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ORIGINAL ARTICLE

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Lactate determination in exercise testing using an electrochemical analyser: with or without blood lysis?

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Abstract The practical use of lactate electrochemical analysers in exercise testing has not been adequately examined. Initial studies have reported differences in lactate concentration between that measured spectrophotometrically and that measured electrochemically. The study described here was undertaken to compare, using the statistical technique of Bland and Altman (1986), two widely available methods of measuring lactate using lysed and non-lysed blood samples and the lactate thresholds derived from the measured lactate values using a log-log transform technique. Thirteen normal, healthy young adults (11 male) undertook progressive exercise tests to exhaustion. Arterialised venous blood samples were taken each minute and the lactate concentration therein was measured both spectrophotometrically and electrochemically and either with or without lysis of the blood samples. The lactate concentrations measured in lysed blood using both methods (182 pairs) were in close agreement. The electrochemical values obtained using non-lysed blood were systematically lower than spectrophotometric values (206 pairs), the difference becoming progressively greater at higher lactate concentrations. Results for the lactate threshold comparisons are given as mean difference (limits of agreement with 95% probability). Lactate thresholds (12 pairs) derived from lysed blood lactate concentrations measured spectrophotometrically and electrochemically were not significantly different −30 (240) ml $O_2 \cdot min^{-1}$. Lactate thresholds (11 pairs) derived from lysed spectrophotometric and non-lysed electrochemical measurements were also not significantly different +20 (250) ml $O_2 \cdot min^{-1}$. Thus, despite the difference in the measured lactate concentrations, the derived lactate thresholds are in agreement and, therefore, electrochemical analysers can be used for lactate threshold determination using the log-log transform technique without sample lysis.

Key words Lactate threshold · Exercise test · Blood sample · Electrochemical · Spectrophotometric

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Introduction

The circulating blood lactate levels produced in response to an exercise stress test are widely used to assess aerobic fitness. Several methods of evaluation have been devised including techniques that depend upon absolute lactate concentrations (Kindermann et al. 1979) and techniques that depend upon the pattern of change in lactate concentration (lactate profile) that occurs during progressive exercise (Beaver et al. 1986; Hughson et al. 1987). These techniques have all been developed using lysed whole blood lactate measurements obtained spectrophotometrically. The most prominent technique is the log-log transform (Beaver et al. 1986), which is only dependent upon the relative changes in blood lactate concentration.

With the development of electrochemical instruments, an attractive alternative method of lactate measurement became available to exercise physiologists. The technology offered greater convenience and speed of analysis (i.e. results were available before the subject left the exercise laboratory). However, the values obtained from the electrochemical instruments were lower than those obtained using traditional lysed whole-blood spectrophotometric measurements (Weil et al. 1986; Bishop et al. 1992). Pre-treatment with a lysing agent that could be tolerated by the components of the enzyme-electrode was suggested as a way of obtaining

agreement between the electrochemical method and the traditional spectrophotometric method (Söutter et al. 1978). Lactate concentrations obtained by the electrochemical method using both lysed and non-lysed blood have been compared with the traditional spectrophotometric method (Soutter et al. 1978; Weil et al. 1986; Bishop et al. 1992; Foxdal et al. 1992). In those studies, agreement between methods was assessed by the statistical techniques of correlation and linear regression, both of which have been shown to be inappropriate for method comparison (Bland and Altman 1986). As a result of these reports, the apparent lack of agreement in the absolute lactate concentrations between lysed and non-lysed blood has given rise to the recommendation that blood samples be pre-treated by lysing before measurement (Bishop et al. 1992). This requirement, however, detracts significantly from the convenience of using electrochemical instruments. Foxdal et al. (1992) proposed a modification of the lactate analyser (YSI 2300GL) dilution buffer to incorporate a lysing agent (TritonX-100), and reported a good correlation between lactate concentration in haemolysed blood and the lactate concentration in deproteinised blood measured using a spectrophotometric-enzymatic method.

Despite a difference in absolute lactate concentrations between lysed and non-lysed blood samples, it is possible that the relative changes in lactate values that occur throughout an incremental exercise test may be similar when spectrophotometric and electrochemical methods are used. In practice this would mean that techniques used to evaluate exercise lactate profiles which are dependent only upon detecting the relative changes in lactate concentration would benefit from the speed and convenience of the electrochemical method without requiring prior lysis of the blood samples.

The study presented here was undertaken to examine the effect of using different methods of assaying blood lactate levels on the lactate thresholds derived using the log-log transform technique (Beaver et al. 1986). Lactate thresholds derived from measurements made electrochemically, with and without a lysing agent, were compared with the lactate thresholds derived using the traditional spectrophotometric method which has been the method most commonly used in exercise physiology studies. The technique of Bland and Altman (1986) was used to evaluate the method comparisons.

Methods

Thirteen normal healthy young adults (11 male) volunteered and gave written informed consent. They undertook 1 or 2 (separated by 7 days) progressive exercise tests to exhaustion, giving 23 exercise tests in total. Arterialised venous blood samples were taken each minute, resulting in a total of 388 blood samples. Approximately 2 ml of each blood sample was placed in a pre-chilled glass tube (Grey Vacutainer, Becton Dickinson, Meylan Cedex, France) containing 12.5 mg sodium fluoride and 10 mg potassium oxalate, and kept on ice. Every blood sample was split into two aliquots and assayed for lactate using a spectrophotometric method and using an electrochemical lactate analyser, respectively. For 12 of the 23 exercise tests, the blood samples for the electrochemical assay were pre-treated with TritonX-100 to lyse the blood prior to aspiration of the sample.

The spectrophotometric method uses lactate dehydrogenase for conversion of lactate to pyruvate and measures the increase in absorbance at 340 nm due to NADH formation, and was performed using an automated spectrophotometric analyser (Cobas Mira Bio, Roche, Basle, Switzerland) with commercially available reagents (Sigma Diagnostics Lactate Reagent Kit, cat. no. 826-UV). The reagents were prepared according to the manufacturer's instructions and blood samples were lysed by dilution (1:3) in 8% (w/v) perchloric acid before analysis. Standard and quality assurance materials were also similarly diluted in perchloric acid.

The electrochemical analyser (YSI 2300GL, Yellow Springs Instrument, Ohio, USA) used for lactate measurement includes lactate oxidase in the electrode and functions by measuring the amperometric signal that is generated by the oxidation of the hydrogen peroxide that is formed when lactate is converted to pyruvate. When samples were lysed prior to aspiration, they were diluted 1:2 in YSI buffer (YSI #2357, Yellow Springs Instrument) with TritonX-100 (BDH Chemicals, Poole, UK) ($10\ g \cdot l^{-1}$) added. As 25 μl of the lysed sample is aspirated and diluted in 600 μl of buffer within the instrument, the final TritonX-100 concentration in the diluted blood that comes into contact with the membrane is low ($0.20\ g \cdot l^{-1}$).

Lactate threshold determination

Lactate thresholds were determined by a reviewer in a blind manner for each series of lactate measurements using the log-log transform technique (Beaver et al. 1986). The blinded review was carried out amongst a larger collection of thresholds being determined by the same reviewer so as to minimise the risk of recognising the pairs of lactate series. After a log-log transformation, the reviewer examined each plot of log lactate concentration versus log oxygen consumption (log $\dot{V}O_2$) to identify the change point (CP) at which the log of the lactate concentration began to rise steeply (see example shown in Fig. 1). To calculate the precise value for the lactate threshold, the series of data points were split about the CP into a lower and an upper set, with the CP data point included in both sets. Least squares regression lines were fitted to the two sets of data points, with the intersection of the two regression lines taken as the derived threshold. The threshold is expressed in terms of oxygen consumption by taking the anti-log of the log $\dot{V}O_2$ at the intersection point (Fig. 1).

Statistical analysis

Agreement was assessed by the technique of Bland and Altman (1986) which examines the difference between pairs of measurements. Both the absolute lactate concentrations measured and the derived thresholds were compared. A comparison was also made between the degree of agreement between the two sets of electrochemically derived thresholds and their respective spectrophotometrically derived thresholds. This was undertaken using the *F*-ratio test to compare the two resultant variances. Finally, a simple comparison of the selected CPs between the pairs of thresholds was made.

Results

Comparison of lactate values in blood samples taken during exercise testing

The 182 pairs of measurements made from lysed blood using the spectrophotometric and the electrochemical methods were analysed in the Bland and Altman (1986)

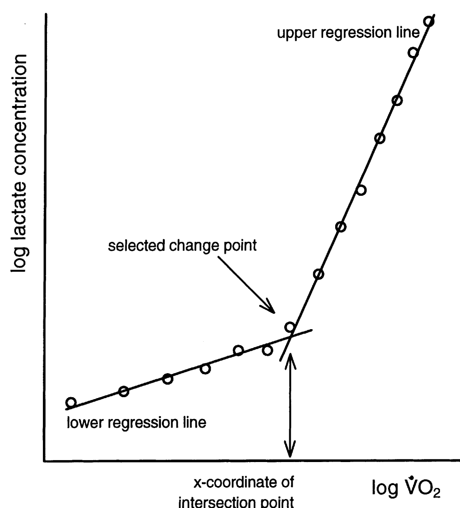


Fig. 1 Diagram of the procedure used to determine the lactate threshold. A logarithmic transformation was applied to each lactate concentration and to its corresponding oxygen consumption ($\dot{V}O_2$). Each *open symbol* represents a single blood sample taken throughout the progressive exercise test. The data points were examined in a blind manner by a reviewer and a change point (*CP*) was selected at the point were the lactate concentration started to rise steeply. The data points are separated into a lower and an upper set with the CP belonging to both sets. Least squares regression lines were fitted to both sets of data and the intersection point of the two lines taken as the derived threshold. This value is expressed in terms of $\dot{V}O_2$ by taking the anti-log of the *x* co-ordinate

manner. The difference between each pair of measurements (spectrophotometric method/electrochemical method) was plotted against their mean, as shown in Fig. 2A. The scatter of the differences increased with the size of the mean, indicating that the data required logarithmic transformation before further analysis could be undertaken. A plot of the differences between the log value of each pair of measurements versus the mean of each transformed pair together with the limits of agreement (95% probability) is shown in Fig. 2B. Reverse transformation indicated that on average, the electrochemical method yielded a value that was 0.99 times that of the spectrophotometric method, which is not significantly different from a ratio of 1.00 ($P > 0.05$, one-group *t*-test). The limits of agreement (95% probability) for the electrochemical values were 0.84–1.16 times those of the spectrophotometric values.

The 206 pairs of measurements made using the lysed blood for spectrophotometric assay and the non-lysed blood for electrochemical measurement were also plotted in the Bland and Altman (1986) manner, as shown in Fig. 3. Since the differences were nearly all negative, rather than randomly distributed about zero, and the size of the differences tended to increase with the size of the measurement, this indicated that the two methods do not agree and that no further analysis of method agreement could be undertaken. This lack of agreement does not preclude the use of these non-lysed electrochemical measurements for lactate threshold determination. The data shown on Fig. 3 do, however, suggest

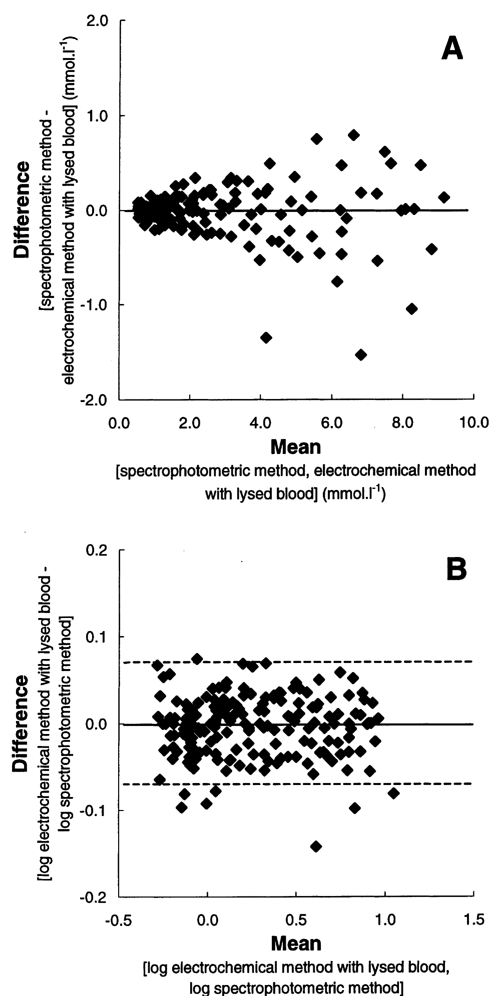


Fig. 2 Bland and Altman (1986) plot (A) of the lactate concentration measured in lysed blood by two methods – spectrophotometric and electrochemical (**A**). The difference between each pair of measurements (spectrophotometric/electrochemical) is plotted against their mean ($n = 186$). The scatter of the differences increases with the size of the mean. Bland and Altman (1986) plot (B) after logarithmic transformation of the lactate concentrations presented in **A**. The scatter is now independent of the size of the mean. *Solid line* mean difference, *dashed line* limits of agreement (95% probability)

that there is a relationship between the two methods. On average, the non-lysed electrochemical values were 0.85 times those of the lysed spectrophotometric values.

Comparison of derived lactate thresholds

The Bland and Altman plot for the 12 pairs of thresholds derived from the spectrophotometric and the lysed electrochemical lactate values obtained is shown in Fig. 4A. The mean difference was -30 ml $O_2 \cdot min^{-1}$, not significantly different from zero ($P > 0.05$, one-group *t*-test). The standard deviation of the differences was 120 ml $O_2 \cdot min^{-1}$, giving 95% limits of agreement of (240) ml $O_2 \cdot min^{-1}$.

The plot for 11 pairs of thresholds derived from the spectrophotometric lactate method using lysed blood

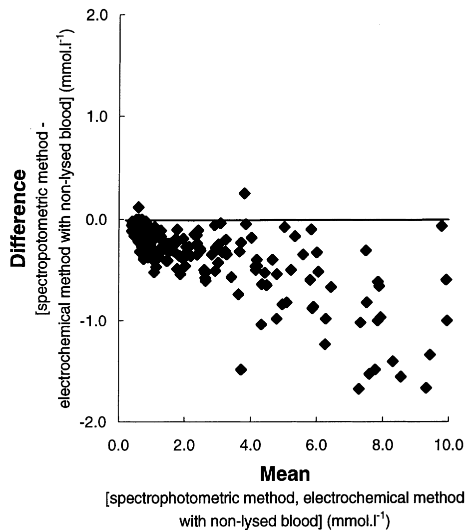
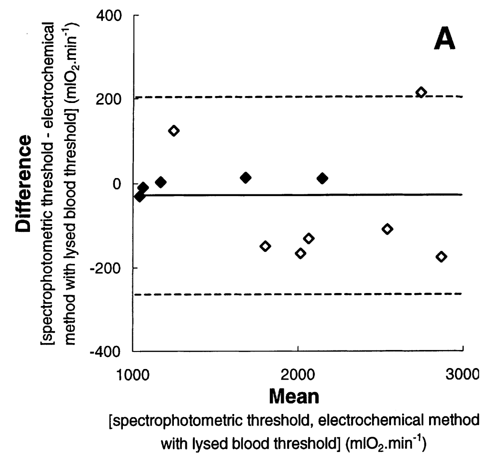


Fig. 3 Bland and Altman (1986) plot of the lactate concentration measured in lysed blood by the spectrophotometric method and non-lysed blood by the electrochemical method. The difference between each pair of measurements (spectrophotometric/electrochemical) is plotted against their mean (n = 206). It can be seen that the differences are nearly all negative rather than being randomly distributed about zero, and that the size of the differences tends to increase with the size of the measurement. This indicates that the methods do not agree and no further analysis can be undertaken as the limits of agreement would be meaningless



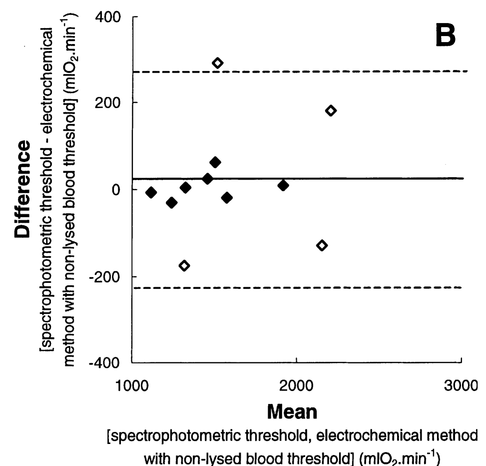


Fig. 4A, B Bland and Altman (1986) plot (A) comparison of 12 pairs of lactate thresholds determined separately from the lactate concentrations in lysed blood measured by spectrophotometric and electrochemical methods (**A**). Bland and Altman (1986) plot (B) comparison of 11 pairs of lactate thresholds determined separately from the lactate concentrations in lysed blood measured spectrophotometrically and in non-lysed blood measured electrochemically. *Solid line* mean difference, *dashed line* limits of agreement (95% probability), *closed symbol* change point selected at the same blood sample (see text for details)

and the lactate values measured electrochemically in non-lysed blood is shown in Fig. 4B. The mean difference was +20 ml $O_2 \cdot min^{-1}$, not significantly different from zero (P > 0.05, one-group t-test). The standard deviation of the differences was 130 ml $O_2 \cdot min^{-1}$, giving 95% limits of agreement of (250) ml $O_2 \cdot min^{-1}$.

The variances of the differences for the two threshold comparisons were not significantly different from each other (P > 0.05, F-ratio test). This, by implication, means that the standard deviations and 95% confidence limits are also not significantly different from each other. We also examined the CPs determined by analysis of the individual log-log plots in a blind manner. For the non-lysed electrochemical plots, the CP chosen by the reviewer was the same as the CP in the corresponding spectrophotometric plot in 7 out of 11 cases. For the lysed electrochemical plots, this value was 5 out of 12 cases. In all of the remaining plots, the chosen CP did not differ by more than a single blood sample either way with respect to the CP chosen in the corresponding plot. The smallest differences, shown in Fig. 4A,B all belong to pairs of threshold determinations where the CP was chosen at the same blood sample.

Discussion

The main findings of this study are: (1) the agreement of the absolute lactate values between the spectrophotometric and the electrochemical method using lysed blood was confirmed using the more rigorous Bland and Altman technique (1986) and, more importantly, (2) despite the lack of agreement of the absolute lactate values measured by the spectrophotometric method and the electrochemical method using non-lysed blood, the corresponding lactate thresholds derived from the measurements are in agreement. These findings indicate that the relative changes in the lactate profile are preserved across the lactate methods. This is of great practical significance, in that lysis of blood is therefore not required, when using electrochemical instruments for the determination of a lactate threshold by the log-log transform technique of Beaver et al. (1986).

It should be emphasised, however, that any technique that is used to evaluate lactate profiles which is dependent upon absolute values for lactate measurements, for example fixed 2- or 4-mmol $\cdot$ l^{-1} concentrations (Kind-

ermann et al. 1979) or the exponential curve technique (Hughson et al. 1987), will require lysis of the blood samples before lactate measurement.

The log-log transform technique for threshold determination only depends upon the relative and not the absolute changes in the lactate profile, and our comparative methodological data using lysed and non-lysed blood provides practical evidence of this. Our finding of the preservation of the relative changes in the lactate profile across methods is also supported by Smith et al. (1997) in eight subjects. Agreement was reported between the lactate thresholds derived using the log-log transform technique from lysed whole blood and from plasma samples, both measured spectrophotometrically. This was in spite of the difference observed between the absolute lactate values.

Electrochemical analysers are either direct-read (undiluted sample) or indirect-read (diluted sample; Fogh-Andersen and D'Orazio 1998). Since direct-read analysers do not dilute the aspirated sample, the electrode measures the concentration of the solute in plasma water. Until recently, all of the commercially available indirect-read analysers operated with isotonic buffer for the dilution of blood within the instrument before exposure to the electrode. Since isotonic buffers preserve the integrity of erythrocyte membranes, not all of the lactate contained within them will diffuse out down the concentration gradient in the time scale of the analysis, and the concentration of lactate in whole blood is influenced by erythrocyte volume fraction. Pre-lysis of the blood sample prior to aspiration or during analysis overcomes this problem. Lactate analysers that operate with a hypotonic buffer so as to cause lysis of the aspirated sample within the instrument, prior to measurement of the lactate concentration, have now been developed and should prove useful in the exercise physiology setting. Indirect-read multi-sensor arrays which include a lactate electrode with electrolyte or blood gas measurements will most likely continue to use an isotonic buffer to prevent haemolysis, especially if potassium determination is required, and may result in the provision of dedicated lactate analysers for either clinical monitoring or exercise physiology applications. The observation that the use of the log-log transform technique for lactate threshold determination is independent of the analytical method used is of great practical value to the exercise physiologist.

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