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EVALUATION OF LACTATE CONCENTRATION FROM 30 SECOND WINGATE

TEST USING THREE SAMPLE SITES

A Thesis submitted to the Graduate College of Marshall University

In partial fulfillment of the Requirements for the degree of Master of Science in Exercise Science

College of Health Professions School of Kinesiology

by

Bethany Christian

Approved by

Dr. Matthew Comeau, Committee Chairperson Dr. William Marley Dr. Terry Shepherd

Marshall University

Huntington, West Virginia

December, 2011

DEDICATION

I would like to dedicate this thesis to my father Craig Christian.

ACKNOWLEDGMENTS

I would like to thank several people who without their help this study could not have been conducted. First, Dr. Matt Comeau: thank you for all your hard work and dedication to this project. I appreciate your diligence. I could have not done this without you! Second, Michelle Copolo, you have been my right hand man in this study! I appreciate all that you have done! Dr. Terry Shepherd, thank you for your assistance in all the testing. Your presence made all the difference. Next, I would like to acknowledge Dr. Marley. Thank you for your guidance and knowledge. It is this you have provided that has enabled me to conduct such a study. To the College of Health Sciences: funding for this study would have been impossible without the gracious contributions made. Thank you! Last but certainly not least, the undergraduate assistants and testing subjects.

To all the undergraduate helpers: having your help in the lab ensured the precision and accuracy needed for this study. Thank you! Last, I would like to give a big thanks to the participants of the study. Without them, this study could have not been made possible.

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ABSTRACT

It is common for athletes to use blood lactate for the determination of performance as well as for the development of training volumes. Depending on the mode, duration, and intensity, the sample site at which blood lactate is drawn becomes important. Due to the debate of sample site, research has been ongoing. There has yet to be a collective investigation examining three sample sites and maximal anaerobic exercise while considering the role of inactive muscle. The purpose of this study was to gain better understanding of blood lactate concentrations at various sample sites after maximal effort exercise. Eight anaerobically trained males completed a 30 second Wingate test. Blood samples were collected after resting supine for 30 minutes before the test, directly after the test, and again 10 minutes post. All three analyses were significant (p < 0.05) with the sample site X sample time interaction, sample time main effect, and sample site main effect being reported, respectively: Wilks' $\Lambda = 0.004$, F (4,4) = 274.33, p = 0.000, Wilks' $\Lambda = 0.039$, F (2,6) = 74.111, p = 0.000, and Wilks' $\Lambda = 0..213$, F (2,6) = 11.066, p = 0.010. Post-hoc paired t-tests on computed mean differences between samples sites for each sample time revealed statistically significant differences (p < 0.05) between several time intervals. Further analysis also revealed significant differences (p < p0.05) in the sample site main effect between the toe and finger. To avoid misinterpretation caused by the pooling of lactate in non-exercising muscles, blood samples should be collected from a site closer to the active muscles.

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Chapter 1

INTRODUCTION

Measuring exercise performance is a major topic of interest in the world of exercise physiology as well as in athletics. Assessing lactate threshold during exercise has been a well investigated method of evaluating athletic performance. By determining blood lactate levels, training status of a given athlete can be used to manipulate a training prescription. Over the years, there has been much debate concerning whether sampling site of extracted blood lactate mirrors accurate data collection (Comeau, Lawson, Graves, Church, & Adams, 2011b; Feliu, Ventura, Segura, Rodas, Riera, Estruch, Zamora, & Capdevila, 1999; Forsyth & Farrally, 2000). The revisiting of the passive sink phenomenon has brought about concern as to whether current practices of sampling from one site, typically the finger or ear, is the most accurate representation of blood lactate concentration ([La⁻]). What differences, if any, are manifested by the chosen sample site considering the role of inactive muscles on lactate uptake? It was the nature of this question that prompted this investigation utilizing a 30 second maximal effort bout of exercise.

STATEMENT OF THE PROBLEM

The problem of the study was to determine the influence of non-active muscle on [La⁻]. The problem was to apply the concepts of Baker et al. (2002) to a maximal anaerobic test using three different sites to assess [La⁻] in order to determine the role sample site plays.

PURPOSE OF THE STUDY

Over the years, the importance of blood sampling site has become a major

concern. The ear lobe, great toe, finger and forearm veins and arteries have been some of the common sites utilized to sample blood to determine the onset of blood lactate accumulation (OBLA) and lactate threshold. Depending on the sample site and timing after exercise, blood lactate levels can vary. In recent studies, assumptions have been made to the varying lactate levels at different sites. It has been shown that the lactate is taken up by non-active muscles during intense aerobic and anaerobic exercise (Poortmans, Delescaille-Vanden Bossche, & Leclercq, 1978). Poortmans et al.'s theory of non-active muscles serving as a "passive sink" suggests this assumption. A study conducted by Comeau et al. (2011b), concluded that lactate is pooled in the upper extremity during lower extremity exercise in cyclists who exercised to exhaustion. Therefore, samples extracted from an inactive muscle group may elicit a higher [La] then blood extracted from an area close to active muscles used during exercise. If non-active muscle plays a significant role in the uptake of lactate, sampling site selection is critical. Given the misunderstanding of sampling site, role of inactive muscle, and flawed data that could result in inaccurate training programs, the main objective of this study was to further the investigation of sample site on blood lactate concentration before, during, and after a 30 second Wingate test using blood samples collected from the great toe, ear lobe, and fingertip

SIGNIFICANCE OF THE STUDY

The debate over sample site is just one facet of distinguishing the gold standard of retrieving and accessing [La⁻] after strenuous exercise. Another variable to be considered is the intensity and duration of activity (Lindinger, Heigenhauser, McKelvie, & Jones, 1990). The pattern at which lactate appears or disappears from the blood is crucial. It

has been documented that removal of lactate from the blood by non-active muscle is reversed soon after exercise subsides (Karlsson, Bonde-Petersen, Henriksson, & Knuttgen, 1975; Poortmans, et al., 1978). With this proposition, it is obvious the uptake of lactate from non-exercising muscle is reversed during recovery. Blood lactate accumulation and release is controlled by metabolic conditions of muscle such as blood plasma shifts. Dassonville et al. (1998) proposed that [La⁻] could be influenced by the ergometer and the exercise protocol. Blood lactate distribution may occur at a later time during recovery after plasma distribution has reached equilibrium. With exhaustive exercise performed at a higher intensity, a prolonged and hyperbolic plasma lipid and lipoprotein is generated. A study by Rettalick et al. (2007) suggested that short duration, high intensity aerobic exercise manifests notable plasma volume changes. The results implied that a 30 second burst of high intensity cycling using resistive forces induced an average change in plasma levels of 8.2%.

Baker et al. (2002) reported significant differences in [La⁻] as determined by blood samples taken from the ear lobes when resistive exercise was preformed. These differences in [La⁻] as determined by the samples were dependent on whether the subject gripped the handle bars or not. These findings are taken into much consideration when determining the upper body's role in the accumulation of blood lactate at various blood sampling sites before, during, and after resistive cycling.

The focus of this study was to provide an accurate assessment of the relationship between the upper body and the lower body in respect to blood lactate during maximal anaerobic exercise. Correcting for misleading results could provide new insight into athletic performance and contribute toward better understanding of lactate utilization

during exercise.

DELIMITATIONS

The study was delimited to:

- Eight healthy subjects who are currently participating in resistance training or some type of anaerobic exercise.
- Subjects were advised not to consume caffeine or any other stimulant 12 hours prior to testing
- 3. Subjects were advised not to eat 4 hours prior to testing.
- Each subject was briefed on experimental procedures and informed consent documents were signed.
- All testing was done using the same Lode Excalibur Sport cycle ergometer (Groningen, The Netherlands)
- 6. Body measurements were determined by skin fold along with height measuring to the nearest 0.1cm and weight to the 0.1kg, respectively.
- 7. Blood lactate concentration was obtained at rest, immediately after exercise, and every ten minutes after exercise for 30 minutes using standard capillary tubes and lancets and then stored in YSI Total blood L-lactate preservative tubes (#2315, Yellow Springs Incorporated, Yellow Springs, Ohio) containing a preservative and anti-coagulant.

LIMITATIONS

The study was limited to the following:

- 1. The sample size used in this study was small (N=8).
- 2. Although exercising was prohibited 24 hours prior to testing, exact pre-exercise

conditions cannot be controlled.

- 3. Subjects were advised not to consume any food 4 hours prior to testing, but whether the subject conformed to the request cannot be thoroughly controlled.
- 4. Resting lactate sample could lead to discomfort and or apprehension of the testing, which could affect performance.
- 5. Environmental elements such as temperature, humidity, and room distraction could influence the study.
- 6. Various amounts of anaerobic training of each subject prior to the testing.
- Distinguishing inactive muscle of the upper body is difficult. Further investigation is needed to examine which body tissues are the recipients of lactate (Retallick, et al., 2007).

ASSUMPTIONS

It was assumed that all of the subjects completed the health questionnaire to the best of their ability, were healthy, and gave a maximal effort performance. Furthermore, it was also assumed that outside stressors including medication did not counteract testing. It is assumed that cycling is a lower body activity leaving the upper body more inactive.

HYPOTHESIS

- H₀: There will not be a significant difference in [La⁻] between the three samples sites after a 30 second Wingate test.
- H₁: There will be a significant difference in [La⁻] between the three samples sites after a 30 second Wingate test.

DEFINITION OF TERMS

- Adenosine diphosphate (ADP) the end-product that results when ATP loses one of its phosphate groups located at the end of the molecule .The conversion of these two molecules plays a critical role in supplying energy for many processes of life.
 (McArdle, Katch, & Katch, 2010)
- *Adenosine triphosphate (ATP)* Considered by biologists to be the energy currency of life. It is the high-energy molecule that stores the energy needed for life (McArdle, et al., 2010)
- *Lactate threshold (LT)* The highest oxygen consumption or exercise intensity with less than a 1.0 mmol per liter increase in blood lactate concentration above the preexercise level (McArdle, et al., 2010)
- *Mean Power (MP)* is the average power output over the entire 30 seconds. It represents local muscle endurance throughout the test.
- *Onset of blood lactate accumulation (OBLA)* Blood lactate shows a systematic increase equal to 4.0 mmol (McArdle, et al., 2010)
- *Oxygen debt* Oxygen uptake above the resting state following exercise based on lactic acid theory. (McArdle, et al., 2010)
- Peak Power (PP) is the highest mechanical power achieved at any stage of the test (usually 3-5 seconds). It represents the explosive characteristics of a person's muscle power. (Force x Total Distance)
- Rate of Fatigue is the drop in power from Peak Power to the lowest power and is presented in % (Highest 5 second peak power minus the lowest 5 second power multiplied by 100) (Baker, et al., 2002)

Chapter 2

REVIEW OF LITERATURE

Muscle lactate over the years has had many different assumed roles during exercise (Laughlin, Korthuis, Duncker, & Bache, 1996). Fletcher and Hopkins (1907) correlated the relation between lactate acid formation and oxygen (O₂) deficiency. Using frogs they found that the amount of lactate produced increased under anaerobic conditions compared to aerobic. In light of Fletcher (1907), further studies have been conducted deepening the understanding of lactate related to the metabolic shift from aerobic to anaerobic metabolism during exercise (Robergs, Ghiasvand, & Parker, 2004). Robergs et al. (2004) details the role and utilization of ATP (adenosine triphosphate) as energy and the formation of lactate during exercise. When an ATP molecule is split for energy, it is broken down into an ADP and inorganic phosphate molecule with the release of one hydrogen ion (Robergs, et al., 2004; Roth, 1991). Exercise causes the need to break down ATP for energy at the rate it is needed (Karlsson, et al., 1975)

Therefore, increased intensity coincided by an accumulation of protons (hydrogen ions) occurs in the muscle due to a much greater involvement of the phosphagen and glycolytic energy systems (Faude, Kindermann, & Meyer, 2009). When the amount of required energy needed to fuel muscle surpasses the resources supplied by the oxidative pathway, glycoloysis proceeds to anaerobic glycoloysis causing large amounts of lactate to be produced (el-Sayed, George, & Dyson, 1993a). This initial increase of blood lactate is known as onset of blood lactate accumulation (OBLA). OBLA occurs when the concentration of blood lactate reaches approximately 4 mmol·L⁻¹. As [La⁻] increases, due to the buildup of hydrogen ions, a decline in muscle pH occurs, a consequence of acidosis

in the muscle (Roth, 1991). During intense exercise, the rate of lactate production exceeds the rate of removal causing muscle fatigue. This concept is known as lactate or anaerobic threshold (Tanaka, Matsuura, Kumagai, Matsuzaka, Hirakoba, & Asano, 1983).

The original concept of muscle lactate during exercise suggested by Fletcher and Hopkins proposed the theory of limiting oxygen and delivery resulting in muscle anerobiosis (Brooks, 1986). The production of lactic acid, muscle glycogenolysis, and glycolysis are all motivated by the theory of O_2 Debt. Nevertheless, numerous studies have since been conducted showing that lactate is not just a waste product caused by the lack of O_2 (Donovan & Brooks, 1983). The production of lactate is a process that occurs during exercise but also can occur in the resting individual (Brooks & Gaesser, 1980). The production of lactate is not just related to the oxygen availability but more so to the metabolic rate (Faude, et al., 2009). Moreover, Brooks (1986) concluded that lactate production is a result of muscle glycogenolysis and glycolysis, but, during exercise, lactate serves the maintenance of blood glucose, and a shuttle carrying oxidizable substrate (in the form of lactate) out of the muscle.

LACTATE ACCUMULATION AND EXERCISE

Many have a distorted view of lactate with misconceptions that lactate causes a direct negative effect on athletic performance. Many researchers have concluded that any limiting influence on muscle to contract is due to the increase in hydrogen ions (acidosis) during the break down of ATP (Cairns, 2006; Robergs, et al., 2004; Tesch, 1980). As stated earlier, when hydrogen ions increase the muscle pH decreases resulting in acidosis. This increase of acidity is the cause of muscle fatigue during maximal exercise, not the

presence of lactate. New investigations have challenged this belief of muscle fatigue. Cairns (2006) hypothesized that acidosis played a little role on muscle fatigue during exercise and it may even increase performance. There have also been assumptions that the inorganic phosphate is produced during the breakdown of ATP causing muscle fatigue during intense activity (Westerblad, Allen, & Lannergren, 2002). Even so, other considerations have been proposed concerning acidity and muscle fatigue. Further investigation of acidosis during exercise is needed to confirm or deny these claims. Some researchers differ in opinion. Kelly et al. (2002) suggested oxidation and glycogen synthesis represented only minor pathways for lactate disposal (6-15%). Although the fate of muscle lactate has been thought to be oxidized and redistributed, further research is needed to clarify these perspectives

METABOLIC RESPONSE

Metabolic response to stress is dependent to the amount of stress that is applied to the environment (Laughlin, et al., 1996). That being said, a correlation between lactate and intensity is relevant in the examination of muscle lactate during exercise. Kelley et al. (2002) proposed that the rates of various muscles play a part in determining the rate of lactate accumulation during exercise. During steady-state exercise, it is thought that most muscle lactate is oxidized by muscles (Brooks, 1986). In accordance with this belief, Gladden et al. (1991) found that lactate shuttled by the canine skeletal muscle increased when the metabolic rate of the muscle was increased. As lactate forms within the active muscle during low intensity exercise, lactate is synthesized into glycogen or oxidized to maintain the state of low blood [La⁻] (Lindinger, et al., 1990). When the stress or demands are increased on the muscle, the amount of fuel from oxidative pathways

becomes limited and the uptake of lactate out of the muscle decreases.

PASSIVE SINK

It is thought that, during exercise, lactate is primarily shuttled out of the muscle to organs, active muscle, and non-active muscles (Poortmans, et al. 1978). This being said, the fate of lactate along with the role of non-active muscles has been an ongoing concern. In a study conducted by Poortmans et al. (1978), lactate uptake by non-active muscles was observed. Poortmans et al. (1978) theorized that non-active muscle plays a role in lactate removal during exercise, but soon stops shortly after exercise has ended. This theory has been thought of as "passive sink." "Passive sink" was demonstrated by subjects during graded leg exercises when net uptake of lactate in inactive muscles increased during progressive exercise and then decreased directly after. The reported uptake was proportional to the blood [La]. More studies have been done furthering Poortmans's conclusions. Comeau et. at. (2011b)examined the relationship between blood [La⁻], varying sample sites, and non-active muscle to further the understanding of the determination and analysis of lactate during exercise. It was concluded that nonactive muscles indeed serve as a passive sink, which causes significant differences in lactate accumulation depending on the location of the active muscle (2011b). Sampling blood lactate from sites that are not close to the working muscle could result in inaccurate blood [La] level due to the pooling of lactate in areas of inactive muscles.

SAMPLE SITE

Common sample sites to extract blood lactate include the forearm, earlobes, finger tips and the great toe (Dassonville, et al., 1998). Considering inactive muscle has a proven role as a passive sink for lactate uptake during exercise, sample site at which [La⁻]

is taken becomes a concern (Comeau, et al., 2011b; Lindinger, et al., 1990; Poortmans, et al., 1978). Ongoing debate has lingered for some time regarding accuracy of sample sites used when sampling blood for the determination of lactate. Considering the influence of non-exercising muscles, blood [La⁻] may differ from sample site depending on the mode of exercise (Comeau, et al., 2011b; el-Sayed, et al., 1993a; Feliu, et al., 1999). Furthermore, if non-active muscles act as a passive sink for lactate, then blood samples extracted from sites closer to the non-active muscle may have higher [La⁻] than those sites near the active muscle (Comeau, et al., 2011b)

Many studies have examined validity of blood sample sites. Dassonville et al. (1998) examined three sample sites (index finger, ear lobe, and forearm) during upper and lower ergometry and treadmill run. It was concluded that lactate values can fluctuate by sample site depending on the mode of exercise. Parallel investigations by Foxdal et al. (1991) and Robergs et al. (2004) investigations did not result in different amounts of blood [La⁻] when using a 4 mmol·L⁻¹ OBLA while cycling and running on a treadmill. Results from el Sayed et al. (1993b) yielded significant differences in fingertip and venous blood from the forearm during treadmill running with increased intensity. These results demonstrate the lactate-intensity relationship. The greater the intensity, the greater the lactate difference between sample sites.

One particular study that is inconsistent with the previous findings tested blood [La⁻] from the ear lobe, finger, and toe in rowers. Forsyth and Farrally (2000) found that there was no significant difference in lactate accumulation at the various sites. Garland and Atkinson (2008) also elicited these results when testing rowers. The higher blood [La⁻] at the toe versus the earlobe opposes the muscle passive sink theory, but it is

believed to be because rowing is a full body activity that activates many muscles, and this could be the reason for their findings.

Considering that more athletes are now using lactate variables to evaluate fitness and manipulate training, the precise determination and assessment of lactate from exercise is crucial to better assist the athlete. The precise determination of blood sample site for a given activity has progressed over the years, but still more research is needed to provide set protocols for blood lactate evaluation.

SAMPLE TIME

Just as sample site is crucial, so is the timing of extraction after exercise. At rest, the normal range for blood lactate is 0.5 to 2.2 mmol·L⁻¹(Gollnick, Bayly, & Hodgson, 1986). The average [La⁻] after a maximal effort exercise is 20- 205 mmol·L⁻¹, but elite athletes can range higher (Hermansen & Stensvold, 1972). Stone et al. (1987) found that trained individuals compared to non-trained produced a significantly greater amount of blood lactate during intense exercise (squats).

TIMING

Poortmans et al. (1978) proved that blood lactate rapidly declines after exercise is completed. A study done by Lindinger et al. (1990) suggested that the rate at which lactate is removed is proportional to the halftime at the end of exercise. Given this information, it can be assumed that blood [La⁻] directly after exercise and following exercise varies depending on the time at which it was sampled. Depending on the mode of exercise and sample site, extracted blood borne parameters could be altered. Comeau et al. (2011b) analyzed the upper body's role on lactate production from a lower body exercise along with the sample site and timing of the sample. Results included a greater

rate of decline in lactate from the finger samples than the toe sample after the completion of a lower body intense exercise, and significant blood [La⁻] changes up to twenty-five minutes post exercise. Training has also proven to increase the rate at which lactate is removed after aerobic and anaerobic exercise (Gollnick, et al., 1986). Furthermore, appropriate extraction time of a blood lactate sample is necessary to provide a true representation of [La⁻] after exhaustive activity.

LACTATE AS AN INDICATOR OF PERFORMANCE

The determination of blood lactate is a very common tool used by athletes in order to better assess fitness. Lactate can be an extremely useful instrument in prescribing exercise intensities, provided precise knowledge is accessible from the physiologist as well as understanding from the athlete. The point at which lactate concentration increases to 4 mmol·L⁻¹ of blood or OBLA, represents the point at which lactate production and removal are at equilibrium (el-Sayed, et al., 1993a). This point is considered to be the intensity required to move oxidative energy supply to anaerobic pathways (Rusko, Rahkila, & Karvinen, 1980). Because this increased lactate production coincides with acidosis, lactate measurement is an exceptional indicator for the metabolic condition of the cell (Robergs, et al., 2004) with OBLA and performance being directly correlated (el-Sayed, et al., 1993a; Karlsson, et al., 1975). Using OBLA, training intensity can be adapted to elicit metabolic changes within the muscle (Sjödin, Jacobs, & Svedenhag, 1982).

 VO_2max is often mentioned as an indicator of an athlete's fitness. The VO_2max is a good general indicator of endurance fitness and has been known as the gold standard measurement of aerobic ability. Although VO_2max has been used as reliable source of

performance, lactate measurements obtained from the blood serves as a more sensitive measure of an athlete's fitness during exercise (Davis, Frank, Whipp, & Wasserman, 1979). Koutedakes et al. (1990) presented that, after five months of endurance training, the VO₂max did not change significantly, but the running speed at a set lactate concentration of 2.5 mmol·L⁻¹ did increase significantly from 4.66 m·s⁻¹ to 5.09 m·s⁻¹. The investigation of Koutedakes et al. (1990) signifies the advantages of lactate testing by showing the sensitivity of lactate to expose changes in fitness compared to the VO₂max test.

SUMMARY

The previous research highlights some of the positive uses of lactate from a performance stand point, but it also addresses many of the difficulties with regard to getting the best and most accurate data when sampling blood lactate.

Chapter 3

METHODS

SUBJECT RECRUITMENT

Eight subjects with exercise history volunteered to assist in this study. All of the subjects use anaerobic training as a source of exercise (2-3/week). All were ACSM risk stratified and considered to be low risk. Proper instructions, procedures, and risks of the experiment were given prior to exercise testing. Each completed an informed consent and was notified that withdrawal from testing at any time could be granted. This study received IRB approval (See Appendix A). The subjects were instructed not to exercise for 24 hours prior to testing nor consume food or caffeine 4 hours prior to testing.

INSTRUMENTATION

In order to conduct this study, the following instruments were used: Lode Excalibur Sport Ergometer (Groningen, The Netherlands), YSI 2300 STAT plus-lactate analyzer (Yellow Springs, OH), Lange skinfold calipers (Cambridge Maryland), and weight scale (Seca Alpha, Model 770).

STUDY DESIGN

Due to the ongoing debate involving the site at which blood lactate should be measured in order to correctly determine [La⁻] because of the upper body's role as a "passive sink," this study was designed to measure [La⁻] from both the upper body, lower body, and a non-extremity site after a maximal lower body exercise. In order to better understand sample site and the role it plays in [La⁻], samples were obtained from the great toe, the index finger, and the ear lobe. Blood samples were taken after a 30 minute rest prior to exercise, directly after exercise, and every ten minutes after for 30

minutes to determine [La⁻] associated with the patterns of the initial lactate appearance and disappearance.

PROCEDURE

Prior to testing, the subject's height and weight were measured to the nearest 0.1 cm and 0.1 kg using an electronic scale and a measuring tape. Body fat of each subject was taken using the three site ACSM guidelines with a Lange skinfold caliper. These three sites were the chest, abdomen, and thigh. The subjects rested in a supine position for 30 minutes on a cushioned table, the blood samples were drawn from the index finger of the non dominant hand, the earlobe, and the greater toe on the ipsilateral side. This procedure was repeated directly after the ergometer test and again every 10 minutes during a 30 minute active recovery.

After the subject was seated on the Lode Excalibur Sport Bike, the handlebar was adjusted both horizontally and vertically depending on subject. Moreover, the saddle was manipulated for horizontal, vertical and angle adjustment (Dotan & Bar-Or, 1983). Each subject was adjusted according to body stature to ensure a slight flex (5-10 degrees) of the knee after a revolution. Toes were held in place with toe straps connected to the pedals. The standard Wingate protocol was used, which consisted of the subject being seated with hand on the handlebars in a gripping manner. Using the same format as Baker et al. (2002), a standard leg-cycle ergometry protocol was used. A one minute programmed warm up at 60 revolutions per minute was instructed, a 3 second countdown to the start of the 30 seconds of testing was given, following a maximal 30 second effort against a fixed resistive lode of 75 g·kg⁻¹ of total body mass. After completion of the 30 second bout, subjects completed an active cool down against 50 W of resistance at

approximately 60 rpm.

BLOOD SAMPLING AND ANALYSIS

Subjects rested in a supine position for 30 minutes on a cushioned table, blood samples were drawn from the index finger of the non dominant hand, the earlobe, and the greater toe on the ipsilateral side. This procedure was repeated directly after the ergometer test and every 10 minutes during an active cool down. Each sample site was wiped with a 70% isopropyl alcohol swab before blood extraction. Upon lancing the site, the initial blood drop was wiped away and then two 50 µL capillary tubes were filled. The amount of time to collect each sample was minimal (45-90 seconds). The individual blood samples collected for analysis were transferred to and stored in YSI Total blood Llactate preservative tubes (#2315, Yellow Springs Inc., Yellow Springs, Ohio) containing a preservative and anti-coagulant. Total blood lactate concentration was determined using a YSI 2300 STAT plus-lactate analyzer (Yellow Springs, OH) with a cell lysing agent buffering system.

STATISTICAL ANALYSIS

All results were reported as means \pm standard deviation. A two-way, 3 X 3 (site x sample time) within-subjects repeated-measures ANOVA ($p \le 0.05$) was used to determine significance differences between the [La⁻] obtained from the three sample sites at rest, immediately post-exercise, and 10 minute post-exercise during an active recovery. Paired samples t-tests ($p \le 0.05$) were used to calculate pair wise differences for each time variable for every possible time combination to determine where differences occurred. Paired samples t-tests (p < 0.05) were utilized to analyze main effect differences. The data were analyzed using SPSS version 17.0 (SPSS, Inc., Chicago, IL).

Chapter 4

RESULTS

Descriptive statistics for all subjects are displayed in Table 1. A two-way (3 X 3) repeated-measures ANOVA was used to determine the effect of sample site and sample time on whole blood lactate concentration ([La⁻]). The sample site X sample time interaction effect, as well as the sample site and sample time main effect, was tested using the multivariate criterion of Wilks' lambda (Λ). All three analyses were significant (p < 0.05) with the sample site X sample time interaction, sample time main effect, and sample site main effect being reported, respectively: Wilks' $\Lambda = 0.004$, F (4,4) = 274.33, p = 0.000, Wilks' $\Lambda = 0.039$, F (2,6) = 74.111, p = 0.000, and Wilks' $\Lambda = 0.213$, F (2,6) = 11.066, p = 0.010. Figure 1 displays the interaction and main effect responses. Posthoc paired t-tests on computed mean differences between samples sites for each sample time revealed statistically significant differences (p < 0.05) between all time intervals as displayed in Figures 2-4 with the exception of the finger and ear 10 minutes postexercise. Paired t-tests also revealed significant differences (p < 0.05) in the sample site main effect between the toe and finger only (Figure 5).

Age (y)	25.8	<u>+</u>	6.5
Height (cm)	182.4	<u>+</u>	4.9
Weight (kg)	97.4	<u>+</u>	18.4
Body Fat (%)	13.2	<u>+</u>	5.4

Table 1. Descriptive statistics of subject (mean \pm SD).

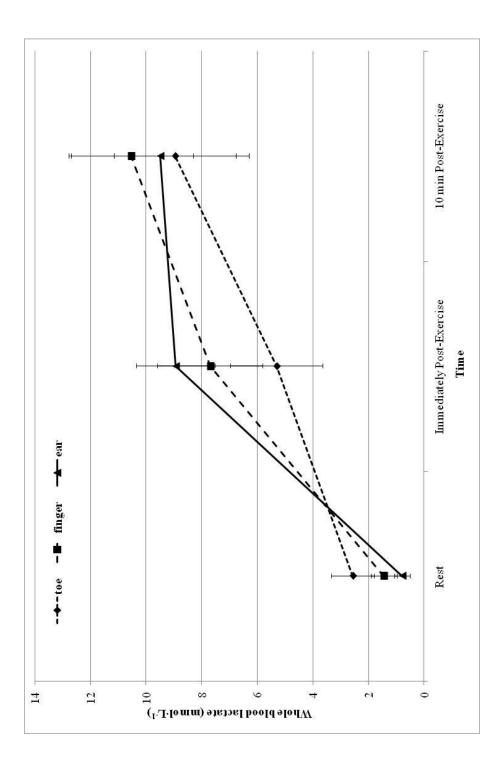
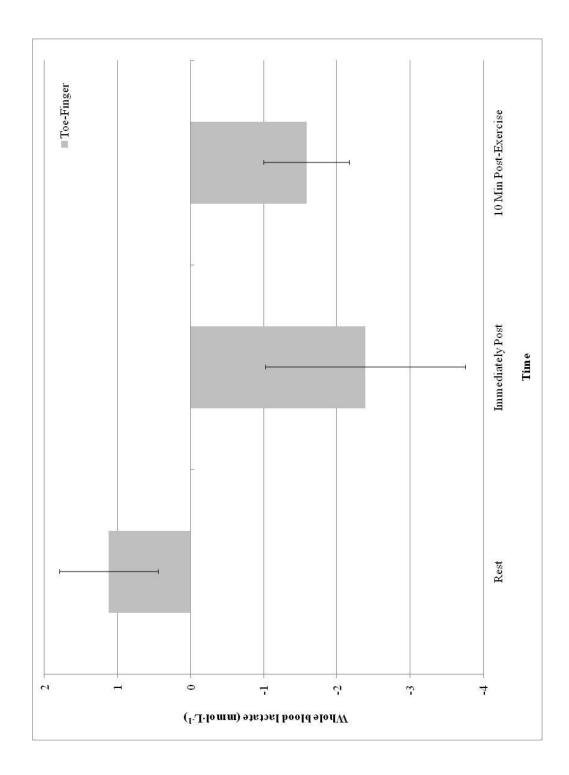
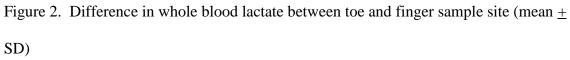


Figure 1. Sample site by time interactions for whole blood lactate (mean \pm SD)





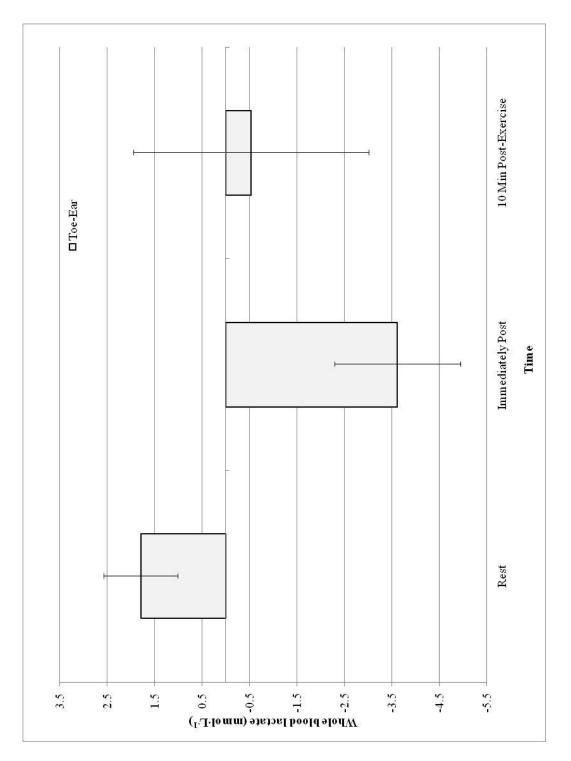
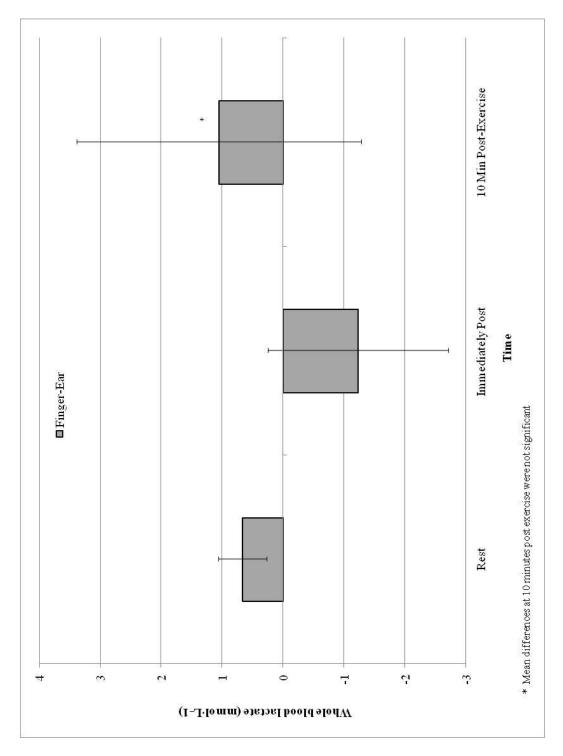
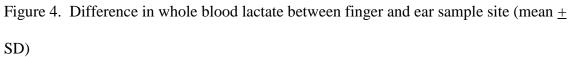


Figure 3. Difference in whole blood lactate between toe and ear sample site (mean \pm SD)





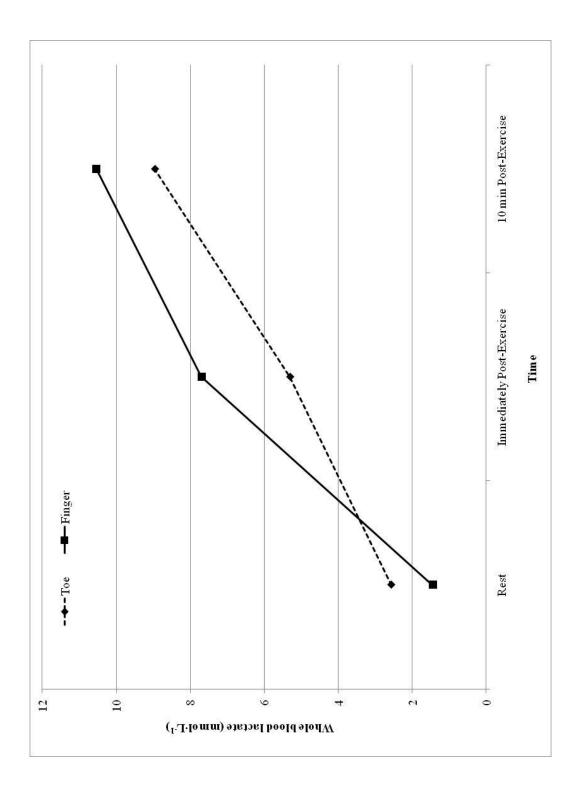


Figure 5. Sample site main effects for toe and finger (mean \pm SD)

Chapter 5

DISCUSSION

Blood lactate sample site is crucial in the determination of athletic performance and the better establishing of training volumes and outcomes (Comeau, Adams II, Church, Graves, & Lawson, 2011a; Comeau, et al., 2011b). Numerous studies have suggested that lactic acid is pulled toward inactive muscles during exercise (Ahlborg, Hagenfeldt, & Wahren, 1976; Brooks, 1986; Buckley, Scroop, & Catcheside, 1993; Catcheside & Scroop, 1993; Comeau, et al., 2011b; Dassonville, et al., 1998; Kelley, et al., 2002; Poortmans, et al., 1978).

In the world of physiology, it is known that muscle lactate is a by-product of anaerobic metabolism. During strenuous exercise, an excessive amount of muscle lactate is produced. As intensity and time increase, so does blood lactate (Figure 1). Our results mimic the findings of previous research (Comeau, et al., 2011b; Poortmans, et al., 1978). Higher lactate values were sampled from the finger compared to samples pulled from the toe during a lower extremity exercise (Figure 1). Although the [La⁻] measured in our study is larger than that reported by Forsyth et al. (2000), there was no significant difference between the finger and ear as sample sites throughout the measurement period, which is similar to their findings. Dassonville et al. (1998) also concluded that lactate values differ depending on sampling site (ear, forearm, and finger) and also recognized that the mode of exercise can affect results (ergometers, treadmill, and an arm crank).

Kelley et al. (2002) were some of the first to truly acknowledge the definition of passivity in muscle when looking at lactate metabolism in canine skeletal muscle. Therefore, the muscles that are not primarily engaged in the exercise in question will

serve in a passive sink role. Hence, the sampling site with the consideration of active and non-active muscle needs to be considered. As can be seen in Figure 1, the finger and ear are both serving as lactate reservoirs during exercise. Although Forsyth and Farrally (2000) found no difference in lactate levels from the ear, finger, and ear of rowers, these findings could be the result that rowing is considered more of a full body exercise compared to a cycle test. If both the arms and legs are active during the exercise bout, as they would be when rowing, there is no pooling of lactate in the inactive muscles.

Baker et al. (2002) found differences in blood [La⁻] sampled from the ear lobe when comparing cyclists who gripped compared to those who did not grip the handlebars and stated that gripping of the handle bar increased the [La⁻]. Gripping the handle bars may have been the reason for the increased [La⁻] observed in the ear and finger samples in the immediate post-exercise blood sample. Given the overall size of the musculature in the lower extremity versus that in the upper extremity, the toe [La⁻] was still the lowest throughout the sampling time period. This interpretation leads us to believe that the upper extremity and possibly the upper body as a whole were serving in a passive sink capacity.

With these findings we can assume that passive sink phenomenon is the main contributor of the sample site differences at a specific time. Non-active muscle plays a leading role in the uptake of muscle lactate. Therefore, sample site selection is critical in the determination of accurate blood lactate concentrations (See Figure 1). When assessing the given relationship between the upper body and lower body during exercise, sample site should be selected in a location near the active muscle group.

Depending on whether the exercise is a lower extremity or upper extremity and depending on where the sample is obtained, results can be misinterpreted. Extracting lactate samples from further away from the active muscles may result in elevated blood lactate compared to samples close to active muscles. The interpretation of inaccurate data could cause faulty intensity outcomes along with skewed training protocols.

PRACTICAL APPLICATION

These results should be taken into consideration for further lactate research and in the measurement of lactate to predict athletic performance. Sample sites closest to the active muscle should be used for true representation of blood lactate.

Appendix A

IRB



w w w . m a r s h a l l . e d u

Office of Research Integrity Institutional Review Board 401 11th St., Suite 1300 Huntington, WV 25701 FWA 00002704

IRB1 #00002205 IRB2 #00003206

November 8, 2011

Matthew Comeau, PhD School of Kinesiology

RE: IRBNet ID# 198974-2 At: Marshall University Institutional Review Board #1 (Medical)

Dear Dr. Comeau:

 Protocol Title:
 [198974-2] The Effects Of A 30 Second Cycle Sprint Performance On Blood Lactate Concentrations Sampled From Three Sites

 Expiration Date:
 November 8, 2012

 Site Location:
 MU

 Submission Type:
 Continuing Review/Progress APPROVED Report

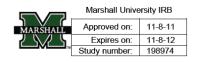
 Review Type:
 Expedited Review

The above study and informed consent were approved for an additional 12 months by the Marshall University Institutional Review Board #1 (Medical) Chair. The approval will expire November 8, 2012. Continuing review materials should be submitted no later than 30 days prior to the expiration date.

If you have any questions, please contact the Marshall University Institutional Review Board #1 (Medical) Coordinator Trula Stanley, MA, CIC at (304) 696-7320 or stanley@marshall.edu. Please include your study title and reference number in all correspondence with this office.

Appendix B

Informed Consent



Informed Consent to Participate in a Research Study

The Effects Of A 30 Second Cycle Sprint Performance On Blood Lactate Concentrations Sampled From Three Sites

Matthew J Comeau, PhD, ATC, CSCS, Principal Investigator

Introduction

You are invited to be in a research study. Research studies are designed to gain scientific knowledge that may help other people in the future. You may or may not receive any benefit from being part of the study. There may also be risks associated with being part of research studies. If there are any risks involved in this study then they will be described in this consent. Your participation is voluntary. Please take your time to make your decision, and ask your research doctor or research staff to explain any words or information that you do not understand.

Why Is This Study Being Done?

The purpose of this study is to determine the effects of a 30 second cycle sprint performance on blood lactate concentrations

How Many People Will Take Part In The Study?

About 15 people will take part in this study. A total of 20 subjects are the most that would be able to enter the study.

What Is Involved In This Research Study?

Before you begin the study, the following would occur

- Report to the Human Performance Laboratory in Gullickson Hall
- Read consent form and sign if willing to participate
- Height, weight, and age will be recorded.

During the study, you will:

- Lie supine and rest for 30 minutes
- A very small sample of blood will be obtained from the earlobe, ring finger, and great toe of the non-dominant side
- A Wingate test will be performed, which is a 30 second sprint on a cycle ergometer against a resistance equal to 7.5% of your body weight.

• You will remain seated for 30 minutes after the sprint performance and blood samples will be obtained every 5 minutes

How Long Will You Be In The Study?

You will be in the study for about approximately 1 to 1.5 hours.

You can decide to stop participating at any time. If you decide to stop participating in the study, we encourage you to talk to the investigators or study staff to discuss what follow up care and testing could be most helpful for you.

The study doctor may stop you from taking part in this study at any time if he/she believes it is in your best interest; if you do not follow the study rules; or if the study is stopped.

What Are The Risks Of The Study?

Being in this study involves some risk to you. You should discuss the risk of being in this study with the study staff.

You should talk to your study doctor about any side effects that you have while taking part in the study.

Risks and side effects related to the sampling of blood: pricking sensation in the ear, finger and toe.

Risks and side effects related to the sprint performance: possible light headedness and nausea

Are There Benefits To Taking Part In The Study?

If you agree to take part in this study, there may or may not be direct benefit to you. We hope the information learned from this study will benefit other people in the future. The benefits of participating in this study may be: to determine the effects of a 30 second cycle sprint performance on blood lactate concentrations.

What About Confidentiality?

We will do our best to make sure that your personal information is kept confidential. However, we cannot guarantee absolute confidentiality. Federal law states that we must keep your study records private. Nevertheless, certain people other than your researchers may also need to see your study records. By law, anyone who looks at your records must keep them completely confidential.

Those who may need to see your records are:

• Certain university and government people who need to know more about the

study. For example, individuals who provide oversight on this study may need to look at your records. These include the Marshall University Institutional Review Board (IRB) and the Office of Research Integrity (ORI). Other individuals who may look at your records include: *the federal Office of Human Research Protection*. This is done to make sure that we are doing the study in the right way. They also need to make sure that we are protecting your rights and your safety.

If we publish the information we learn from this study, you will not be identified by name or in any other way.

What Are The Costs Of Taking Part In This Study?

There are no costs to you for taking part in this study. All the study costs, including any study medications and procedures related directly to the study, will be paid for by the study. Costs for your regular medical care, which are not related to this study, will be your own responsibility.

Will You Be Paid For Participating?

You will receive no payment or other compensation for taking part in this study.

What Are Your Rights As A Research Study Participant?

Taking part in this study is voluntary. You may choose not to take part or you may leave the study at any time. Refusing to participate or leaving the study will not result in any penalty or loss of benefits to which you are entitled. If you decide to stop participating in the study we encourage you to talk to the investigators or study staff first to learn about any potential health or safety consequences.

Whom Do You Call If You Have Questions Or Problems?

For questions about the study or in the event of a research-related injury, contact the study investigator, Matthew Comeau, PhD at 696-2925 8:00 am - 5:00 pm. You should also call the investigator if you have a concern or complaint about the research.

For questions about your rights as a research participant, contact the Marshall University IRB#1 Chairman Dr. Henry Driscoll or ORI at (304) 696-7320. You may also call this number if:

- You have concerns or complaints about the research.
- The research staff cannot be reached.
- You want to talk to someone other than the research staff.

You will be given a signed and dated copy of this consent form.

SIGNATURES

You agree to take part in this study and confirm that you are 18 years of age or older. You have had a chance to ask questions about being in this study and have had those questions answered. By signing this consent form you are not giving up any legal rights to which you are entitled.

Subject Name (Printed)	
Subject Signature	Date
Person Obtaining Consent	Date
Principal Investigator	Date
Witness (If not applicable, omit this line)	Date

Appendix C

Raw Data

Subject	Toe Resting Lactate	Finger Resting Lactate	Ear Resting Lactate
	$(\text{mmol}\cdot\text{L}^{-1})$	$(\text{mmol}\cdot\text{L}^{-1})$	$(\text{mmol} \cdot \text{L}^{-1})$
1	2.02	1.87	0.97
2	4.29	2.34	1.03
3	2.49	0.89	0.49
4	2.08	1.47	1.16
5	2.06	1.50	0.36
6	2.03	1.27	0.95
7	2.78	1.07	0.65
8	2.84	1.21	0.69
Subject	Toe Lactate	Finger Lactate	Ear Lactate Immediately
	Immediately Post	Immediately Post	Post Exercise
	Exercise (mmol· L^{-1})	Exercise (mmol· L^{-1})	$(\text{mmol}\cdot\text{L}^{-1})$
1	7.79	12.15	10.85
2	8.08	8.06	10.45
3	4.84	6.96	6.79
4	4.05	6.61	9.68
5	4.82	6.39	9.05
6	3.82	7.33	8.52
7	4.30	7.59	8.83
8	4.82	6.53	7.31
Subject	Toe Lactate 10 Min Post	Finger Lactate 10 Min	Ear Lactate 10 Min Post
_	Exercise (mmol· L^{-1})	Post Exercise	Exercise (mmol· L^{-1})
		$(\text{mmol} \cdot \text{L}^{-1})$	
1	11.30	12.15	11.70
2	12.60	14.40	14.80
3	8.09	10.90	10.20
4	6.38	7.86	8.22
5	9.23	11.05	11.30
6	7.52	8.71	8.58
7	9.80	11.20	4.60
8	6.79	8.10	6.58

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Resume of

Bethany Christian

Bethany Christian

1562 Washington BLVD APT B Huntington, WV 25703 (304) 639-4067 Christian49@marshall.edu

SUMMARY OF QUALIFICATIONS

- Solid academic and clinical background in Exercise Science.
- Exceptional communication skills; proven ability to work well with varied populations.
- Outgoing and personable; great motivational attitude.
- Strong work ethic; financed most of college expenses through full time work CPR Certification
- Personal Training Certification by ACSM in progress

EDUCATION

- **B.A. Exercise Science** May 2009 Marshall University, Huntington, WV. GPA: 3.6, Major GPA: 3.85
- **M.A Exercise Physiology** In Progress Marshall University, Huntington, WV G.P.A 3.5

CLINICAL EXPERIENCE

Exercise Physiology Graduate Assistant Present

Marshall University, Huntington, WV

- Exercise Physiology Lab Course Instructor
- Supervise and perform exercise testing on athletes as well as the community
- Assist department professors

Clinical Experience (480hours) August 2008 – May 2009

Nichols Chiropractic, Ashland, KY

- Supervised chiropractic exercise sessions; routine exams.
- Provided general equipment care and maintenance.

Research Experience June 2010

Marshall University Camp New You

• Assisted principle investigators with developing an effective intervention to improve the health and fitness levels of at-risk adolescents.

RELEVANT WORK EXPERIENCE

Exercise Specialist (150) March 2002 – January 2008

Marshall University Fitness Center, Huntington, WV

- Supervised use of exercise equipment; assisted in fitness testing.
- Served as a Personal Assistant, aiding clients in exercise
- Developed exercise prescriptions; assisted with education sessions