Determination of Blood Lactate Concentration: Reliability and Validity of a Lactate Oxidase-Based Method

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ABSTRACT

Int J Exerc Sci 2(2): 83-93, 2009. The measurement of blood lactate has long been used as marker of exercise intensity and training status. We compared a commercially available lactate oxidase spectrophotometric method (LO) to determine blood lactate levels to two previously validated methods, the lactate dehydrogenase spectrophotometric method (LDH), and the YSI 1500L Sport lactate analyzer (YSI). First we established a series of calibration curves over physiological range of lactate values (1-15 mM·l-1 for the spectrophotometric assays and 1-30 mM·l-1 for the YSI) with high correlations ($0.986 < r < 0.999$). Aerobically trained participants (n = 11) performed repeated exercise bouts of varying intensities on a cycle ergometer. Capillary blood samples (n = 189) were collected from the earlobe and the blood lactate concentration was determined using each of the three methods. Differences in blood lactate concentrations between the three methods were analyzed using ANOVA (a=0.05). Although all three experimental protocols yielded similarly shaped lactate curves, the actual values for the LDH method was significantly lower than the HPO and the YSI at every intensity level (bias $p<0.05$). We conclude that the HPO method is a reliable and valid method to determine blood lactate concentrations spectrophotometrically. All three methods can provide useful within-subject lactate curves, although we caution against interchangeable use of the three methods.

KEY WORDS: blood lactate, lactate, testing, training, lactate threshold.

INTRODUCTION

Measurements of blood lactate concentrations are common in exercise and clinical settings, as they can reveal information about the participant's fitness status or the patient's clinical state. Concerns about accuracy, cost, and ease of use have resulted in the development of several methodologies for the determination of blood lactate levels. For years all such measurements were conducted using an LDH-based enzymatic analysis on a spectrophotometer (5). The need for faster feedback and the ability to conduct field research led to the development of automated analyzers designed to increase the speed of blood lactate analysis at lower operating costs. More recently, portable hand held units were developed to make blood lactate analysis easier and faster in the clinical setting and feasible for field research with athletes. Generations of these devices have revolutionized the use and value of lactate for training of athletes and in the care of patients (6, 10). Unfortunately, the operating costs associated with these devices (i.e. cost of lactate strips) may be too high, which is why the cheaper (yet

more time-consuming) spectrophotometric analyses have not lost their appeal.

In order for any particular methodology to become accepted for use in the laboratory or clinic, the technique must be shown to be reliable and valid. Whenever a new technique to measure blood lactate concentrations has been introduced, studies have been undertaken to establish reliability and validity of that method (5, 7, 9). Most investigators obtained blood samples and determined lactate concentrations using the new technology and at least one previously validated experimental technique. The results were then compared using primarily Pearson-product moment correlations and standard error as measures of reliability, and t-tests and regressions for validity.

In this study we report on a spectrophotometric method for determination of blood lactate levels, recently introduced by Trinity Biotech (in the U.S.). Although the first patent for this technique was issued in 1979 to the Eastman Kodak company, the technique has been primarily used by affixing the enzymes onto a membrane attached to an electron probe, similar to the previously validated YSI analyzers (5, 7, 9). Our review of the available literature revealed no studies that independently examine issues of reliability and validity for this particular spectrophotometric assay. Thus, we examined the reliability and validity of this oxidase-based enzymatic method against the well-established lactate dehydrogenase-based method and the YSI 1500 Sport analyzer. Our purpose was to examine these methods for possible error or bias, and identify any potential differences between them.

METHOD

This work was performed in accordance with the ethical standards stated in the Helsinki Declaration and all protocols and procedures were approved by the Willamette University Institutional Review Board prior to the study. Blood samples (n = 189) were collected from college-age recreational athletes (n = 11) who were determined to be free of injury using the PAR-Q, and signed the appropriate informed consent forms. These athletes were recruited for a separate study. Briefly, participants attended five exercise sessions on separate days where, following appropriate warm-up of self-selecting intensity and duration, they rode a properly calibrated computerized Monark 839E cycle ergometer at prescribed and often overlapping intensities. The position of the saddle (height and angle) and handlebars was standardized for each participant, and was kept consistent for each of their bouts. Each participant identified the cadence they were most comfortable with, and maintained this cadence throughout all the tests. The first exercise bout was an incremental VO_{2max} test (starting intensity 100W, 40W increments every 2 minutes, to voluntary exhaustion). Blood samples collected at the end of each stage established the lactate curve, and the lactate deflection point was identified as the first increase from baseline in the work/blood lactate curve. The second test was designed to establish the lactate threshold (LT) and consisted of 5-min stages with intensity increasing by 10W from 30W below to 30W above the aforementioned lactate deflection

point. Once again, The LT was determined as a deflection point in the work/blood lactate curve. The last three bouts took place 3 days apart, and consisted of steady-state exercise of approximately 30min each, at intensities corresponding to 95%, 100% and 105% of the intensity corresponding to the LT.

Determination of [Lactate]$_{blood}$

Within 30 sec from the end of every stage during the first two tests, and every five minutes during the three longer bouts we simultaneously obtained two capillary blood samples from the participant's earlobe. One whole blood sample of 25 µL was injected into an automated YSI 1500L Sport unit for immediate analysis. The second blood sample of 50 µL was injected into an eppendorf tube containing 100 µL of 7% perchloric acid (PCA) and was subsequently mixed and centrifuged, and the supernatant was extracted and stored at 0 °C for later spectrophotometric analysis (Genesys-10, Fisher Scientific). These analyses utilized a previously validated LDH-based procedure (Sigma Scientific) and a newer lactate oxidase enzymatic procedure (LO) marketed in the U.S.A. by Trinity Biotech, whose reliability and validity have yet to be determined by independent study.

YSI 1500L Sport

Lactate levels were measured with an the YSI 1500L Sport electron probe according to the following reaction that converts lactate levels anode electron current (www.ysilifesciences.com):

Prior to data collection, we performed calibrations and linearity checks with YSI standard lactate solutions in concentrations ranging from 1-30 mmol·L^{-1} diluted from 5 mmol·L^{-1} or 30 mmol·L^{-1} standard stock solutions (see fig. 1A). We also performed a linearity test before each exercise session, using the 5 mmol·L^{-1} and 30 mmol·L^{-1} standards as recommended by the manufacturer. After calibrating the instrument, whole blood samples of 25 µL were injected into the sample chamber using a calibrated syringe pipette, and the blood lactate concentration of the sample was determined within one minute.

Trinity Biotech

The lactic acid concentration was determined using a commercially available spectrophotometric colorimetric assay (www.trinitybiotech.com/) according to the following reaction:

Lactate reagent (pH 7.2) was reconstituted in vials from reagent powder (400 U/mL lactate oxidase, 2400 µL/L peroxidase, and chromogen precursors) by adding 10 mL de-ionized water. All samples were read at λ= 540 nm.

Prior to the preparing the samples we generated a calibration curve using standards of known concentrations (see fig. 1B). Lactate concentrations ranging from 0-14 mmol·L^{-1} were created from stock standards, and 25 µL of each standard was added to 1 mL lactate reagent in separate cuvettes. We determined through a pilot study that 14 mmol·L^{-1} is the maximum optical density registered by the spectrophotometer. Each standard dilution was analyzed in duplicate and incubated for a total of 15 minutes; absorbance values were recorded every 5 minutes. Duplicate absorbance values were averaged and then used to calculate lactate values.

The blood samples were analyzed in duplicate using 1 mL lactate reagent with 25 μL supernatant fluid of centrifuged blood samples. In order to check the stability of the samples we recorded their absorbance values every 10 minutes for a total duration of one hour. Regression equations established from the calibration curves were used to determine the sample lactate concentration.

Sigma Diagnostics

The lactic acid concentration was determined using spectrophotometric colorimetry according to the following reaction:

Samples were analyzed using a commercially available procedure (Sigma-Aldrich, www.sigmaaldrich.com) by adding 25 μL of supernatant into 1 mL of the reagent cocktail (a 25 mL cocktail contained 8 mL of 1 M glycine, 0.4 mL of ~400U/mL lactate dehydrogenase, 0.6 mL of 0.1 mM NAD, 0.4 mL of 20 M hydrazine, and 15 mL de-ionized water was made daily) and read in the spectrophotometer, this time at λ= 340 nm.

Prior to the preparing the samples we generated calibration curves using standards of known concentrations (see fig. 1C). For the spectrophotometric assays lactate concentrations ranging from 0 - 14 mmol·L^{-1} were created from stock standards, and 25 μL of each standard was added to 1 mL lactate reagent in separate cuvettes. We determined through a pilot study that 14 mmol·L^{-1} is the maximum optical density registered by the spectrophotometer. Each standard dilution was analyzed in duplicate and incubated for a total of one hour; absorbance values were recorded every 10 minutes. Duplicate absorbance values were averaged and then used to calculate lactate values.

Statistical Analyses

Validity of Measurements

In order to identify potential differences in blood lactate values obtained from each of the three methods we used an ANOVA (α=0.05). In addition, the strength of agreement between the different methods was determined with Pearson product moment correlations. The degree of agreement between methods was determined using Bland-Altman analysis (2), where the difference in value between two devices is plotted against the mean of these measurements. Limits of agreement (1.96 x SD) were also calculated and plotted.

Intra-Investigator Reliability

For the Sigma and Trinity methods each sample was analyzed in duplicate. The agreement between the two samples was analyzed using a Pearson product correlation and the relative standard deviation. It was determined *a priori* that if the difference in the resulting estimate of lactate concentration was greater than 0.2 mmol·L^{-1} the samples would be analyzed again in triplicate. Given the inherent delay in the determination of blood lactate samples by the YSI, it was not feasible or practical to analyze these samples in duplicate.

Instrument Reliability

In order to assess the instrument reliability we randomly selected blood samples (n=23 for the LDH method, n=21 for the LO method, range 1.12 – 13.21 mmol·L^{-1}) and

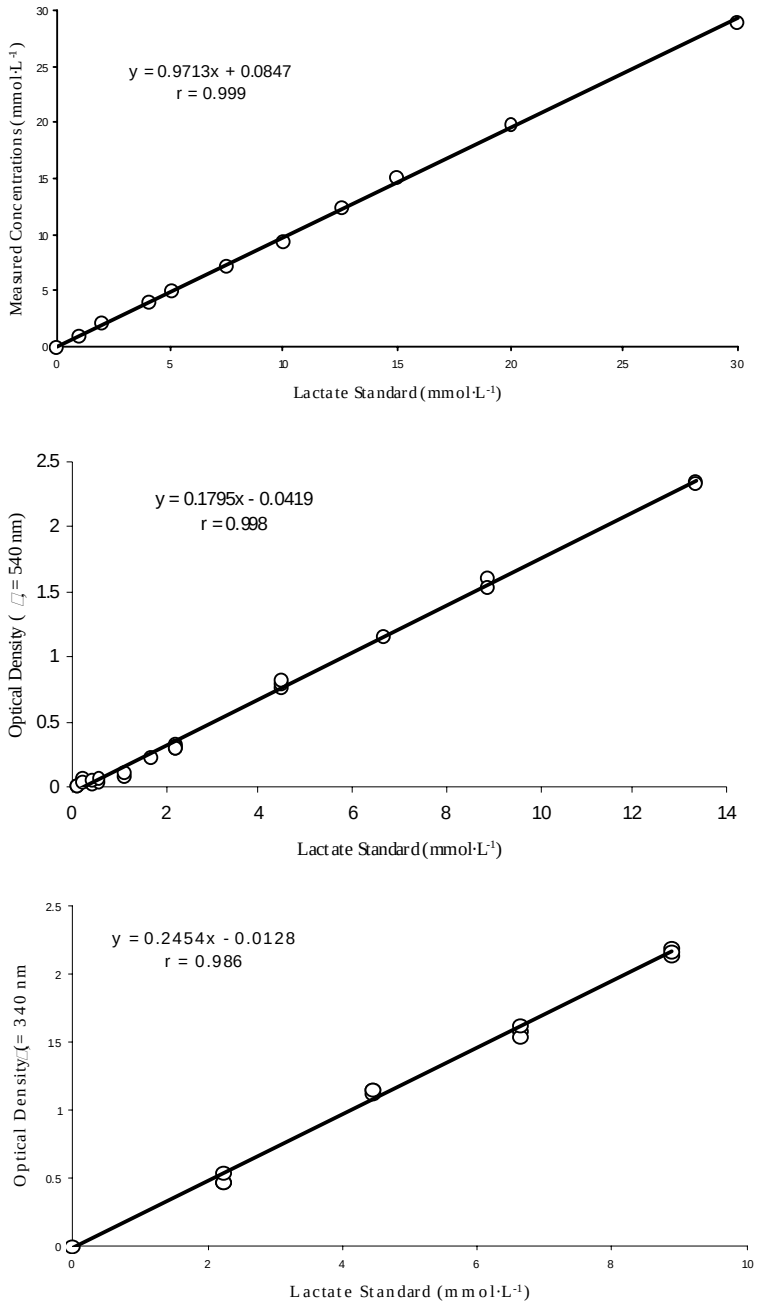

Figure 1. Composite calibration curves for the YSI 1500 Sport lactate analyzer (panel A), LO procedure (λ = 540 nm) (panel B), and the LDH procedure (λ = 340 nm) (panel C), showing the agreement line and the regression equation for each curve. The YSI analyzer is linear to 30mmol·L-1 and the spectrophotometer is linear to the maximum range of absorbance. These curves represent several standard curves generated on separate days, without day-to-day variability.

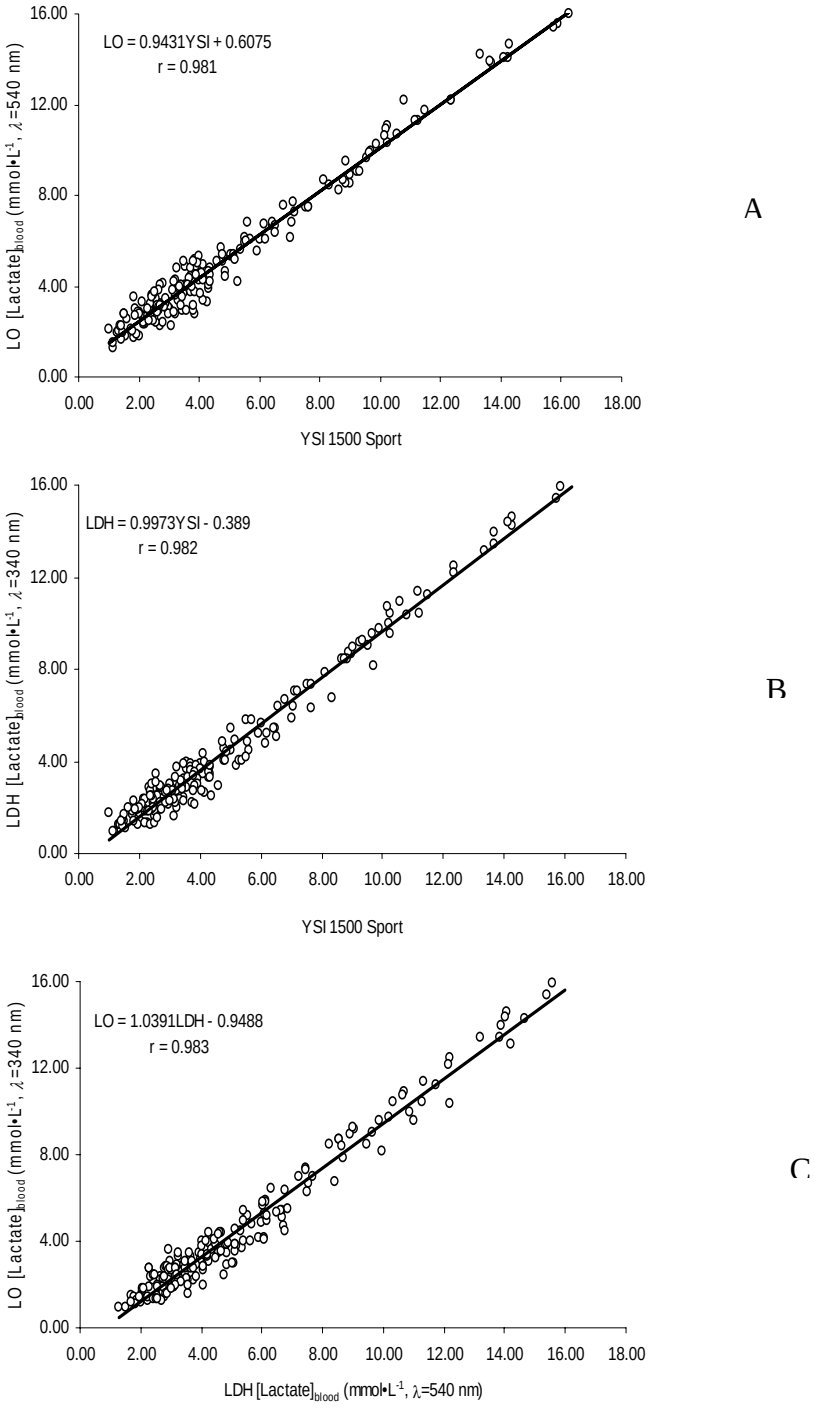

Figure 2. Correlations for each of the three possible combinations of procedures. This figure only includes samples where all three procedures yielded lactate values (n = 190 sets of samples). An additional 14 samples were not included as data from one or more procedures are missing. The regression line is shown as a solid line.

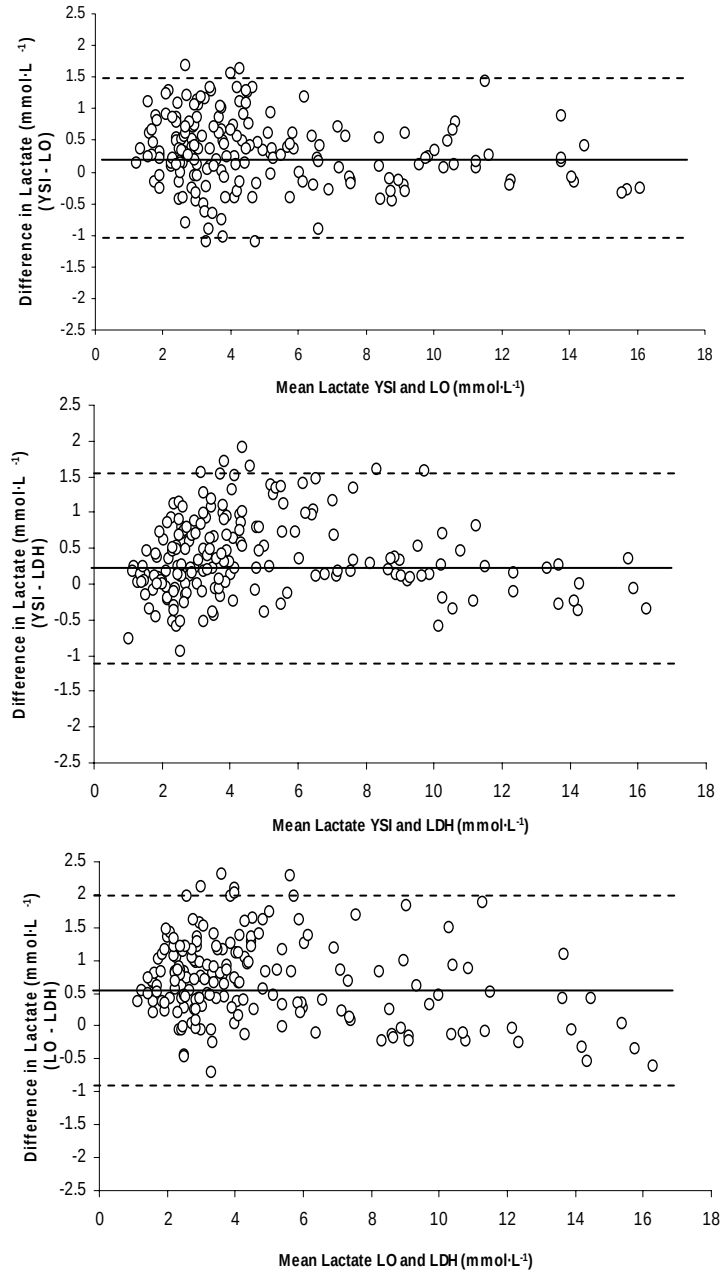

Figure 3. Limits of agreement, based on Bland and Altman (1986). Reliability of measurement is determined as a function of the mean lactate between a) the YSI and LO procedures (Panel A, bias = -0.385, 95% agreement limits 0.848 to -1.618 mmol?L^{-1}, standard error = 0.243 mmol?L^{-1}); b) the YSI and LDH procedures (Panel B, bias = 0.581, 95% agreement limits 1.930 to -0.768 mmol?L^{-1}, standard error = 0.248 mmol?L^{-1}); and c) the LO and LDH procedures (Panel B, bias = 1.021, 95% agreement limits 2.059 to -0.018 mmol?L^{-1}, standard error = 0.246 mmol?L^{-1}).

repeated the analyses. Student T-tests were used to compare the two measurements for differences. It was again predetermined that if the difference in the resulting estimate of lactate concentration between the two measurements was greater than 0.2 mmol·L^{-1} the samples would be analyzed again in triplicate.

RESULTS

The first order of business was to establish that all three procedures are valid for the measurement of blood lactate. For this purpose we constructed several standard curves for each procedure, all of which yielded very high correlations over a broad range of physiological values (1-30 mmol·L^{-1} for the YSI and 1-15 mmol·L^{-1}for the LDH and LO procedures) predicted to be observed in this population of aerobically trained athletes (see figure 1).

The slopes of the regression curves were very similar for the two spectrophotometric techniques (LDH and LO), whereas the slope for the YSI was higher, perhaps reflecting differences in the instruments (i.e. units) and/or the standards used. In addition to providing a good estimate of lactate concentrations, these curves served as confirmation of the analytical techniques utilized.

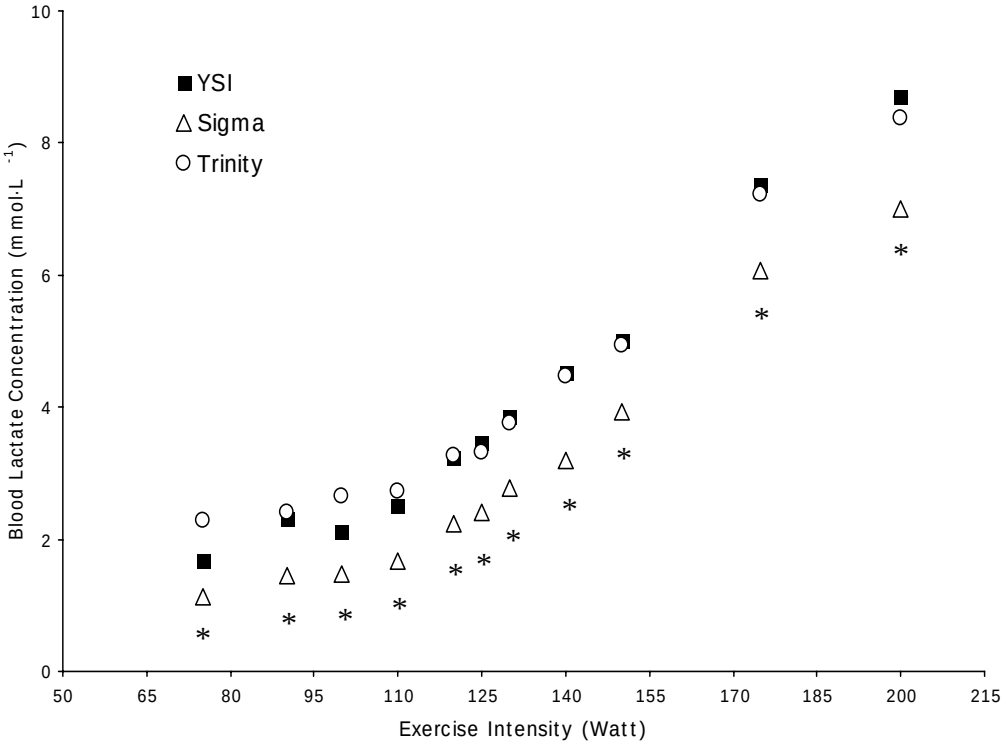

Figure 4. Composite lactate curve for one participant during several bouts of exercise of progressively increasing intensity, using the three different methods of blood lactate analysis. Since the participants performed several repeat bouts, each data point represents multiple (2-6) measurements for each intensity.

Agreement of Measurements

A total of n=204 sets of samples (i.e. one for the YSI and one for the two spectrophotometric analyses as described above) were collected but only the 189 complete sets of samples were used in this study, where every procedure yielded a lactate concentration for each sample. A small number of samples (n=15) did not yield lactate values for one or more procedures due to equipment or experimenter error (i.e. an error message on the YSI or accidentally dropped eppendorf tube). The relationships between the measurements obtained from the three experimental procedures yielded significant correlations (see figure 2). The strength of the agreement (mean ± 2 standard deviations) between the three methods is presented in the Bland-Altman plots (figure 3).

All three blood lactate determination protocols yielded similarly shaped and highly correlated lactate curves (see example from an incremental test in figure 4) for each experimental session, and an ANOVA revealed no differences between the 3 methods.

Intra-Investigator Reliability

Very high correlations (Sigma: r =0.99, Trinity: r =0.99) were calculated between the duplicate samples (data not shown, 0.23 $mmol \cdot L^{-1}$< SEM < 0.24 $mmol \cdot L^{-1}$ for the three methods). There were minimal differences between the two identical samples used to determine lactate

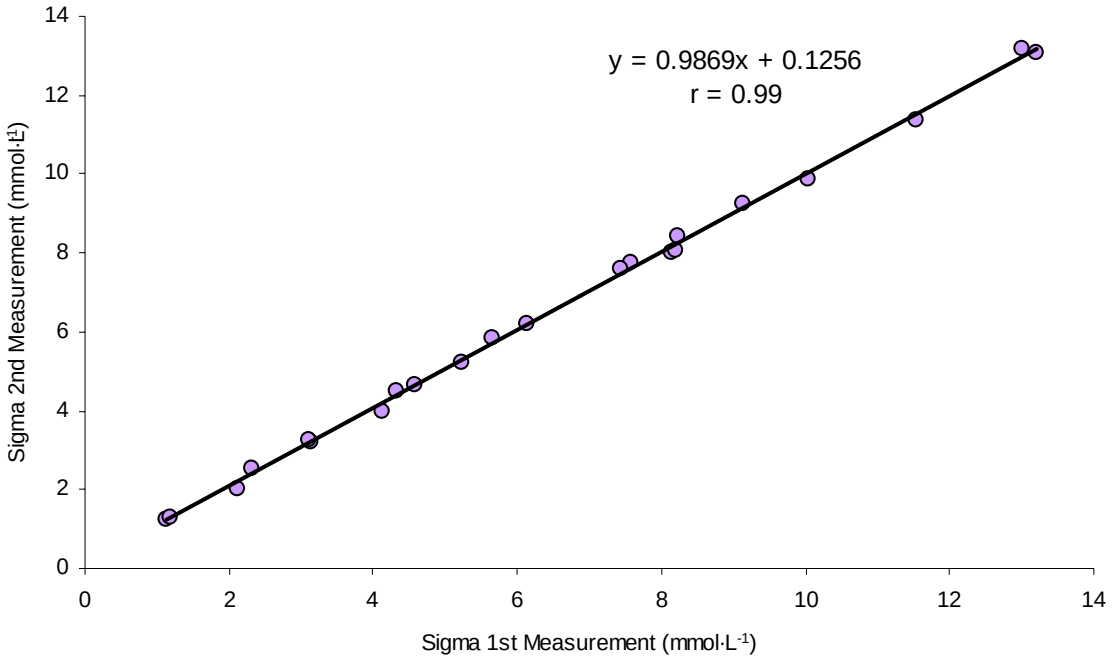

Figure 5. A repeat analysis of random samples (n = 23) using the Sigma protocol indicates good agreement between the two analyses. A similar analysis using the Trinity method (n = 21) yielded similar results (r = 0.99, data not shown). There were no differences between the two sets of values from the same blood sample analyzed at different days.

concentrations; the relative standard deviation ranged from 1.2% (LDH) to 1.4% (LO). Repeat analyses in triplicate as was stipulated *a priori* were not necessary.

Instrument Reliability

Very high correlations were calculated between the first and second analyses of the same sample (for example, see figure 5 for the Sigma procedure). The T-tests revealed no differences between the two assays which were conducted 1-3 days apart. The samples were stored at 0 °C between the two measurements, and on ice during the analysis.

DISCUSSION

The purpose of our study was to evaluate the reliability and validity of the lactate oxidase-based method for lactate determination (LO), a commercially available assay. We compared this method to the well-established protocol using the LDH-based spectrophotometric analysis (LDH) and a previously validated lactate oxidase-based system using an electrode probe (YSI). Independent validation of an experimental technique is a necessary first step before the technique can be used in the measurement of blood lactate concentrations. The aim of this study is consistent with established practices (9), and our results indicate that the LO test is a valid and reliable method to determine blood lactate concentration.

In determining the validity of the LO method we carefully established linearity over a broad range of physiological values using appropriate lactate standards. We also demonstrated high degree of linearity with the two reference methods (LDH and YSI). Given the potential for day-to-day variations in any given assay, we generated a new standard curve for each procedure on a daily basis, and we concluded that the differences between identical assays were insignificant, as shown in the composite standard curves (figure 1).

Subsequently, we compared the results from the LO procedure to the previously validated LDH and YSI procedures. The high correlations between the three methods (see figure 2) provide strong support for the use of the LO-based assay as an acceptable experimental procedure to determine lactate concentrations in blood. Such high correlation values have been reported previously (1). However, exclusive reliance on Pearson's r values may mask potential differences in the absolute lactate values obtained from the different methodologies. For this reason we used the Bland-Altman plots (figure 3), which revealed that there was no obvious pattern of disparity between method and blood lactate concentration.

Our methodology and findings are in agreement with similar reports, as differences in blood lactate concentrations measured with different methods are not uncommon. Some have even created algorithms to convert data from one device to another to allow for comparisons of data across different methods (8). Our observations do not differ from those of others (1, 3-5, 7) in that the different techniques yielded different lactate curves. We are also in agreement with previous studies that identification of the lactate threshold (using a variety of methods such as Log-log transformation) yields the same

exercise intensity for all methods (data not shown). Furthermore, when compared to the early stages the differences in the lactate values between the three methods were not statistically different at higher intensities, indicating that there is no disparity at higher lactate concentrations. In agreement with other studies, we conclude that comparisons of lactate values between different methodologies are not appropriate (3, 5).

The decision as to the selection of a protocol to measure blood lactate concentration is based on a combination of factors such as reliability, validity, cost, convenience, and ease. We conclude that the lactate oxidase method is a reliable and valid technique, and its operating costs were less than the YSI and the LDH methods. This method is an appropriate alternative for laboratory, field, and clinical testing where costs are a higher priority than convenience and speed of results.

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