ABSTRACT

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The purpose of this study was to evaluate the validity of the Accusport™ portable blood lactate analyzer in cold environments as compared to a reference method (Yellow Springs Instruments enzyme electrode system [YSI]). It was hypothesized that the Accusport™ would not be accurate in cold environments (cold 1 = 5-10°C and cold 2 = < 0°C). Subjects consisted of male and female well-trained nonathletes. A VO_2 max test, as well as 2 additional tests were performed on an electrically braked cycle ergometer. Blood lactate and heart rates were measured using both the YSI and Accusport™ at rest and during the last 30 seconds of each testing stage. Blood samples of > 50 µl were drawn from the fingertip and separated for analysis. A 25 µl sample was transferred to a buffer tube for YSI analysis in the lab at room temperature. The additional 25 µl sample was drawn for Accusport™ analysis under room temperature, cold 1, and cold 2 conditions. There was a consistent relationship between BL concentrations measured using the Accusport™ in all temperature environments measured in this study. However, BL concentrations achieved using the Accusport™ were about 25% greater than the reference method.

A COMPARISON OF THE YELLOW SPRINGS INSTRUMENTS AND THE ACCUSPORT™ PORTABLE LACTATE ANALYZER FOR MEASURING BLOOD LACTATE IN COLD ENVIRONMENTS

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THESIS FINAL ORAL DEFENSE FORM

Candidate: Jodi L. Franklin

We recommend acceptance of this thesis in partial fulfillment of this candidate's Requirements for the degree:

Master of Science in Exercise and Sport Science-Human Performance

The candidate has successfully completed the final oral defense.

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This thesis is approved by the College of Health, Physical Education, and Recreation.

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INTRODUCTION

Background

The pattern of accumulation of blood lactate (BL) is highly related to performance ability in endurance athletes [3,5,7,9,10]. Additionally, changes in the pattern of BL accumulation reflect adaptations to training much more effectively than changes in VO_{2max} [2,5,10,11,14]. Accordingly, the measurement of BL has been used to develop and monitor training programs for athletes [7,10,11].

Numerous methods have been used to determine BL concentrations, including blood sampling from different sites including capillary, venous, or arterial [6,8], using lysed or unlysed whole blood and plasma [1]. Instrumentation for testing lactate in the laboratory setting primarily includes enzyme electrode systems (Yellow Springs Instruments [YSI]). Recently, portable enzyme driven systems have become available which allow coaches and physiologists to benefit from conducting tests in the field.

Since portable lactate analyzers are enzyme driven, they are potentially temperature sensitive. Accordingly, there may be some inaccuracy between BL testing in a laboratory environment as compared to the field, particularly in colder environments. However, the magnitude of potential problems during testing in cold environments is not established. The manual for one popular portable BL analyzer, the Accusport™, lists workable temperature ranges for the analyzer (> 20°C) which do not include colder environments (20°C to < 0°C). Accordingly, the purpose of this study was to

systematically evaluate the performance of the Accusport™ under varying temperature conditions.

METHODS AND PROCEDURES

Subject Selection

The subjects for this study were 13 males and females who were apparently healthy, well-trained non-athletes. All subjects were informed of potential health risks and provided a written informed consent prior to participation. The testing protocol had been approved by the University of Wisconsin-La Crosse IRB. Descriptive subject characteristics are shown in Table 1.

Table 1. Individual subject characteristics (mean +/- standard deviation)

Variable	Male (n = 7)	Female (n = 6)
Height (cm)	178.9 ± 9.2	163.9 ± 6.6
Weight (kg)	90.7 ± 13.1	60.0 ± 6.6
Age (years)	23.0 ± 2.4	20.8 ± .75
Peak Power Output (Watts)	258 ± 51	215 ± 36
Peak VO_2 (liters x min^{-1})	3.40 ± .40	2.62 ± .28
Peak VO_2 (ml x min^{-1} x kg^{-1})	38.3 ± 8.3	43.9 ± 3.9
HR (beats x min^{-1})	179 ± 16	187 ± 15
Power Output (Watts) @ 5 mmol	240 ± 59	145 ± 36
HR @ 5 mmol	169 ± 22	181 ± 13

Procedures

Subjects performed an incremental test to fatigue in order to determine maximal oxygen uptake (VO_{2peak}) and to establish their individual BL profile. The test was conducted using an electrically braked cycle ergometer (Lode, Groningen, The Netherlands). Oxygen uptake was measured using open spirometry (Q-Plex, Quinton Instruments, Seattle, WA) throughout the test. Peak oxygen uptake (VO_{2peak}) was defined as the highest full minute VO_2 observed during the test. Power output was initially 50 Watts for all males, 40 Watts for all females weighing 60 kg or more, and 30 Watts for females under 60 kg and incremented by this amount every 5 min until fatigue. Heart rates were recorded at rest and during the last 30 s of each stage using radiotelemetry (Polar Electro Oy, Port Washington, NY). Capillary blood was taken from a fingertip at the conclusion of each exercise stage and analyzed for BL after lysing the erythrocytes using an enzyme electrode system (YSI Sport, Yellow Springs Instruments, Yellow Springs, OH). Reference blood lactate concentrations of 2.5 and 5.0 mmol/l were defined by interpolation to represent the aerobic and anaerobic thresholds as suggested by McLellan and Skinner [11], Kindermann et al. [10], and Beneke [2].

Subsequently, each subject performed two exercise sessions using the same incremental testing protocol as used during the initial test. However, the test was ended one stage below that achieved during the maximal effort 198 $\pm$ 35 and 165 $\pm$ 31 watts for males and females respectively. Blood lactate and heart rates were measured using both the YSI and the Accusport™ at rest and during the last 30 s of each stage.

Blood samples of > 50 µl were drawn from the fingertip and placed into a capillary tube. A 25 µl sample was transferred to a buffer tube for YSI analysis in the lab at room temperature. An additional 25 µl sample was placed onto the test strip of the Accusport™ for analysis under the same temperature. Additional 25 ml samples were placed on the strips of the Accusport™ analyzers that were in stable colder environments (cold 1 = 5-10°C, cold 2 = < 0°C).

Statistical Analysis

Statistical comparisons were made to test the hypothesis that cold environments would bias toward low blood lactate concentrations. Comparisons of the mean calculated power outputs at interpolated blood lactate concentration at rest, one-third, two-thirds, and peak workload were made using repeated measures analysis of variance. A Scheffe' test was used for post hoc comparisons.

RESULTS

There was a significant effect of exercise intensity on blood lactate concentration (see Figure 1, Table 2). There was also a significant effect attributable to analyzer with the Accusport™ giving significantly higher values for blood lactate concentration at all points during exercise than the YSI (see Figure 1). There was no significant effect attributable to temperature (see Figure 1, Table 2).

There was a generally consistent relationship between BL concentrations measured using the Accusport™ and the reference method, although the correlation between simultaneously determined blood lactate concentrations was not strong ($R^2 = .5511$) (see Figure 2). The regression relationship indicated that the BL

concentrations achieved using the Accusport™ were about 25% greater than the reference method.

Figure 1. Mean blood lactate concentrations for analyzers at all temperature ranges.

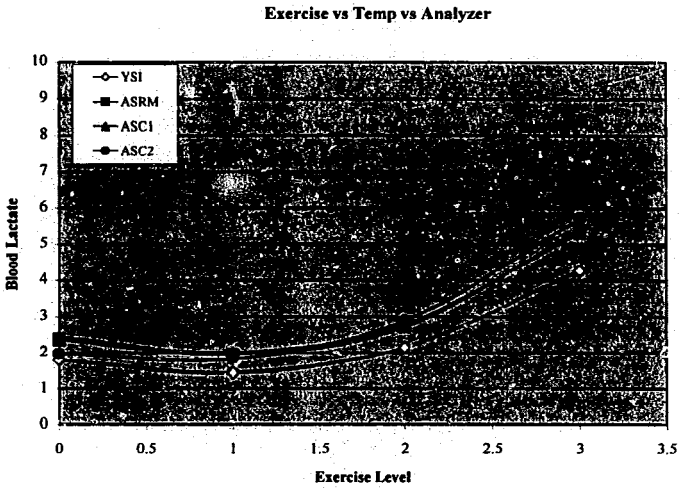
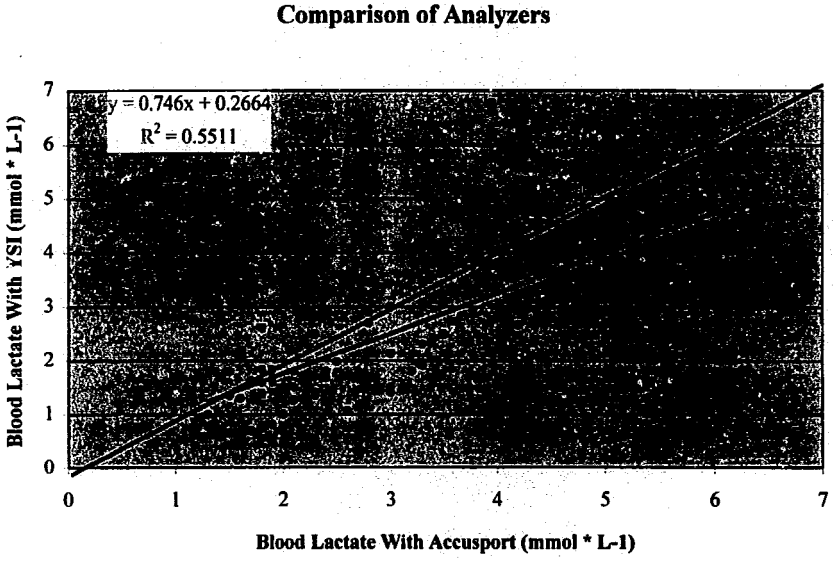


Table 2. Mean (± sd) blood lactate concentrations in the various power output-Temperature-analyzer combinations.

Exercise	YSI	ASRM	ASC1	ASC2
0	1.79 ±	2.37 ±	2.42 ±	1.98 ±
1	1.45 ±	1.96 ±	2.01 ±	1.82 ±
2	2.15 ±	2.71 ±	2.78 ±	2.88 ±
3	4.27 ±	5.41 ±	5.32 ±	5.72 ±

Figure 2. Correlation between simultaneously determined blood lactate concentrations.



DISCUSSION

The primary purpose of this study was to determine the accuracy of the Accusport™ portable lactate analyzer for use in measuring blood lactate in cold environments. We compared measured blood lactate concentrations at three different temperature ranges with the Accusport™ (room temperature (~20° C), cold 1 (~10° C), and cold 2 (< 0° C)) versus the YSI as a reference standard for blood lactate measurements. In all temperature conditions, blood lactate was measured at rest and at power outputs representing one-third, two-thirds, and at the peak individual workload (which was one stage less than the power output achieved during a screening incremental maximal test). There were no significant differences under various

temperature conditions. However, there was a difference in analyzers with the Accusport™ giving consistently greater blood lactate concentrations compared to the reference method.

In contrast to the present results, previous studies have reported equivalent blood lactate concentrations when a given blood sample is analyzed using the Accusport™ and the YSI [4,12]. The present data suggest systematic differences between analyzers. However, the relationship between blood lactate concentrations measured using the Accusport™ and YSI is regular enough that a regression equation for converting Accusport™ to YSI has been generated.

While the present study determined a significant difference between the YSI and the Accusport™ analyzers, a similar study found no analyzer effect [12]. Part I of that study resulted in a significant difference between analyzers. They attributed this difference to variations in sample size. Part II of the Naik et al. [12] study then controlled for sample size by drawing capillary blood into a capillary tube and pipetted 25μl onto the Accusport™ testing strips. It was then revealed that mean lactate values measured with the Accusport™ were significantly lower than those obtained using the YSI. However, the final part to this study determined that blood lactate values were significantly similar upon analysis using whole blood and controlling for sample size.

The differences found between YSI and Accusport™ analyzers during the present study cannot be explained. However, the methodology used during the present study was similar to Naik et al [12], in that controlled sample sizes and capillary whole blood was used. The main difference in blood handling used by the present study was that whole

blood was used for injection into the Accusport™ and lysed whole blood was used for injection into the YSI.

The YSI manual describes usage of whole blood within a capillary tube as impractical [15]. By storing capillary blood in a NaF-Triton buffer at a 1:3 cocktail/whole blood ratio, samples can be stored for extended periods of time without change in lactate concentration. In order to correct for dilution of the sample, blood lactate readings are to be multiplied by three. However, samples measured with the Accusport™ must be prepared for immediate analysis since the Accusport requires use of capillary whole blood only. Previous studies [1,13] further acknowledge the use of lysed blood in conjunction with the YSI for accurate analysis. In that study, no statistical difference was determined between lysed whole blood measured with the YSI as compared to the Accusport™.

The present study utilized two Accusport™ analyzers for blood lactate measurement at every incremental cycling stage for individual subjects. Statistical analysis suggested strong reliability between both Accusport™ analyzers. In addition, previous studies comparing the Accusport™ with other blood lactate analyzers [4,12] were done within controlled laboratory temperatures. The present results, collected at various temperatures, demonstrated that the Accusport™ is consistent throughout a comparatively wide temperature range. To our knowledge, there have been no other studies done comparing the performance of the Accusport™ (or other enzyme driven systems) within various temperature conditions.

In summary, the results of this study suggest that there may be systematic inaccuracies in the performance of the Accusport™. However, it does not seem to be sensitive to comparatively wide temperature variations. Although use in cold environments is possible, the present data suggest a correction factor should be used to accurately compare blood lactate to testing done between the YSI and the Accusport™.

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APPENDIX A

INFORMED CONSENT

INFORMED CONSENT FOR "The Comparison of the Yellow Springs Instruments and the Accusport™ Portable Lactate Analyzer for Measuring Blood Lactate in Cold Environments"

I_____, give my informed consent for my participation in this study which is designed to validate the use of the Accusport™ in cold environments (<0°C).

(1). I have been informed that participation in this study will include a total of three incremental exercise sessions outdoors on a stationary bicycle at temperatures of, ~20°C, ~10°C, and <0°C. This study will also include a preliminary incremental test on the stationary bicycle to determine ones individual profile of lactic acid in the blood. Measurements will include; blood samples taken from a fingertip, gas analysis, and VO_{2peak} which is the maximum amount of oxygen that can be consumed during exercise.

(2). I have been informed that the purpose of this study is to validate the accuracy of the Accusport™ portable lactate analyzer in cold environments. I understand that there may be some discomfort cycling in cold temperatures. There may also be some discomfort from the fingertip pricks resulting in sore fingers.

(3). I have been informed that the benefits that I may expect from participation in this study include knowledge of my maximal oxygen consumption and blood lactate profile.

(4). I have been informed that there are no "disguised" procedures in this experiment. All procedures can be taken at face value.

(5). I have been informed that I am not obligated to remain in this study and am free to withdraw from the experiment without penalty.

(6). I have been informed that the investigator will be available during and after exercise sessions to answer any questions regarding the research or procedures. Concerns may be addressed to Jodi Franklin (investigator) at (608) 782-5957 or Carl Foster (faculty supervisor) at (608) 785-8687. Questions regarding the protection of human subjects may be addressed to Dr. Garth Tymeson, chair, University of Wisconsin-La Crosse, IRB for the protection of human subjects at (608) 785-8155.

_____ _____

Investigator Date Participant Date

APPENDIX B

REVIEW OF RELATED LITERATURE

REVIEW OF RELATED LITERATURE

Introduction

In recent years, there has been a growing interest in the measurement of blood lactate as an evaluation of an athlete's training status or performance and the effectiveness of their training programs. Wasserman and McIlroy [17] introduced the concept of anaerobic threshold as a means for evaluating work capacity. They reported that during incremental exercise a person could work beyond the point at which lactate began to accumulate for only a limited period of time. Wasserman et al. [18] later defined this accumulation of blood lactate as the anaerobic threshold, which they interpreted as a marker of the workload at which the cardiovascular system fails to supply adequate oxygen to the bodily tissue. They have linked this accumulation of blood lactate to the point at which pulmonary ventilation becomes disproportionate to oxygen uptake. The concept remains highly controversial [2,3].

The concept of anaerobic threshold as a means for detecting athletic performance has become widespread [6,12,13,14]. The anaerobic threshold presumptively represents the last point where the lactate concentration in the blood can reach an equilibrium between lactate production and lactate clearance [2,17]. By measuring blood lactate to determine the anaerobic threshold, researchers and coaches have a training tool to evaluate and predict athletic performance. [11,15,16].

The concept of anaerobic threshold has been widely debated [2,17]. Strong disagreements exist as to how much anaerobic metabolism really occurs during exercise [2,3]. The concept that is agreed upon, however, is that lactic acid accumulation in the blood or muscle can be attributed to the dynamic balance between the rate of lactate production and lactate removal. Although debate still exists regarding anaerobic threshold, the accumulation of lactate in the blood is related to the capacity to perform prolonged exercise. The anaerobic threshold can be described as a relationship between maximal lactate steady state and the highest exercise intensity that can be sustained for extended periods of time [4].

The measurement of ones anaerobic threshold is important for understanding late adaptations to training as the anaerobic threshold can change beyond the point of where VO_{2max} fails to increase [9,10]. The anaerobic threshold therefore, represents a potentially superior method for measuring endurance capacity [8,10,12,21]

Several theories have evolved since the concept of anaerobic threshold was first introduced, all used to describe variables related to blood lactate accumulation. Such concepts as the onset of blood lactate accumulation and individual lactate threshold are similar to the concept of anaerobic threshold in that they refer to exercise intensity at which a given lactate accumulation occurs. The term onset of blood lactate accumulation (OBLA) developed by Jacobs [10] is defined as the point where a blood lactate concentration reaches 4 mmol x l^{-1}.

Additionally, Stegmann et al. [22] have noted that steady-state blood lactate concentrations may vary between individuals. Thus, the concept of individual anaerobic threshold (IAT) was introduced.

Blood Handling Techniques

Despite the widespread use of measuring blood lactate, determining the site for sampling has varied. These sites include; artery [19,21], capillary [12], and venous [3,15]. It is well established that different sites of blood sampling affect blood lactate concentrations [5,21]. Measurements made in order to establish the relationship between plasma and blood lactate concentrations were analyzed using the Yellow Springs Instruments. Foxdal et al. [5] measured blood samples from both venous and capillary sites. This study determined that standardization of blood sampling and handling are necessary in order to make direct comparisons against results obtained from different studies. According to Bishop et al. [1], blood lactate levels at various concentrations could be predicted from plasma with $R^2 > .95$. However, blood drawn from the capillary has been shown to be the most practical [1,5,10].

In addition to the varied use of blood sampling sites, a number of methods have been used in the handling of blood samples. These include treatment with various anti-clotting agents, dilution with the use of lysing agents, and separation of plasma by centrifugation [1,5,7]. Williams et al. [19] and Yeh et al. [20] found that the variations in blood handling techniques might influence the comparisons made during testing. For this reason, it is suggested that one method is chosen and used for the duration of a study. Bishop et al. [1] compared lysed and unlysed blood with YSI analyzers and compared

them to another enzyme electrode system (Boehringer Mannheim [BMM]). Analysis revealed a statistical difference between YSI unlysed and BMM, while no differences were found between YSI lysed blood and BMM. In order to achieve accuracy, Bishop et al. [1] and Williams et al. [19] suggest that a lysing agent used to rupture erythrocytes would be the most accurate blood-handling device.

Summary

Both the YSI and the Accusport™ have been shown to be accurate and useful tools for the measurement of blood lactate concentrations. Additionally, problems with blood handling techniques and site sampling have been reviewed to address any variation between tests. The fact that enzyme electrode systems are temperature sensitive (YSI operators manual), leads to concerns that although the Accusport™ performs accurately in laboratory settings as compared to the YSI, it may not, perform similarly in cold environments ($< 0°C$).

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