

USING A GENERAL PURPOSE SPREADSHEET SOFTWARE PACKAGE TO ESTIMATE EXPONENTIAL PLUS CONSTANT MODEL FITS FOR BLOOD LACTATE CONCENTRATION VERSUS WORK RATE DATA

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ABSTRACT

The objective of this analysis was to evaluate the accuracy of a standard spreadsheet software package to estimate best-fit parameters for an exponential plus constant model ($y=a+b.e^{cx}$) applied to blood lactate concentration versus work rate data. During an incremental cycle test, blood lactate concentrations were measured in six endurance-trained athletes. A spreadsheet (Microsoft® Excel) and a dedicated curve-fitting software programme (GraphPad Prism®) were used to obtain model coefficients, model fit parameters, interpolated work rate at a fixed blood lactate concentration ($WR_{4\text{mmol/l}}$) and work rate at a curve gradient of 1 mmol/l per watt ($WR_{dy/dx=1}$). Model coefficients and model fit parameters were identical to the sixth decimal place for four subjects and differed by ≤ 0.000006 in two subjects. $WR_{4\text{mmol/l}}$ differed by < 0.003 watts in five subjects, while a difference of < 1 watt was found for one subject. $WR_{dy/dx=1}$ differed by 0.0018 watts in only one subject. These findings suggest that a general purpose spreadsheet software package can be used by sport and exercise physiologists to accurately determine model coefficients, model fit parameters, $WR_{4\text{mmol/l}}$ and $WR_{dy/dx=1}$.

Key words: Blood lactate; Modelling; Parameter fits; Spreadsheet.

INTRODUCTION

Twenty years ago Stanley *et al.* (1985) established that the rate of blood lactate appearance and disappearance ($\mu\text{mol/min/kg}$) and consequently blood lactate concentration (mmol/l) increases exponentially with increasing work rate. Since then, several papers have presented convincing evidence that the exponential plus constant model ($y=a+b.e^{cx}$) provides a better fit than transformed linear models (Hughson *et al.*, 1987; Campbell *et al.*, 1989; Dennis *et al.*, 1992; Tokmakidis & Leger, 1992). Moreover, not only have these reports demonstrated statistically better fits (Hughson *et al.*, 1987; Campbell *et al.*, 1989; Dennis *et al.*, 1992; Tokmakidis & Leger, 1992), but there is also a compelling body of evidence supporting the contention that a continuous rise in blood lactate accumulation with increasing work rate is physiologically more plausible than “threshold” models (Brooks, 1985; Dennis & Noakes, 1998; Gladden, 2004). In contrast, the linearized “threshold” models invoke the “oxygen deficit” paradigm such that at a certain point “anaerobic” metabolism is activated which leads to eventual fatigue (Wasserman, 1984; Weltman, 1995).

Prior to modern computing capabilities, linearized models (transformation of non-linear data to a general linear form) were the method of choice for researchers in general when dealing with any non-linear or continuous data (Davies & Hicks, 1992; Motulsky & Christopoulos, 2003). The popular use of linear methods to describe non-linear blood lactate vs. work rate data within the Sport and Exercise Sciences (Beaver *et al.*, 1985) was likely the result of two significant factors. Firstly, linearized “threshold” models purported a physiological underpinning (Wasserman, 1984; Beaver *et al.*, 1985). Secondly, because of the simplicity of the calculations required for the solving of linearized “threshold” models, the calculations could be performed by hand or by writing relatively simple computer programmes (Van Wyk, 1984). As an example of the popularity of linear transformation of non-linear data or equations, hand-held calculators transform non-linear equations into general linear form to obtain model coefficients (Hewlett-Packard, 1988).

The advent of powerful desktop computers and accompanying software has provided researchers with the necessary analytical tools to describe non-linear data using appropriate numerical methods which do not rely on the inappropriate linearization of data. However, to estimate the model coefficients (a , b , c) for the non-linear equation $y=a+b.e^{c \cdot x}$, sport and exercise physiologists have had to either write customised computer programmes incorporating more complex numerical methods (Hughson *et al.*, 1987; Myers *et al.*, 1994; Tokmakidis & Leger, 1992) or employ dedicated curve-fitting software (Dennis *et al.*, 1992). Consequently, if investigators do not have the expertise to write computer programmes nor the funds to purchase the required dedicated software, they cannot apply the exponential plus constant model to blood lactate vs. work rate data. However, commercially available spreadsheets offer iterative tools, which can solve for unknown x-values given a linear or non-linear equation and find optimal values for model coefficients. Spreadsheets are thus ideally suited to implement the methodology outlined by Hughson *et al.* (1987) in order to obtain the best-fit model coefficients for the exponential plus constant model.

Therefore, the objective of this analysis was to compare the accuracy of the output of a common spreadsheet software package (Microsoft® Excel) to the output of a dedicated curve-fitting software package (GraphPad Prism®) when estimating the exponential plus constant model for non-linear blood lactate concentration vs. work rate data. Since commercial curve-fitting software programmes often differ in the algorithms used, yet produce very similar outputs (Motulsky & Christopoulos, 2003), we hypothesized that the methods we employed would produce *practically* and *statistically* insignificant differences.

METHODS

Subjects and procedures

Six endurance-trained male athletes (cyclists, triathletes), with at least three years of competitive, provincial and/or national experience, were recruited. Anthropometric measurements and an incremental cycle test to volitional exhaustion were completed during a single visit. During the incremental cycle test micro-blood samples were obtained from the subject's earlobe for the determination of blood lactate concentration. All subjects provided signed informed consent prior to participating in the study. Ethics approval was obtained from the University of Pretoria.

Anthropometry

Stature and body mass were measured to the nearest 0.1 cm and 0.1 kg, respectively. For the purpose of calculating power output (see next section), a combined subject-bicycle mass was also obtained. To determine the percentage body fat, four skinfold thicknesses (triceps, biceps, subscapular, suprailiac) were measured, in triplicate to the nearest 0.1 mm, using a Harpenden skinfold caliper (Siri, 1956; Durnin & Wormersley, 1974).

Incremental cycling protocol

Subjects completed an incremental cycling protocol to volitional exhaustion (Hagberg *et al.*, 1978, Hagberg *et al.*, 1981). The incremental protocol involved cycling on a motorized treadmill (Quinton, Tiernay Electrical Co., Seattle, WA, USA) using the subject's own bicycle at a constant speed (32.2 km/hr) and increasing the gradient by increments of 0.5%, starting at 0% gradient. To ensure sufficient time for a steady-state blood lactate concentration to be reached the stage duration was increased from the original 1 min stage duration of Hagberg *et al.* (1978) to 5 min stage durations. A 10 min ride at 0% gradient served as familiarization and warm-up. No restrictions were placed on preferred gear ratios or pedal cadences. At the end of each stage and at the end of the incremental test the subject stopped pedalling and held onto the treadmill side-railings while a small blood sample was obtained from the subject. An assistant, straddling the treadmill belt, steadied the subject during the blood collection phase by holding onto the bicycle seat. Once the subject started pedalling, the assistant removed any support. The assistant remained available during the test to assist should the subject have lost his balance. However, no such incidents occurred during the test.

Power output was calculated using the formula,

$$Power = (mass \times velocity \times \sin \theta) + (0.185 \text{ kp} \times velocity) + (0.000434 \text{ kp} \cdot \text{min/m} \times velocity^2)$$

where *Power*=kilopond-meters/minute (kpm/min), *mass*=mass of cyclist and bicycle (kg), *velocity*=treadmill belt velocity (m/min) and *sin θ*=angle of the treadmill bed (deg) (Hagberg *et al.*, 1978). For the purposes of this analysis, power was expressed in watts (1 kpm/min=0.1634442 W). Prior to testing, the metered treadmill speed and inclination were verified. Firstly, to verify the metered treadmill speed, the actual treadmill belt speed was determined by measuring the belt length and the time it took to complete 30 revolutions at a reading of 32.2 km/hr. Secondly, the treadmill inclination was verified by comparing metered treadmill angles to manual measurements using a protractor and weighted line.

Determination of whole blood lactate concentration

Prior to the test, a vasodilator (Forapin) was placed on the right earlobe of the subject (the earlobe was chosen simply for ease of sampling). The earlobe was then hyperemized, and a resting blood sample collected in a calibrated capillete (0.02 ml) and emptied into an Eppendorf test tube containing a standard reagent solution (Eppendorf, Hamburg, Germany). The same collection procedure was performed at the cessation of each workload and at the termination of the incremental cycle test. Blood samples were collected within 30-60 seconds at the end of the stage and test. Electro-enzymatic analysis of whole blood samples was performed with an ESAT® 6661 Lactate Analyzer (Eppendorf, Hamburg, Germany) (Clark, 1979; Clark *et al.*, 1984). Whole blood lactate concentration was expressed as mmol/l.

Statistical analysis

All curve-fitting analyses were performed using Microsoft® Excel 2002 (version 10.2614.2625) and GraphPad Prism® for Windows (version 4.03). The latter was considered the “gold standard” against which to test Excel’s output. For the purposes of this analysis output was defined as:

- model coefficients (a , b , c) for the exponential plus constant model ($y = a + b.e^{c.x}$),
- goodness-of-fit parameters (the coefficient of determination or R^2 , absolute residual sum of squares or RSS),
- work rate (W) at a curve gradient of 1 mmol/l per watt ($WR_{dy/dx=1}$),
- and work rate (W) at a fixed blood lactate concentration of 4 mmol/l ($WR_{4mmol/l}$).

The two latter outputs, $WR_{dy/dx=1}$ and $WR_{4mmol/l}$, represent practical measures for quantifying the rate of blood lactate accumulation when evaluating athletic ability (elite vs. trained) or adaptation to endurance training (Hughson *et al.*, 1987; Tokmakidis & Leger, 1992; Dennis & Noakes, 1998). The four outputs were generated from the blood lactate concentration vs. work rate data for each subject using both Excel and Prism.

Microsoft® Excel

A numerical method (quasi-Newton method), found in Excel’s Solver Tools, was used to implement the model coefficient estimation method of Hughson *et al.* (1987), and to calculate $WR_{4mmol/l}$. The work rate at a gradient of 1 mmol/l per watt was calculated by using the first derivative of the exponential plus constant model (see Figure 1) and solving for x ,

$$WR_{dy/dx=1} = \frac{\ln\left(\frac{dy}{dx} / b.c\right)}{c}$$

where dy/dx = curve gradient = 1 mmol/l per watt and b and c are model coefficients (Hughson *et al.*, 1987). Model fit parameters (R^2 and RSS), were calculated in Excel using standard methods (Hughson *et al.*, 1987). (Note: the Microsoft® Excel spreadsheet used in this analysis can be obtained from the first author).

GraphPad Prism®

Model coefficients were estimated using the Prism Non-linear regression (Curve fit) function. As with the Excel analysis, once the model coefficients were optimized, the $WR_{dy/dx=1}$ was calculated by using the first derivative of the exponential plus constant model and solving for x . The $WR_{4mmol/l}$ was calculated by selecting the “Unknowns from a standard curve” option in Prism’s Non-linear regression (Curve fit) function. Model fit parameters (R^2 and RSS) were computed by Prism using standard methods (Motulsky & Christopoulos, 2003).

To evaluate the outputs of the two software programmes, simple differences (Excel minus Prism) were calculated for model coefficients (a , b , c), model fit parameters (R^2 and RSS), $WR_{dy/dx=1}$ and $WR_{4mmol/l}$. These differences were tested for significance using paired t-tests. Appropriate statistical software (SPSS for Windows 11.0.1) was used for inferential statistics and significance was set at $p < 0.05$.

RESULTS

Descriptive statistics for subject characteristics are reported in Table 1.

TABLE 1. SUBJECT CHARACTERISTICS

Age (years)	26.0 (2.9)
Body mass (kg)	77.6 (4.9)
Stature (cm)	180.8 (6.0)
Percentage body fat (%)	13.6 (3.7)
Peak work rate (watts)	342 (38)

n=6, data reported as mean (standard deviation)

The blood lactate concentration vs. work rate data were well described by the continuous exponential plus constant model such that 98% and above of the variation in the blood lactate concentration could be explained by the work rate (Figure 1).

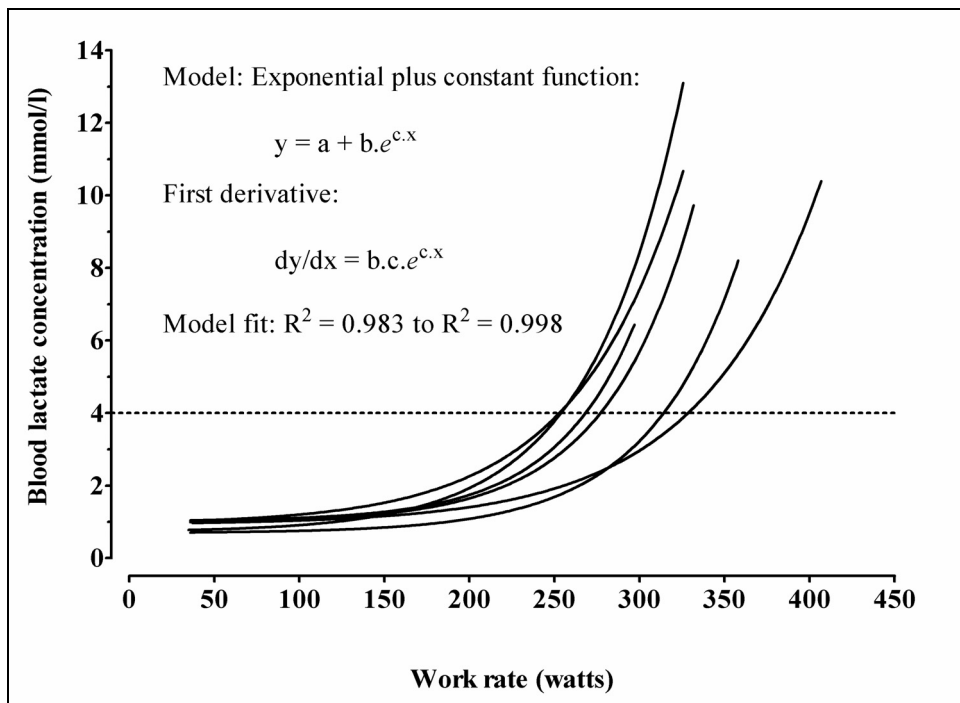


FIGURE 1. LINES OF BEST-FIT FOR AN EXPONENTIAL PLUS CONSTANT MODEL APPLIED TO BLOOD LACTATE CONCENTRATION VS. WORK RATE DATA FOR SIX ENDURANCE-TRAINED MALE SUBJECTS

There were no significant differences ($p>0.3$) for model coefficients (a , b) or model fit parameters (RSS), up to the sixth decimal place (Table 2). For model coefficient c and model fit parameter R^2 there were no differences (row differences equalled zero). Only two subjects (3 and 6) showed absolute differences of ≤ 0.000006 in model coefficients or model fit parameters (Table 2).

TABLE 2. BEST-FIT VALUES ESTIMATED FOR THE EXPONENTIAL PLUS CONSTANT MODEL APPLIED TO BLOOD LACTATE CONCENTRATION (MMOL/L) VS. WORK RATE (WATTS) DATA USING A GENERAL PURPOSE SPREADSHEET SOFTWARE PACKAGE (EXCEL) AND A DEDICATED CURVE-FITTING SOFTWARE PACKAGE (PRISM)

Subject	Excel					Prism				
	Model constants			Model fit parameters		Model constants			Model fit parameters	
	a	b	c	R^2	RSS	a	b	c	R^2	RSS
1.	0.918392	0.027781	0.014336	0.992953	0.620940	0.918392	0.027781	0.014336	0.992953	0.620940
2.	0.687277	0.009503	0.018634	0.997227	0.140393	0.687277	0.009503	0.018634	0.997227	0.140393
3.	1.015016	0.011691	0.020668	0.982648	0.442451	1.015015	0.011691	0.020668	0.982648	0.442451
4.	0.925818	0.056001	0.015842	0.998532	0.125842	0.925818	0.056001	0.015842	0.998532	0.125842
5.	0.963549	0.014464	0.019301	0.996801	0.224518	0.963549	0.014464	0.019301	0.996801	0.224518
6.	0.714368	0.030468	0.018446	0.992183	1.129400	0.714374	0.030467	0.018446	0.992183	1.129399

a , b and c are constants for the exponential plus constant model ($y=a+b.e^{c.x}$), R^2 =coefficient of determination, RSS=residual sum of squares, difference between spreadsheet (Excel) vs. dedicated curve-fitting software (Prism) ≤ 0.000006

The differences in model coefficients did not significantly influence $WR_{4\text{mmol/l}}$ ($p=0.3680$) or $WR_{dy/dx=1}$ ($p=0.3632$) (Table 3). In fact, differences in $WR_{4\text{mmol/l}}$ were only apparent from the third and fourth decimal places for five of the six subjects (Table 3). Only subject 6 demonstrated an absolute difference in $WR_{4\text{mmol/l}}$ of 0.9968 watt which affected a unit change due to rounding (Table 3).

TABLE 3. WORK RATES (WATTS) AT FIXED BLOOD LACTATE CONCENTRATIONS AND CURVE GRADIENTS FOR BLOOD LACTATE CONCENTRATION VS. WORK RATE DATA ESTIMATED USING A GENERAL PURPOSE SPREADSHEET SOFTWARE PACKAGE (EXCEL) AND A DEDICATED CURVE-FITTING SOFTWARE PACKAGE (PRISM)

Subject	Work rate at 4 mmol/l ($WR_{4\text{mmol/l}}$)			Work rate at 1 mmol/l per watt ($WR_{dy/dx=1}$)		
	Excel	Prism	Difference	Excel	Prism	Difference
	1.	328	328	0.0020	546	546
2.	314	314	0.0026	464	464	0.0000
3.	268	268	0.0013	403	403	0.0000
4.	253	253	0.0029	444	444	0.0000
5.	277	277	0.0002	424	424	0.0000
6.	254	255	-0.9968	406	406	-0.0018

values are expressed in watts, difference calculated as Excel values minus Prism values

There were no differences in $WR_{dy/dx=1}$ for five of the six subjects up to the fourth decimal place. A difference was apparent in only one subject from the third decimal place.

DISCUSSION

This analysis shows that a general purpose spreadsheet software package produced outputs practically identical to that of a dedicated curve-fitting software package. Therefore, to apply the exponential plus constant model to blood lactate concentration vs. work rate data, sport and exercise physiologists can employ readily-available spreadsheet software to determine model parameters and work rates at specific gradients and blood lactate concentrations to a high degree of accuracy.

The objective of this analysis was not to exhaustively detail and compare the mathematical algorithms of the two software programmes we used. Rather, we needed to demonstrate that the method of Hughson *et al.* (1987) coupled with Excel's Solver Tools produced outputs that could be considered *practically* nearly identical, albeit not exactly equal, to a "gold standard". Prism's non-linear regression algorithms use iterative methods which include matrix algebra and optimization techniques such as the Levenberg-Marquardt method to minimize the sum-of-squares for a particular set of model coefficients (Motulsky & Christopoulos, 2003). The iterative approach of Hughson *et al.* (1987) coupled with the use of the quasi-Newton method in Excel's Solver Tools, although different in approach to Prism, will produce accurate and valid outputs because, as with Prism, the algorithm is geared towards minimizing the sum-of-squares. It is important to note that commercially available curve-fitting software programmes do not all use the same method to minimize the sum-of-squares yet produce outputs that are practically identical (Motulsky & Christopoulos, 2003). Therefore, the criterion for our implementation of the approach of Hughson *et al.* (1987) would be that the Excel output should be *practically* and *statistically* insignificant from that of a trusted curve-fitting programme. As such, our results show that in comparison to a more sophisticated software programme, the implementation of a non-linear regression method (Hughson *et al.*, 1987) using a general purpose spreadsheet software programme can be considered valid for continuous blood lactate concentration vs. work rate data.

The method of Hughson *et al.* (1987) uses an appropriate transformation of the x-axis ($e^{c \cdot x}$) to obtain an estimate for the model coefficient c . Unlike the transformation of the y-axis, transforming the x-axis does not affect the best-fit model parameters (Motulsky & Christopoulos, 2003). Moreover, the x-axis transformation of Hughson *et al.* (1987) only spreads the data along the x-axis and minimizes the sum of squares of the vertical distance of the data points from the regression curve. Importantly, unlike y-axis transformation, x-axis transformation does not violate the assumption of a Gaussian distribution of data points about the regression line, nor the assumption of homoscedasticity (scatter of residuals is constant for every x-value). It should be noted that by dividing or multiplying all the y-values by a constant does not alter the best-fit curve. However, this does not hold true when the y-values are converted to their logarithms, square roots or reciprocals. By transforming y-values through logarithms, square roots or reciprocals the relative position of the data points are altered and this results in a different curve. Only if the data scatter about the curve is not Gaussian should y-transforms be considered to improve the curve fit (Motulsky & Christopoulos, 2003).

The log-log model commonly employed to determine a “threshold” in blood lactate concentration vs. work rate data obtained from incremental testing, requires that both the x-axis and y-axis be transformed. Once the two segments have been identified, usually by visual inspection, it is relatively simple to obtain two linear functions ($y=m.x+c$) for the two segments and then to determine where the two segments intersect (Tokmakidis & Leger, 1992). This methodology could be excused prior to access to personal computers but present day desktop computers and software programmes are extremely powerful such that the proper numerical techniques can be applied to non-linear data sets (Davies & Hicks, 1992; Motulsky & Christopoulos, 2003). Instead of trying to identify physiologically questionable “thresholds” in blood lactate concentration vs. work rate data (Brooks, 1985), sport and exercise physiologists should rather report blood lactate accumulation as gradients at particular points on the exponential blood lactate concentration vs. work rate relationship (Hughson *et al.*, 1987; Tokmakidis & Leger, 1992).

Investigators have also employed linear interpolation or linear extrapolation methods to obtain the work rate at a particular blood lactate concentration e.g. 4 mmol/l (Olbrecht *et al.*, 1985, Ribeiro *et al.*, 1990; Weltman, 1995). This method necessitates the assumption of linearity between the two data points, used as reference, and the interpolated or extrapolated data point (Maglischo *et al.*, 1984, Olbrecht *et al.*, 1985). This assumption usually holds true because of the increasing curve gradients at increasing blood lactate concentrations. However, investigators have not reported comparisons between linear interpolation or extrapolation and non-linear methods. Therefore, there is uncertainty as to the amount of error produced by linear interpolation or extrapolation. It is likely that investigators have used linear methods primarily because of the simplicity of the linear interpolation or extrapolation methods. The results from this paper suggest that, once a spreadsheet is properly set up, researchers can just as easily use non-linear methods to determine work rates at fixed blood lactate concentrations. Importantly, numerical methods do not make assumptions about the linearity between non-linear data points that linear methods do. Another important advantage of using non-linear methods is that gradients at specific blood lactate concentrations and work rates can be accurately and easily calculated (Hughson *et al.*, 1987; Tokmakidis & Leger, 1992). We would however caution against using non-linear interpolation techniques such as Bezier curves and non-uniform rational B-splines. As with the popular two-point linear interpolation techniques, the blood lactate concentration vs. work rate data should rather be treated as an expression of a biological *system*. In other words, interpolation should ideally be made from the fitted model, and not the local conditions between two data points.

Researchers should be aware that polynomial equations can be applied to blood lactate concentration vs. work rate data (Zhou & Weston, 1997). A major pitfall of this approach is that researchers are tempted to produce higher order curves that produce *mathematically and statistically* excellent fits that follow the data points perfectly, but are *biologically* implausible. In other words, the fitted mathematical model has no relationship to the actual biological processes (Morton, 1989). An extreme example of this approach would be cubic spline curves which go through every data point. It is important to note that many processes in biology yield exponential rate functions (Motulsky & Christopoulos, 2003) so that although polynomials can produce extremely good statistical fits, it is better practice to use biologically and statistically validated models (Dennis *et al.*, 1992; Hughson *et al.*, 1987; Stanley *et al.*, 1985).

Furthermore, polynomial equations are, strictly speaking, considered to be linear equations (Motulsky & Christopoulos, 2003).

Incremental tests can result in constant or decreasing blood lactate concentrations during the early stage of the test, which would reduce the goodness of fit of exponential models (Hughson *et al.*, 1987). In an earlier analysis of the present data set (Cook & Van Wyk, 1991), we found that the mean difference in R^2 values between polynomials and the exponential plus constant model was only +0.007; mean $R^2=0.997$ vs. mean $R^2=0.990$, respectively. Despite constant or decreasing early blood lactate concentrations during an incremental test, the overall fit of the exponential plus constant model still provides extremely good fits (Hughson *et al.*, 1987). It would thus seem prudent, for the majority of incremental testing scenarios, to settle for the most biologically *and* mathematically acceptable model. This would ensure that standard analytical techniques can be adopted to obtain practically relevant indices for measuring athletic ability or adaptation to training. This analysis provides an easily implemented methodology which is theoretically sound and practically relevant.

CONCLUSIONS

In conclusion, it has been demonstrated in this paper, that non-linear model coefficients and useful information, such as non-linear work rate interpolation and curve gradients, are easily and accurately obtained using an appropriately designed, general purpose spreadsheet. Importantly, the use of suitable numerical techniques allows blood lactate concentration vs. work rate data to be interpreted and reported by sport and exercise physiologists as a continuous, exponential function.

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