This is the Author’s version of the paper published as:

Author: R. Duffield, J. Edge, D. Bishop and C. Goodman
Author Address: rduffield@csu.edu.au
J.A.Edge@massey.ac.nz
bishop@motorie.univr.it
Title: The relationship between the VO2 response, muscle and blood metabolites and performance during exhaustive exercise
Year: 2007
Journal: Journal of Science and Medicine in Sport.
Volume: 10
Issue: June
Pages: 127-134
ISSN: 1440-2440
URL: Keywords: accumulated oxygen deficit, cycle to fatigue, muscle metabolites, VO2 kinetics.

Abstract: This study examined the relationship between the VO2 response, particularly the slow component (SC), muscle metabolite changes and performance during very-heavy exhaustive exercise. Sixteen active females performed a graded exercise test to determine VO2 peak and the lactate threshold followed 48 h later by a constant-load cycle test to exhaustion (ET) at 85% VO2 peak intensity. Muscle biopsies and capillary blood samples were obtained before and after the ET to determine changes in muscle ATP, pH, lactate and phosphocreatine and also plasma pH and lactate. Breath-by-breath data from the ET were smoothed using 5-s averages and fit to a three-component exponential model. The mean time to exhaustion (texh) during the ET was 16.8 (± 6.4) min. Results showed no correlation between the SC and texh or any muscle metabolite changes (p>0.05). Significant correlations (p<0.05) were evident between texh and tau; τ0 (r = -0.54), τ1 (r = -0.65), change in (pHb (r = -0.60), [La-b] (r = -0.58) and [La-b] post (r = -0.64).

Furthermore, a negative value resulted when the accumulated oxygen deficit was calculated for the entire duration of the ET. Results showed no association between the amplitude of the SC and texh or to changes in muscle/blood metabolites, suggesting that the SC is not a determinant of high-intensity exercise tolerance. Furthermore, it is possible that a reduced perturbation of anaerobic energy sources, as a result of a faster τ1, may have contributed to a longer texh.
Title: The relationship between the VO$_2$ slow component, muscle metabolites and performance during very-heavy exhaustive exercise.

Running Title: VO$_2$ response during exhaustive exercise in females.
Abstract:

This study examined the relationship between the VO₂ response, particularly the slow component (SC), muscle metabolite changes and performance during very-heavy exhaustive exercise. Sixteen active females performed a graded exercise test to determine VO₂ peak and the lactate threshold followed 48 h later by a constant-load cycle test to exhaustion (ET) at 85% VO₂ peak intensity. Muscle biopsies and capillary blood samples were obtained before and after the ET to determine changes in muscle ATP, pH, lactate and phosphocreatine and also plasma pH and lactate. Breath-by-breath data from the ET were smoothed using 5-s averages and fit to a three-component exponential model. The mean time to exhaustion (t_exh) during the ET was 16.8 (±6.4) min. Results showed no correlation between the SC and t_exh or any muscle metabolite changes (p>0.05). Significant correlations (p<0.05) were evident between t_exh and tau; τ₀ (r = -0.54), τ₁ (r = -0.65), change in (Δ) pH_b (r= -0.60), Δ[La⁻]_b (r= -0.58) and [La⁻]_b post (r = -0.64).

Significant correlations (p<0.05) were also evident between τ₁ and [La⁻]_b post (r= 0.54). Furthermore, a negative value resulted when the accumulated oxygen deficit was calculated for the entire duration of the ET. Results showed no association between the amplitude of the SC and t_ext or to changes in muscle/blood metabolites, suggesting that the SC is not a determinant of high-intensity exercise tolerance. Furthermore, it is possible that a reduced perturbation of anaerobic energy sources, as a result of a faster τ₁, may have contributed to a longer t_exh.

Key Words: accumulated oxygen deficit, cycle to fatigue, muscle metabolites, VO₂ kinetics.

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Introduction

The characteristics of the VO_2 response to step-wise increments in exercise have been well documented [1] and shown to be dependent upon the exercise bout intensity [2]. For exercise in the moderate intensity domain (below the lactate threshold (LT)) not resulting in a net increase in blood lactate concentration ([La]_b), an initial cardio-dynamic phase is followed by a mono-exponential rise in VO_2 to a predicted steady state, normally within 3 min [1]. As commonly reported, exercise intensities above the LT result in VO_2 rising above that predicted and hence a delayed attainment of steady-state VO_2, referred to as the slow component (SC) [3]. As the exercise intensity continues to increase, particularly above Critical Power (maximum exercise intensity sustainable for an extended duration), the VO_2 SC has been shown to become more pronounced and may continue to rise until either VO_2 peak or exhaustion [4].

The mechanisms underlying the SC remain elusive. However, the magnitude of the SC rise during exercise of heavy to severe intensities has been suggested to be a limiting determinant of exercise time to exhaustion (t_{exh}) [5 6]. While these authors did not directly measure the SC in relation to t_{exh}, Fernandes et al. [7] have reported a significant positive relationship between the SC magnitude and t_{exh} at swim velocities associated with VO_2 peak. In contrast, neither Billat et al. [8] nor Demarle et al. [9] reported any association between the SC rise and t_{exh} in either run or cycle to fatigue exercise bouts at work rates associated with approximately 90% VO_2 peak. As yet, the role or relationship of the SC rise in the development of fatigue in very heavy-intensity exercise to exhaustion is
unclear. As such, an investigation into the relationship between the SC and changes in skeletal muscle bioenergetics and the potential association with the onset of exhaustion may provide a further understanding of the limitations to very-heavy exercise.

Previous studies have outlined a range of possible mechanisms responsible for the SC rise in VO₂ above the predicted VO₂ determined by extrapolation of the sub-maximal VO₂–work relationship[3 6 10]. While both central and peripheral factors have been proposed to contribute to the SC, Poole et al. [11] have reported that approximately 86% of the SC excess VO₂ arises from the exercising limb. While Carra et al. [12] have proposed that approximately 27% of the SC rise in VO₂ can be attributed to the increased O₂ cost of Vₑ in higher intensity exercise, the dominant mechanism/s for the excess SC VO₂ seem to be linked to peripheral function. Factors such as the accumulation of by-products of anaerobic metabolism, including [La⁻] and hydrogen ions (H⁺) and other intra-muscular mechanisms, including temperature, epinephrine and norepinephrine levels (which may also have roles in muscle fatigue) have been proposed as mechanisms for the delayed rise in VO₂ [2 4]. However, further research has shown a lack of association between these factors and the magnitude of the SC [5 10 13].

Therefore, due to contradictory results on the relationship of the SC to exhaustion, the aim of this study was to investigate whether there was an association between the magnitude of the SC and tₑₓₘₐₜ. In addition, in order to link pulmonary measures to changes in skeletal muscle metabolites, we tested the hypothesis that the magnitude of the SC was associated with perturbations in muscle and blood metabolites.
Methods

Participants

Sixteen female university students who were physically active (mean ± SD: age 20 ± 3 y, mass 62.3 ± 10.0 kg, VO₂_peak 45.8 ± 6.2 mL·kg⁻¹·min⁻¹) volunteered to participate in this study. Participants were informed of the study requirements, benefits and risks before giving written informed consent. Approval for the study’s procedures was granted by the Institutional Research Ethics Committee.

Experimental overview

All participants completed a familiarisation trial of both the graded exercise test (GXT) and the constant-load, cycle ergometer endurance test (ET) prior to testing. On the day of the ET, a muscle biopsy was taken from the vastus lateralis muscle at rest (prior to warm up) and immediately upon cessation of the exercise test. At least 48 h separated each testing session and tests were conducted at the same time of day. Participants were asked not to consume any food or beverages (other than water) two hours prior to testing and to refrain from alcohol consumption or performing vigorous exercise in the 24 h prior to testing. During both the GXT and ET, pulmonary gas exchange parameters were determined using a breath-by-breath gas analysis system (K4b², Cosmed, Rome, Italy). The gas analysers were calibrated immediately before and verified after each test using a certified gravimetric gas mixture (BOC Gases, Chatswood, Australia), while the ventilometer was calibrated pre-exercise and verified post-exercise using a three litre syringe in accordance with the manufacturer's instructions.
Graded exercise test

The GXT was performed on an electronically-braked cycle ergometer (Lode, Gronigen, The Netherlands) and consisted of graded exercise steps (4-min stages), using an intermittent protocol (1-min break between stages). The test commenced at 50 W and thereafter, intensity was increased by 25 W every 4 min until volitional exhaustion. The test was stopped when the subject could no longer maintain the required power output. Strong verbal encouragement was provided to each subject as they came to the end of the test. Capillary blood samples were taken at rest and immediately following each 4-min stage of the GXT. A hyperaemic ointment (Finalgon, Boehringer Ingelheim, Germany) was applied to the earlobe 5 - 7 min prior to initial blood sampling. Glass capillary tubes were used to collect 50 μL of blood during the GXT and plasma pH and [La\(^{-}\)] concentration was measured using a blood-gas analyser (ABL 625, Radiometer, Copenhagen, Denmark). Both lactate threshold (LT) and VO\(_2\)\(_{\text{peak}}\) were determined from the GXT. The LT was calculated using the modified D\(_{\text{max}}\) method, which is determined by the point on the polynomial regression curve that yields the maximal perpendicular distance to the straight line connecting the first increase in lactate concentration above resting level and the final lactate point [14]. VO\(_2\)\(_{\text{peak}}\) was determined as the highest 30-s rolling average during the GXT.

Constant-load Endurance cycle test:

Participants performed a constant-load cycle test to exhaustion (same ergometer as the GXT) at the absolute power output associated with 85% VO\(_2\)\(_{\text{peak}}\). Participants performed...
4 min of unloaded cycling before a square-wave transition to the individually determined exercise intensity (power output associated with 85% VO₂ peak) until the required power output could no longer be maintained (tᵦᵦᵦ). Transition times from unloaded to 85% VO₂ peak intensity cycling took 2 – 5 s. Breath-by-breath data during the ET was smoothed using 5-s averages to enhance the curve characteristics. A three-component exponential model (equation 1) [15] was used to fit the ET data, using an iterative, non-linear regression technique which was aligned with the start of ET.

\[
VO_2(t) = VO_2(b) + A_0 \cdot (1 - e^{-t/\tau_0}) + A_1 \cdot (1 - e^{-(t - td_1)/\tau_1}) + A_2 \cdot (1 - e^{-(t - td_2)/\tau_2})
\]  

(eq 1).

\(VO_2(t)\) represents the VO₂ at any given time; \(VO_2(b)\) is the unloaded cycling VO₂ baseline value; \(A_0, A_1\) and \(A_2\) are the asymptotic amplitudes; \(\tau_0, \tau_1\) and \(\tau_2\) are the time constants and \(td_1\) and \(td_2\) are the time delays for each respective phase (initial, primary and slow). While multiple transitions are normally required, recently Zoladz et al. [16] have justified the use of a single exercise trial. Based on individual VO₂ – power output regression equations determined from the GXT, the Accumulated Oxygen Deficit (AOD) was calculated for each subject during the ET as the difference between the predicted VO₂ (determined from the GXT) and actual VO₂ for the whole ET [17]. For reasons to be covered in the discussion, AOD was also calculated as the difference between the predicted and actual VO₂ until the VO₂ response reached the predicted VO₂ value.
**Muscle Sampling and Analysis**

On the day of the ET, one incision was made under local anaesthesia (2 x 2.5 mL 1% Xylocaine) into the vastus lateralis of each subject. Following the pre-test biopsy, the incision was then closed with a steri-strip and then subsequently re-used for the post-test biopsy. Pre and post-test biopsy samples were taken with the needle inserted at different angles, with suction applied for both samples. The first muscle sample (50 - 80 mg wet mass per sample) was taken (prior to warm up) during supine rest. The second muscle sample (50 - 80 mg) was taken immediately following the cessation of the ET, while the subject remained on the cycle ergometer. Mean time for the pre-exercise and post-exercise (time from cessation of pedalling) muscle sample to be placed in liquid nitrogen was 6.1 ± 1.8 s and 16.9 ± 5.5 s respectively. The samples were then removed from the biopsy needle and stored at -80º C until subsequent analysis. The frozen muscle samples were weighed on a microbalance (HM202 Lab Supply Pty Ltd, Sydney, Australia) and freeze dried. The freeze-dried muscle was then dissected free from visible blood, fat and connective tissue.

**Muscle buffering capacity; titration method (βm \textsubscript{in vitro})**

Freeze-dried, resting muscle samples (1.8 - 2.3 mg) were homogenised on ice for 2 min in a solution containing sodium fluoride (NaF) (10 mM) at a dilution of 30 mg dry muscle/mL of homogenising solution. The muscle homogenate was then placed in a circulating water bath at 37º C for five min prior to and during the measurement of pH. The pH measurements were made with a microelectrode (MI-415, Microelectrodes Inc,
Bedford, NH, USA) connected to a pH meter (SA 520, Orion Research Inc, Cambridge, MA, USA). After initial pH measurement, muscle homogenates (rest samples) were adjusted to a pH of approximately 7.2 with a sodium hydroxide (NaOH) (0.02 M) solution and then titrated to a pH of approximately 6.2 by the serial addition of 2 µL of hydrochloric acid (HCl) (10 mM). From the fitted titration trendline, the number of moles of H⁺ (per g of dry muscle) required to change the pH from 7.1 - 6.5 was interpolated [18]. This value was then normalised to the whole pH unit for final display as micromoles H⁺ per gram dry muscle per unit pH (µmol H⁺·g muscle dw⁻¹·pH⁻¹) and determined as the participants βm in-vitro. Previous research has shown this method to have a coefficient of variation of 2.2 – 3.0% [18].

Muscle Metabolites

Freeze-dried, rest and post exercise muscle samples (3.5 - 4.5 mg) were enzymatically assayed for [La]ₘₙ, phosphocreatine (PCr), creatine (Cr) and ATP according to the methods of Harris et al. [19]. Muscle metabolite concentrations, with the exception of lactate, were adjusted to the individual highest TCr content, to account for measurement errors arising from the variable inclusion of connective tissue, fat or blood in tissue samples. Anaerobic ATP yield (mMol·kg⁻¹ dw) was calculated according to Bangsbo et al. [20] as Anaerobic ATP yield = ΔATP + ΔPCr + 1.5Δ[La]ₘₙ.
Statistical Analysis

Pearson product-moment correlations were used to establish the strength and direction of the linear relationships between the measured variables. A one-way repeated measures ANOVA was performed to determine if there were significant changes from pre to post-exercise during the ET in blood/muscle metabolites and pH. All statistical analysis was performed using the SPSS statistical package (Version 11.0), with the alpha level for statistical significance set at 0.05.

Results:

The mean (± SD) power output at VO₂ peak and LT of the participants during the GXT was 199 ± 25 W and 132 ± 21 W (66% of power at VO₂ peak) respectively, and the mean time to exhaustion (t_exh) during the ET was 16.8 ± 6.4 min. The mean (± SEM) VO₂(b), A₀, A₁ and A₂ amplitudes were 819 ± 42, 477 ± 68, 1244 ± 79 and 321 ± 24 mL·min⁻¹ respectively. When these amplitude values are summed together (2861± 114mL·min⁻¹), the total is not significantly different from VO₂ peak (2863 ± 386 mL·min⁻¹; p> 0.05), indicating that VO₂ peak was reached at or prior to t_exh. Time constants for each respective phase were 11 ± 2, 37 ± 3 and 93 ± 11 s for τ₀, τ₁ and τ₂ respectively, while t₁ and t₂ were 15 ± 1 and 173 ± 13 s respectively. The VO₂ response of a typical subject during the ET is presented in Figure 1. The mean AOD (± SD) during the ET calculated for the whole duration of the ET was -12.4 ± 36.6 mL O₂ eq·kg⁻¹; however, when calculated until the time point where the predicted VO₂ equalled the modelled VO₂ data, it was 22.6 ± 6.5 mL O₂ eq·kg⁻¹. An example of this is presented in Figure 2.
Muscle buffer capacity, anaerobic ATP yield and pre and post measures of muscle and blood metabolites and pH are presented in Table 1. There were significant (p < 0.05) changes from rest to exhaustion in [La−]m, pHm, [ATP], [PCr] and [Cr]. There were also significant (p < 0.05) changes from rest to exhaustion in [La−]b and pHb.

The results of correlation analyses between selected VO₂ response parameters, t_exh, and selected muscle and blood metabolites are presented in Table 2. No significant correlations were evident between the SC and t_exh (p>0.05). Significant correlations (p<0.05) were evident between t_exh and τ₀ (r = -0.54), τ₁ (r = -0.65), ΔpHb (r= -0.60), Δ[La−]b, (r= -0.58) and [La−]b_post (r = -0.64). Significant correlations (p<0.05) were also evident between τ₁ and [La−]b_post (r= 0.54). While significant correlations were evident, as presented in Table 2, only the correlations of t_exh with τ₁, and t_exh with [La−]b_post provided a moderate explanation of variance (r² = 0.42 and 0.40 respectively). No significant correlations were evident between t_exh or any other VO₂ response parameter or with any other muscle or blood metabolite measure.

Discussion:

The primary aim of the present study was to determine the relationship between the SC and time to exhaustion, and further, if a relationship was present, to determine whether it was linked to changes in muscle metabolites. The main findings of this study show that there were no significant correlations between the SC and t_exh or with any muscle
metabolite changes; however, $\tau_1$ was related to $t_{exh}$ as well as $[La^-]_b$ post. The $t_{exh}$ was also related to $\tau_0$, $\Delta pH_b$, $\Delta[La^-]_b$ and $[La^-]_b$ post. Finally, a negative value resulted when the AOD was calculated for the entire duration of the ET, questioning the validity of the AOD methodology when there is a significant SC.

While the SC rise in $VO_2$ has been proposed as a possible factor limiting exercise tolerance, the research literature varies as to the exact relationship between the two variables [5, 7, 8, 21]. The results of the present study concur with those of both Billat et al. [8] and Demarle et al. [9] in that no relationship was evident between the SC and $t_{exh}$. The exact mechanisms responsible for the onset of either the SC or fatigue ($t_{exh}$) during very-heavy, constant-load exercise are yet to be confirmed [22 23]. However, based on the current results, it would seem that the mechanisms responsible for the SC are unlikely to be directly responsible for very-heavy exercise intensity tolerance.

The mechanisms responsible for the SC rise in $VO_2$ during constant-load exercise are as yet unclear. Recent research has reported that the contribution of the type and pattern of motor-fibre recruitment may contribute to the SC [24]. Furthermore, significant correlations have been reported between % type II fibre distribution and an increased magnitude of the SC [3]. In conjunction, higher $O_2$ costs for the same high-energy phosphate production in type II fibres [25] has further lead to an implication of fibre type distribution and recruitment as a mechanistic cause of the SC. However, current EMG research is inconclusive as to whether fibre type distribution and recruitment patterns or

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alternatively, an increase in the ATP requirement of the recruited fibres (as opposed to recruitment of new fibres) is a cause of the SC rise [26]. Alternatively, Pringle et al. [23] have proposed that fatigued muscle fibres may consume O₂ in order to maintain either Na⁺ - K⁺ pump or Ca⁺⁺ homeostasis, even though they are not contributing to force development, and as such, increasing the O₂ cost for a given work rate. Thus, while the mechanisms are speculative, the SC may occur alongside the fatigue process rather than being directly associated with fatigue in very-heavy constant load exercise. This notion is consistent with the lack of an association between the SC and t_{exh} in this and previous studies.

Currently, the mechanisms underlying fatigue during constant-load exercise are not understood. Historically, La⁻ and H⁺ accumulation, and more recently Na⁺, K⁺ and Ca⁺⁺ perturbations, have been linked with fatigue [22]. These same parameters have also been proposed as being linked to the development of a SC during heavy to very-heavy exercise [5, 10]. Data from the present study indicated that t_{exh} was associated with ΔpH₅₅, Δ[La⁻]₅₅ and [La⁻]₅₅ post. However, no relationship was evident with ΔpH₅₅, Δ[La⁻]₅₅ and [La⁻]₅₅ post, possibly due to the role of Monocarboxylate transporters in regulating the efflux of La⁻ from the muscle by the time the post-exercise biopsy measure was taken. Previous research confirms a lack of a relationship between [La⁻]₅₅ post and t_{exh} [27], while [La⁻]₅₅ post has been reported to be negatively correlated with t_{exh} [28]. These results are similar to the current study and indicate an association between metabolite accumulation in the blood, but not the muscle, and t_{exh}. 

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While there was no association between the SC and $t_{\text{exh}}$, a significant correlation was evident between both $\tau_1$ and $\tau_0$ and $t_{\text{exh}}$. Previous research has reported on the VO$_2$ response of exhaustive constant-load exercise [8,9]; however, to our knowledge, $\tau$ has not previously been reported to be related to $t_{\text{exh}}$. Conceptually, a faster VO$_2$ response reduces the time to peak aerobic functioning and may reduce the reliance on anaerobic metabolism at the commencement of exercise, which in turn, may reduce the early accumulation of possible exercise limiting metabolites. While $\tau_1$ is likely to be influential in reducing the reliance upon anaerobic energy sources during the early (or primary) phases of exercise, it is less likely to be directly responsible for the development of fatigue for exercise durations of approximately 16 min. This may explain the moderate variance in $t_{\text{exh}}$ accounted for by $\tau_1$ ($r^2 = 0.42$). Previously, Demarle et al. [9] have reported a significant correlation between the reduction in AOD and improved $t_{\text{exh}}$ following high-intensity endurance training. However, this data has been the subject of some discussion as only three of six participants improved $t_{\text{exh}}$, while two performed worse, after training [29].

In the present study, no correlations were evident between $\tau$ and either AOD or calculated anaerobic ATP yield. However, a slower $\tau_1$ value was associated with a larger post-exercise [La$^-$]$_b$ value, which in turn was associated with a reduced $t_{\text{exh}}$. [La$^-$]$_m$, which is the predominant value used in the calculation of anaerobic ATP yield, also showed no correlation with $\tau_1$ or $t_{\text{exh}}$. However, this is not surprising as the post-ET biopsy was on average approximately 16 min after the commencement of exercise, allowing time for clearance or further metabolism of La$^-$ [20]. As such, it is not surprising for exercise of
this duration, that no correlation was reported between $\tau_1$ and $[\text{La}^-]_m$ or ATP yield. No other measure, including $\beta_m$, showed any association with $\tau$ or $t_{\text{exh}}$; the current study did find an association between the speed of the $\text{VO}_2$ response ($\tau$) and $t_{\text{exh}}$.

A possible explanation for the lack of correlation between $\tau_1$ and AOD is that it is not valid to determine AOD during prolonged very-heavy exercise. It can be seen in Figure 2 that the excess $\text{VO}_2$ above the predicted $\text{VO}_2$, due to the development of a SC, was greater than the oxygen deficit at the commencement of exercise, resulting in a negative AOD. This is consistent with the recently reported results of Ozyener et al. [30] that during heavy and very-heavy exercise the excess $\text{VO}_2$ from the superimposed SC was large enough to result in negative AOD values. As the conventional method of AOD calculation [19] is dependent on the extrapolation of the sub-maximal steady state $\text{VO}_2$–work relationship, the development of a SC above the predicted $\text{VO}_2$ value will decrease the calculated AOD value. Thus, the assumptions underlying the calculation of AOD for heavy and very heavy exercise from sub-maximal data appear invalid, as the $\text{VO}_2$ response does not follow first-order kinetics and as such, any development of a SC will result in a reduction in the computed AOD.

In conclusion, the SC rise in $\text{VO}_2$ was not related to either $t_{\text{exh}}$ or changes in muscle/blood metabolites, pH or $\beta_m$. However, $\tau$ did show an association with $t_{\text{exh}}$ and post exercise $[\text{La}^-]_b$, which in turn, along with $\Delta\text{pH}_b$ and $\Delta[\text{La}^-]_b$, was related with $t_{\text{exh}}$. It is proposed
that a reduction in the early perturbation of anaerobic energy sources as a result of a
closer $\tau_1$ value may have allowed for a longer $t_{exh}$. Furthermore, the SC rise resulted in the
calculation of negative AOD values, again questioning the validity of traditional AOD
measures in the very-heavy exercise domain.
References:


Table 1: Mean and mean difference ± SD for muscle buffer capacity ($\beta_m$), anaerobic (an) ATP yield and pre and post constant-load endurance test measures of muscle ATP, PCr, Cr, Lactate ([La]$_m$), pH$_m$ and blood measures of lactate ([La]$_b$) and pH$_b$.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Pre-test</th>
<th>Post-test</th>
<th>Mean difference</th>
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<tbody>
<tr>
<td>$\beta_m$ (µmol H$^+$·g muscle dw$^{-1}$·pH$^{-1}$)</td>
<td>169.6 ± 32.9</td>
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<td>-</td>
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<tr>
<td>An ATP yield (mMol·kg$^{-1}$ dw)</td>
<td>-</td>
<td>141.4 ± 51.3</td>
<td>-</td>
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<tr>
<td>ATP (mMol·kg$^{-1}$ dw)</td>
<td>23.0 ± 1.7</td>
<td>19.1 ± 3.3</td>
<td>3.9 ± 2.2</td>
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<tr>
<td>PCr (mMol·kg$^{-1}$ dw)</td>
<td>86.1 ± 16.2</td>
<td>53.3 ± 19.9</td>
<td>**32.9 ± 20.9</td>
</tr>
<tr>
<td>Cr (mMol·kg$^{-1}$ dw)</td>
<td>30.9 ± 16.0</td>
<td>63.7 ± 20.1</td>
<td>*32.8± 18.0</td>
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<tr>
<td>[La]$_m$ (mMol·kg$^{-1}$ dw)</td>
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<td>67.3 ± 25.6</td>
<td>**60.2± 21.3</td>
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<td>pH$_m$</td>
<td>7.10 ± 0.07</td>
<td>6.85 ± 0.07</td>
<td>**0.25 ± 0.09</td>
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<td>[La]$_b$ (mMol·L$^{-1}$)</td>
<td>1.1 ± 0.5</td>
<td>16.3 ± 2.8</td>
<td>*15.4 ± 2.63</td>
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<td>pH$_b$</td>
<td>7.42 ± 0.04</td>
<td>7.17 ± 0.06</td>
<td>*0.26 ± 0.08</td>
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Note: * significantly different from pre-test value (p<0.05)  **(p<0.01)
Table 2: Correlation matrix of the linear relationships between the time to exhaustion (\(t_{\text{exh}}\), \(\dot{V}O_2\) parameters (\(A_1, A_2, \tau_0, \tau_1, \tau_2\)), muscle buffer capacity (\(\beta_m\)), muscle metabolite (\(\Delta\text{ATP}, \Delta[\text{La}^-]_m, \Delta\text{pH}_m, [\text{La}^-]_{m \text{ post}}\)) and blood metabolite (\(\Delta[\text{La}^-]_b, \Delta\text{pH}_b, [\text{La}^-]_{b \text{ post}}\)) values.

<table>
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<tr>
<th></th>
<th>(t_{\text{exh}})</th>
<th>(A_1)</th>
<th>(A_2)</th>
<th>(\tau_0)</th>
<th>(\tau_1)</th>
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<td>(t_{\text{exh}})</td>
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<td>-0.26</td>
<td>-0.54*</td>
<td>-0.65**</td>
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<td>([\text{La}^-]_{m \text{ post}})</td>
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<td>0.05</td>
<td>-0.26</td>
<td>-0.13</td>
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<td>(\Delta[\text{La}^-]_b)</td>
<td>-0.58*</td>
<td>-0.14</td>
<td>0.35</td>
<td>0.46</td>
<td>0.48</td>
<td>-0.34</td>
</tr>
<tr>
<td>(\Delta\text{pH}_b)</td>
<td>-0.60*</td>
<td>0.23</td>
<td>0.49</td>
<td>0.29</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>([\text{La}^-]_{b \text{ post}})</td>
<td>-0.64*</td>
<td>-0.20</td>
<td>0.30</td>
<td>0.43</td>
<td>0.54*</td>
<td>-0.17</td>
</tr>
</tbody>
</table>

Note: * statistically significant (p<0.05).

** statistically significant (p<0.01).
Figure 1: The VO₂ response, tri-exponential fit and residuals for a representative individual subject for the constant-load endurance test.
Figure 2: An example of a “negative” accumulated oxygen deficit, where the excess VO₂ resulting from the slow component (B) rises above the predicted VO₂ from the sub-maximal VO₂ – work relationship. As a result of the excess VO₂, (B) is larger than the oxygen deficit (A).