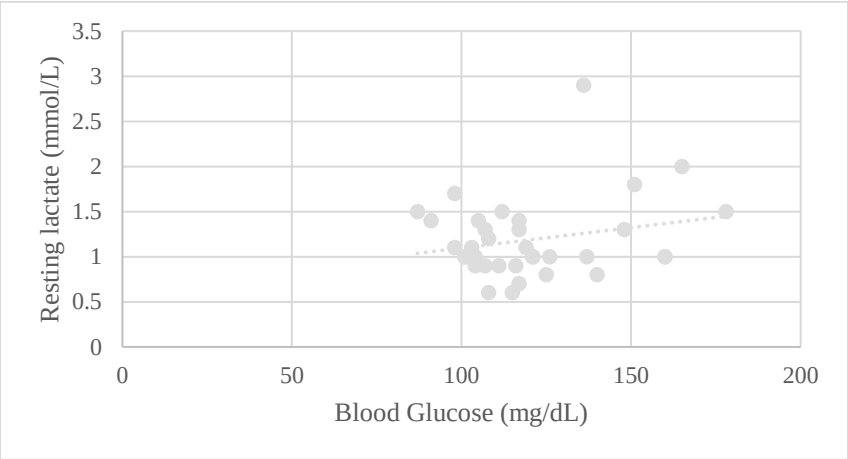
Demographics of participants

Variable	Men (n=17)	Women (n=18)	Total (n=35)
Age (years)	33.8 (10.46)[a]	51.94 (15.32)	52.8 (13.03)
BMI (kg/m²)	35.9 (8.70)	38.81 (11.80)	37.39 (10.36)
Blood glucose (mg/dL)	125.71 (20.81)	115.39 (22.96)	120.4 (22.24)
Resting Lactate (mmol/L)	1.06 (0.32)	1.31 (0.51)	1.19 (0.44)
Prime HR (bpm)	112.24 (14.45)	114.22 (16.54)	107.17 (15.75)
Anaerobic HR (bpm)	123.76 (33.25)	132.39 (18.10)	128.2 (26.52)

Note: [a]Mean(standard deviation)

Figure 1

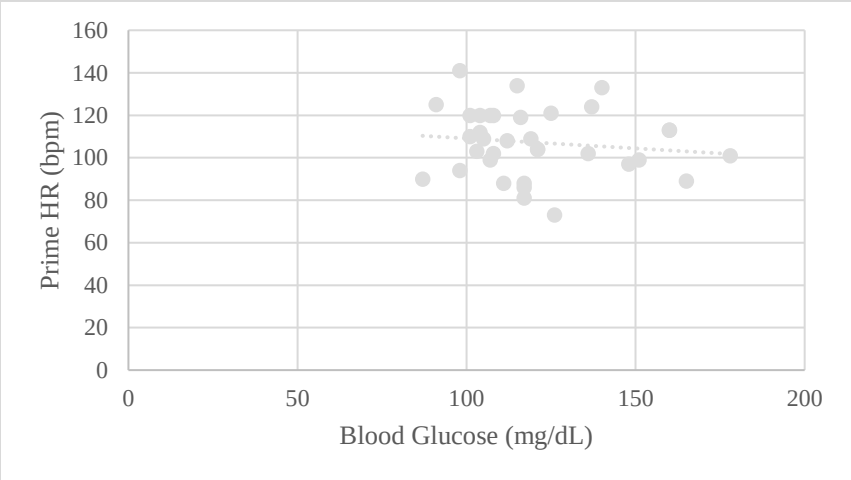
Blood Glucose and Resting lactate (Total Sample Group)



Note: No statistically significant correlation. n = 35, p = 0.19, R squared = 0.05

Figure 2

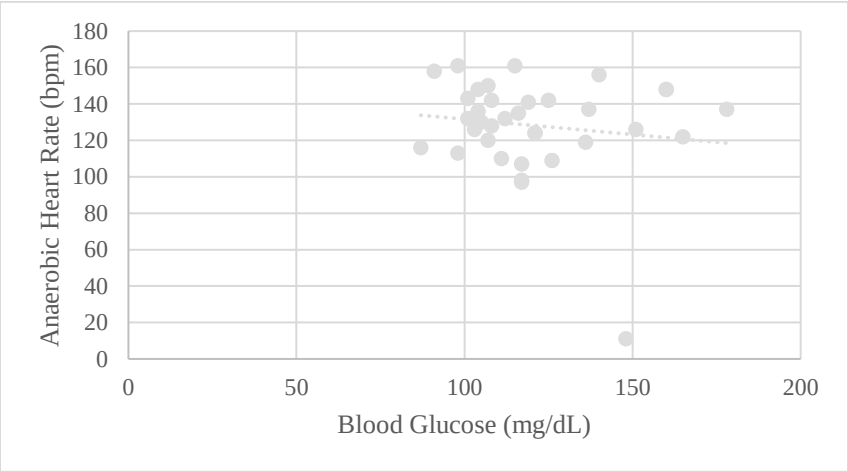
Blood Glucose and HR_{PR} (Total Sample Group)



Note: No statistically significant correlation. n = 35, p = 0.45, R squared = 0.02

Figure 3

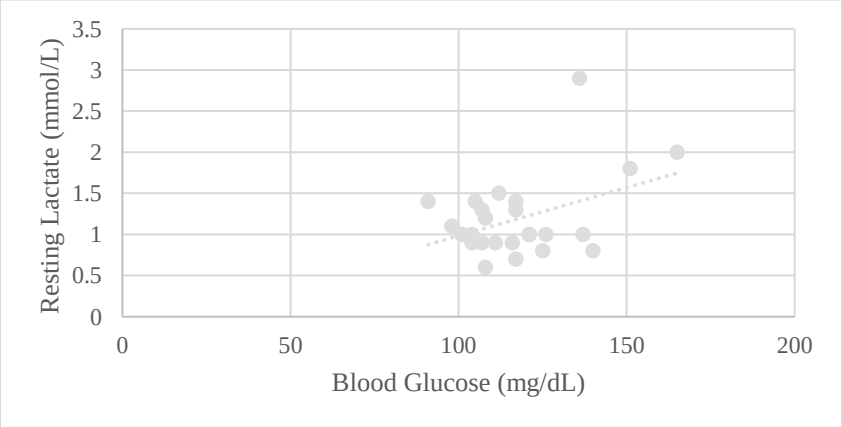
Blood Glucose and HR_A (Total Sample Group)



Note: No statistically significant correlation. n = 35, p = 0.42, R squared = 0.02

Figure 4

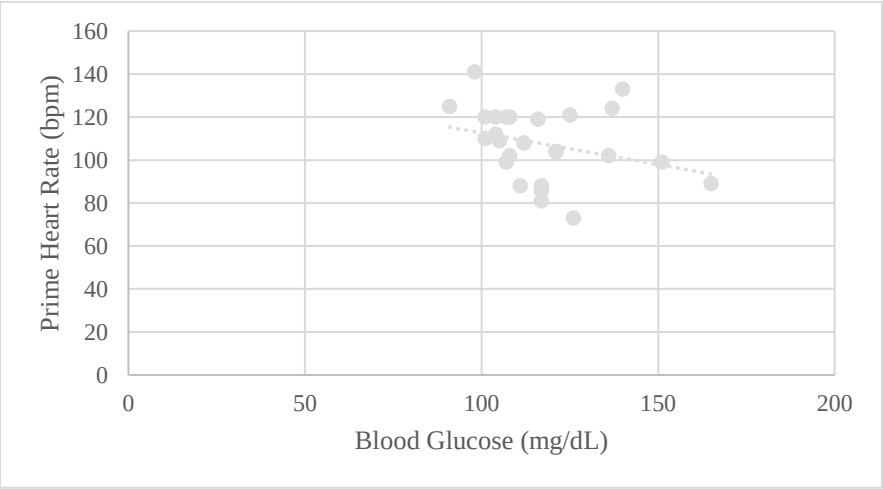
Blood Glucose and Resting lactate (BMI > 30 Group)



Note: p = 0.03. n = 26, R squared = 0.18

Figure 5

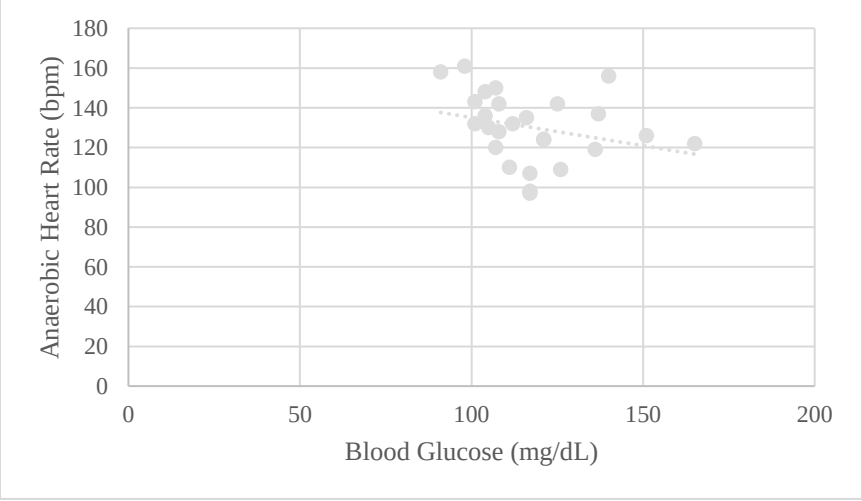
Blood Glucose and HR_{PR} (BMI > 30 Group)



Note: No statistically significant correlation. n = 26, p = 0.13, R squared = 0.09

Figure 6

Blood Glucose and HR_A (BMI > 30 Group)



Note: No statistically significant correlation. n = 26, p = 0.17, R squared = 0.08

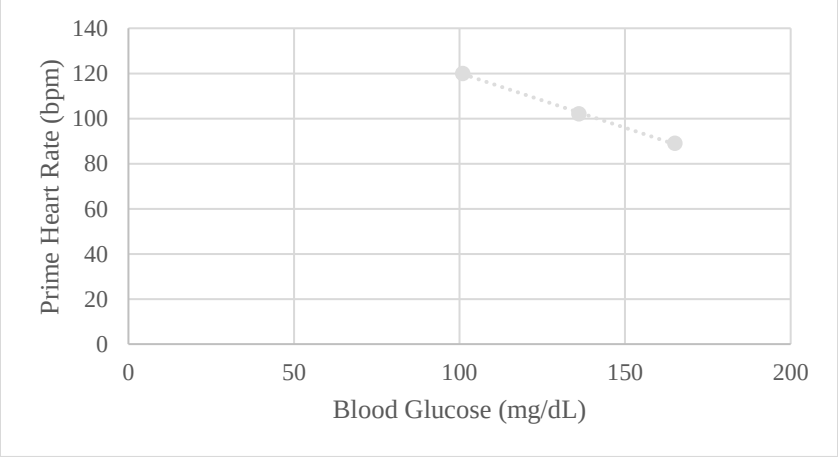
Figure 7

Blood Glucose and Resting lactate (SD Group)

Note: No statistically significant correlation. n = 3, p = 0.61, R squared = 0.33

Figure 8

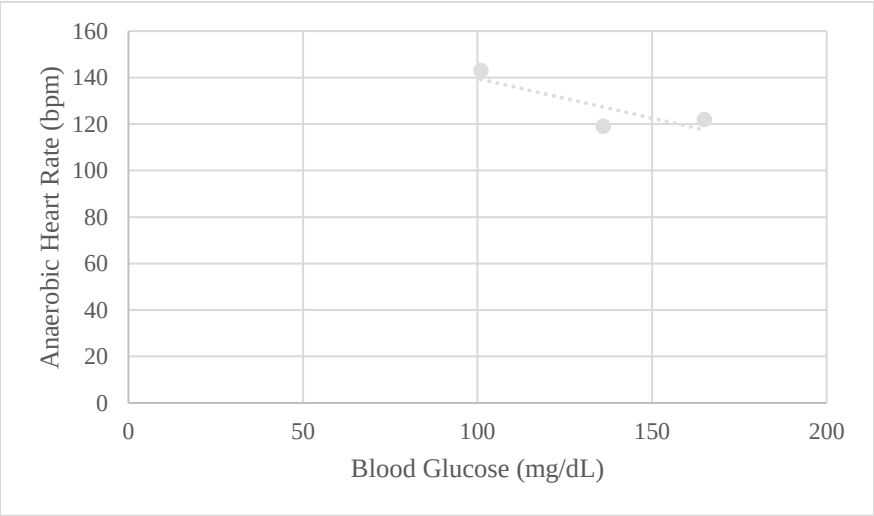
Blood Glucose and HR_{PR} (SD Group)



Note: p = 0.02. n = 3, R squared = 0.99

Figure 9

Blood Glucose and HR_A (SD Group)



Note: No statistically significant correlation. n = 3, p = 0.37, R squared = 0.70

CHAPTER II: REVIEW OF THE LITERATURE

The body can and will utilize different stored fuel sources based on a multitude of factors. Some of the primary factors affecting fuel utilization include training status, exercise intensity, duration of exercise, sex differences, and nutritional status (Purdom, 2018). At submaximal levels of exercise, the body turns to its body fat stores to oxidize for energy. Submaximal exercise can be defined as exercise below 65% of VO2max (Purdom, 2018). As exercise intensity increases over 65% of VO2max, the portion of energy contribution from carbohydrates increases and fat oxidation decreases (Purdom, 2018). In a study by Purdom (2018), the point of MFO was found to occur between 47 and 75% of VO2max. The point of MFO also varies based on conditioning level of an individual as well as diet and sex (Purdom, 2018). With these percentages in mind, if the point of MFO is known for a specific person, then exercise can be performed to keep one at MFO for prolonged fat oxidation given that some of the other variables can be controlled.

Aerobic and Anaerobic Metabolism

Once past MFO, during high intensity exercise, there is a point at which carbohydrate oxidation aerobically can no longer be supported with significant oxygen and an anaerobic burning of carbohydrates will begin (Chen et al., 2017). This anaerobic metabolism results in lactic acid buildup and cannot be maintained. Under normal conditions, lactate produced by muscle is shuttled to the liver and taken up by monocarboxylate transporters (MCT) and converted back into pyruvate by pyruvate dehydrogenase to be oxidized further or converted into glucose (Chen et al., 2017). This buildup of blood lactate occurs more rapidly in the obese at least partially due to changes in lactate-utilization related proteins found in the muscle as well as

the liver. This means that MFO is being exceeded much more rapidly in the obese resulting in the earlier onset anaerobic metabolism (Chen et al., 2017).

In one study by Conceição et al. (2018), authors looked to compare energy system contributions and total energy expenditure after a low intensity exercise with and without blood flow restriction. This study used only sedentary male volunteers. Each volunteer did two exercise tests, one with a cuff to restrict blood flow to the leg and one without the cuff. The exercise protocol for both exams was kept the same. Results of this study showed significant increases in aerobic metabolism, lactic metabolism, and total energy expenditure in the blood flow restricted tests. More significant results from this study include significantly higher HR and ventilation in the blood flow restricted group at minute intervals of 10, 15, 20, 25, and 30 minutes as well as throughout the 15-minute post exercise recovery (Conceição et al. 2018). The increase in lactic acid production in this experiment was probably the most predictable, but the more interesting results are the two latter ones. In mechanically induced hypoxic events, such as the one in this study, the body autoregulates by increasing HR and ventilation to allow for more oxygen to reach the muscles. This displays some of the complexities related to anaerobic and aerobic metabolism.

Disrupted Metabolism

Obesity. Metabolic flexibility is defined as the ability to change substrate oxidation in accordance to hormonal changes (Kim et al., 2018). Obese individuals can express metabolic inflexibility, or the lack of metabolic adjustments in response to hormonal secretions such as insulin (Kim et al., 2018). For some obese individuals, metabolic inflexibility can be evidenced

by lessened fat oxidation during fasted times, impaired suppression of lipid oxidation, and decreased stimulation of glucose oxidation during the fed state (Kim et al. 2018).

Obesity is linked to an increase in certain adipokines associated with inflammation and can result in systemic inflammation, leading to metabolic inflexibility (Uranga & Keller, 2019). This systemic inflammation also results in something referred to as metabolic syndrome. Metabolic syndrome is diagnosed with three of the following 5 criteria: central obesity, elevated BP, reduced glycemic control, low HDL levels, and/or high serum TAG levels. This syndrome can predispose individuals to diabetes, cardiovascular disease, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, cancer, and sleep apnea amongst many others (Uranga & Keller, 2019).

Blood lactate has potential to be used as an indicator of metabolic activity in obese individuals. Research in rats indicates that blood lactate accumulates faster in obese rodents and is associated with poorer performance during exercise (Chen et al., 2017). This again supports the previous statement of obesity leading to higher levels of anaerobic metabolism. Several proteins are required for the proper oxidation of lactate. Two of these subsequent proteins are MCT and lactate dehydrogenase (LDH). It has been observed that obesity in rodents can lead to a decrease in LDH expression (Chen et al., 2017). This decrease in LDH expression leads to the increased blood lactate concentrations in the obese rodents. This subsequently takes them out of MFO faster and reduces their ability to utilize their fat stores.

Adipokines. Since adipose tissue functions as an endocrine organ, increases in the amount of adipose tissue that the body has can lead to large hormonal changes (Singla et al., 2010). One of the most researched hormones from adipose is Leptin. Leptin is an important

adipokine that signals the hypothalamus to suppress food intake (Singla et al., 2010). Leptin production is directly related to the quantity of adipose present in the body, that means the more adipose you have, the more leptin you can make and thus as weight reduction occurs, leptin levels go down. Leptin resistance can occur in much the same way that insulin resistance can occur. Overproduction of leptin and overstimulation of the hypothalamus with leptin long-term can blunt the body's responsiveness to the signal. There are several specific hypotheses for mechanisms of leptin resistance. These mechanisms include impaired leptin transport across the blood-brain barrier, dysfunction of neuronal effector pathways in the brain, and overall reduced leptin-receptor signal transduction. Leptin is an interesting topic of research into obesity and by association, metabolic syndrome. Other notable adipokines include visfatin, apelin, resistin, and adiponectin (Singla et al., 2010).

Adiponectin is another adipokine that serves to enhance the actions of insulin on the liver, suppress fatty acid influx to the liver, improve the uptake of glucose by the liver and skeletal muscle, and improve the oxidation of fatty acids (Singla et al., 2010). While many other hormones produced by adipose tissue increase in production alongside increases in adiposity, adiponectin works in the opposite way. That is, as body adiposity decreases, adiponectin production in the body increases. Adiponectin has much more positive outcomes associated with it than others and low adiponectin levels can lead to an increased risk of cardiovascular disease. Another factor affecting adiponectin production is the presence of type two diabetes mellitus (Singla et al., 2010). It was found in a study by Hotta et al. (2000) that type two diabetics had significantly decreased levels of adiponectin in the blood, independent of BMI.

Three more notable adipokines are visfatin, apelin, and resistin (Singla et al., 2010). The first, visfatin, is increased in concentration with individuals who have abdominal obesity and

type two diabetes mellitus. Visfatin has the ability to mimic the functionality of insulin and results in decreased hepatic glucose production and movement of glucose into peripheral tissues. While some studies do support this function of visfatin, others do not, and it is still subject to research to fully understand how it relates to metabolic changes in obese or diabetic individuals. Apelin is another adipokine that is produced more in the setting of obesity. This adipokine has been associated with vasorelaxation and reduction in arterial blood pressure. Resistin is intuitively named in that it acts the increases resistance of the body to insulin. It has been found that resistin is another hormone increased in obese individuals and is considered pro-inflammatory. In fact, it has been found that stimulation of the inflammation process can result in the release of more resistin. Resistin's role in obesity-mediated insulin resistance and type two diabetes is still up for debate in humans (Singla et al., 2010).

Diabetes and prediabetes. Diabetes has several effects on metabolic flexibility. Some of these effects include excessive hyperlipidemia, insulin resistance, and lipid droplet (LD) accumulation in adipose tissue (Ji et al., 2019). The β-cells of the pancreas secrete insulin during times of high glucose and lipid presence in the blood. High blood concentrations of these nutrients creates an increased need for insulin secretion from the β-cells. If the pancreas is unable to meet the insulin needs of the body then glucose intolerance, and subsequently, overt diabetes can develop (Ji et al., 2019). Diabetes comes with many metabolic and hormonal changes; however, the two most prominent metabolic changes are hyperglycemia and hyperlipidemia (Ji et al., 2019). Both obesity and diabetes can disrupt normal metabolic function enough to change what metabolites we will use for energy, as well as what ratio of aerobic to anaerobic metabolism we will be performing at various training intensities.

In a study conducted by Gaster et al. (2004) a group of 10 obese type 2 diabetic patients and a group of 10 obese non-diabetic control subjects were assessed on their capacity to oxidize fatty acids. The core finding of this study was that the myotubes cultured from the type 2 diabetic patients displayed reduced oxidation of palmitate and increased esterification of palmitate into phospholipids. The authors offer up several potential mechanisms for this observation: decreased fatty acid uptake into the cell, reduced acetyl CoA flow into the mitochondria, altered morphology or number of mitochondria, and/or decreased fatty acid oxidation and carbon dioxide production all of which are supported by the literature (Gaster et al., 2004).

From studies such as these, it is clear that disease states such as type 2 diabetes can be associated with general oxidative suppression and reduced mitochondrial function. More specifically, increased number of reactive oxygen species (ROS) secondary to hyperglycemia can lead to decreased mitochondrial biogenesis and thus lower respiratory efficacy; however, that is not always the case (Lund et al., 2019). This, along with increased glucose concentration is hypothesized to push more activity in the glycolytic pathway resulting in more lactic acid production in these individuals (Lund et al., 2019).

Weight loss. In a study by Metz et al. (2007), expression of lactic acid transporters was assessed in individuals and then re-assessed after a 15-week weight loss protocol. Muscle biopsies were obtained three weeks prior to the start of the weight loss program and again four to six weeks after the end of the program. Researchers specifically looked at the MCT1 and MCT4 lactic acid transporters. Post weight loss, there was some change in MCT expression, but it was insignificant. Interestingly, the changes found for MCT1 expression were not consistent from participant to participant; in fact, some individuals had increased expression of MCT1 post weight loss and others had decreased expression. To explain this phenomenon, the researchers

suggest that possibly multiple different changes would be required to elicit increased MCT1 expression, and not solely weight loss. This is quite different from other, similar transporters such as GLUT4 which does increase in expression with weight loss alone. Another interesting finding was that MCT4, a lactate transporter responsible for lactate extrusion from the cell, was expressed more in the muscle cells of the obese individuals more so than the post weight loss individuals. The authors hypothesize these MCT4 results could be secondary to the overall increased level of lactic acid muscle cells of obese individuals which would have to be extruded (Metz et al., 2007). This study suggests that lactate metabolism in the obese population is complicated and possible multifactorial in its reversal to normalcy.

Another study by Zak-Golab et al. (2010) supports the idea of a multifactorial changes being necessary for reversal of some of these lactate oxidation problems. In this study, the effects of weight loss on anaerobic threshold were examined with 59 women having BMIs greater than or equal to 30. The participants were enrolled in a 3-month weight loss program. Post weight loss program, an average weight loss of 12.3 plus/minus 4.2% was achieved which was still insufficient to cause significant changes to lactate threshold. Researchers hypothesize again that either more weight loss is necessary to elicit changes, or that there is more than just weight loss necessary (Zak-Golab et al., 2010).

Metabolite Markers

Lactate. Lactate is a glucose derivative that can be synthesized by the muscles in order to meet energy requirements when the body is insufficiently supplying oxygen to those tissues. Thus, when oxygen supply is low, lactate production will increase. It is important to note that the resting lactate level in the blood is usually around 1mmol/L and that a threshold known as the lactate threshold occurs when blood lactate begins to accumulate significantly, that amount of

lactate depends on the individual's conditioning, but is usually around 4mmol/L (Zakynthinaki, 2015). Lactate is also a key regulator in gluconeogenesis and glycogenolysis and is an important regulator of glucose maintenance (Emhoff et al., 2013).

In modern literature, lactate is thought of as much more than just a wasteful byproduct as once thought (Gladden, 2004). That was the previously held position on lactate commonly held from the 1930s to the 1970s. This opinion started to change substantially in 1984 when the lactate shuttle hypothesis by George Brooks was presented. In short, this hypothesis describes the movement of lactate within the cell. Lactate is now known as a mobile fuel for aerobic metabolism as well as a potential mediator of redox states both in and in-between cells.

During exercise, intramuscular and blood lactate levels rise dramatically. The hydrogen ions associated with lactate are partially the cause of a change in pH. This drop in pH is often what is correlated with decline in muscular force generation. Interestingly, the lactate without its hydrogen does not actually contribute significantly to muscular fatigue. A study by Posterino et al. (2001) found that lactate without its hydrogen ion had less than 5% of an effect on the muscular contractility of mammalian muscle fibers.

Some studies also focus on accumulation of inorganic phosphate as another driving force in muscle fatigue. Another study conducted with muscle fibers found that intense muscular contraction or exercise, quantities of inorganic phosphate increase along with the increasing muscle fatigue. This aspect of muscular figure still needs more research since PCr breakdown occurs in such a limited window of time, The exact cause of muscular fatigue is unclear, but it is evident that these two factors are part of the answer (Gladden, 2004).

The aforementioned hypothesis by Books in 1984 can simply be described as the lactate shuttle (Gladden, 2004). This cell-to-cell lactate shuttling is another important function of lactate

in the body. This widely utilized hypothesis states that lactate formation and its movement through the body is a large part of the coordination and accomplishment of intermediary metabolism in the body as a whole. Once transported through the body, lactate can be used as a carbohydrate fuel for skeletal muscle during exercise (Gladden, 2004).

In addition to uptake of lactate, skeletal muscle can be a large producer of lactate as well (Gladden, 2004). Even at rest, skeletal muscle has a small, slow release of lactate into the blood stream. During low to moderate exercise intensity the lactate produced in muscle fibers can also be taken up by neighboring fibers and oxidized there. Lactate can also leave the interstitial fluid of muscles in use and enter the plasma. Lactate can then enter red blood cells and move to other tissues in the body down the lactate gradient (Gladden, 2004).

Lactate levels are not only influenced by exercise and production under normal conditions in the body (Anderson et al. 2013). Lactic acid is also a commonly overproduced and poorly cleared byproduct of some disease states. When lactic acid is severely elevated, there can be serious hemodynamic consequences resulting in death. Disease states and conditions that can lead to elevated lactic acid levels include shock, cardiac arrest, regional tissue ischemia, diabetic ketoacidosis, seizures, liver failure, mitochondrial disease, cancer, and burns. Certain drugs, medications, or toxins can have the same result on the body. Examples of lactic acidosis causing medications or chemicals include alcohol, cocaine, cyanide, carbon monoxide, linezolid, metformin, epinephrine, propofol, acetaminophen, and theophylline (Anderson et al. 2013). Prolonged, high levels of lactic acid can lead to organ failure and deadly changes in blood pH.

In a study performed by Lund et al. (2018), the authors studied the metabolic relationship between glucose, fatty acids, and lactic acid in human myotubes, or skeletal muscle fibers. Specifically, they chose to investigate the two main lactic acid transporters in skeletal muscles,

MCT1 and MCT4 as well as two additional lactic acid transporters, MCT2 and MCT3. They found that when acute addition of lactate was given to myotubes, meaning four hours of exposure, there was no difference in glucose uptake but when the lactic acid was added in a much higher concentration, oxidation of glucose was decreased. When the myotubes were chronically exposed to lactic acid, meaning 24 hours of exposure, the myotubes tended to have some enhanced glucose uptake and oxidation (Lund et al., 2018). These findings suggest that the muscles' preference for energy substrates changes depending not only the presence of lactate, but also the concentration and duration of time the cells are exposed to the lactate. Studies such as this display the relevance of lactate as something more than just a wasteful byproduct of metabolism as it was once thought.

Similar results were found in a study conducted by Miller et al. (2002). In this study, researchers supplemented lactate into subjects and ran them through 90-minute exercise trials in order to compare both lactate and glucose oxidation at rest and during exercise (Miller et al., 2002). Only exercise-competent subjects were used to allow for more consistently stressed metabolite oxidation. This study found that glucose oxidation was decreased in the presence of lactate and this decrease in glucose oxidation correlated with a decrease in glucose uptake by muscle cells as well as more lactate oxidation (Miller et al., 2002). This study in addition to the aforementioned study suggest that lactate is important in the overall metabolic activity experienced in the muscular tissue during exercise and that its presence influences glucose utilization in some way.

Heart rate. Heart rate and blood lactate levels have an interesting and complex relationship. While exercising at a low-moderate intensity, blood lactate remains close to its resting level and heart rate will increase to a steady rate appropriate to the exercise being

performed (Zakynthinaki, 2015). As intensity increases, the lactate level approaches threshold, and heart rate has a delay in reaching a steady rate. This heart rate delay around lactate threshold is known the slow component of cardiovascular kinetics and has been associated with onset of fatigue. Endurance training will cause an elevation in lactate threshold which will in turn decrease the effects of the slow component of cardiovascular kinetics during some intensities of exercise. This complex relationship means that if individuals are trained appropriately to raise their lactate threshold, then the distance between resting lactate and threshold will increase, and heart rate will rise more appropriately during sub-lactate threshold intensities resulting in a slower onset of fatigue (Zakynthinaki, 2015).

The velocity lactate threshold (VLT) is a well-known standard used for exercise intensity (Shen & Wen, 2019). In a study by Shen & Wen (2019), 84 adults were recruited for a graded exercise study where researchers used blood lactate analyzers and HR monitors to establish VLT prediction models. Initial treadmill speeds were 2m/s and 1.75m/s for men and women respectively. Speed was increased by 0.5m/s every three minutes along with a lactate sample. The test would be stopped when participants reach a blood lactate concentration of 4mmol/L or more, or if HR reached 90% or more of their max HR. The regression results from this study showed that 47-65% of variance could be explained by the models they developed, specifically the models using velocity at high HR showed good predictiveness. This study would suggest that HR could be a good predictor of blood lactate, and subsequently, substrate utilization with certain models (Shen & Wen, 2019).

Method CRA. Method CRA, or Method Cellular Respiration Analytics, is a program and tool that can be used to prescribe exercise routines that optimize aerobic metabolism and result in the best fat oxidation (Method, n.d.). Method CRA involves assigning an individual to a protocol

with an incremental workload increase which is typically performed on an exercise bike. The individual has their blood lactate sampled every three minutes, typically via the fingertip or earlobe, while undergoing the exercise test. The goal of this program is to identify three key points in relation to both blood lactate as well as heart rate.

The first point of note is prime (HR_{PR}). Prime is the heart rate that corresponds to a blood lactate concentration of around 2mmol/L and is associated with maximal aerobic fat oxidation. The next important point is anaerobic (HR_{A}). As stated previously, 4mmol/L blood lactate concentration is sometimes known as the lactate threshold but for the context of Method CRA, it will be called HR_{A}. HR_{A} is still defined the same as lactate threshold, however. The final notable point is peak (HR_{PK}). HR_{PK} is measured at a blood lactate concentration of 6mmol/L and is defined as the point at which exercise cannot be maintained much longer and could result in slight muscular tissue damage due to lactate buildup (Method, n.d.). With these three, very individualized points identified, an individual can be prescribed a precise HR_{PR} to exercise at and maintain optimal fat oxidation to lose weight in the most efficient and expeditious manner.

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