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Alterations in the VO₂-Power Relationship above the Lactate Threshold during a Graded Bicycling Exercise Protocol

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ABSTRACT

**Purpose:** The purpose of this study was to examine the changes in VO₂-power and energy expenditure-power relationships below (Bₖₜ) and above (Aₖₜ) the lactate threshold (LT). **Methods:** Thirty active subjects (15 males, 15 females) performed a graded exercise protocol on a bicycle ergometer. Initial power outputs were determined based upon subject size and training status. Workloads were increased by 30-Watt increments every 4 minutes until exhaustion. Indirect calorimetry and blood lactate measures were performed during each stage. Linear regressions were developed Bₖₜ and Aₖₜ for VO₂-power and energy expenditure-power (EE-power) relationships. Only 18 subjects (13 males, 5 females) had a sufficient number of data points Bₖₜ and Aₖₜ to adequately describe the linear regressions. **Results:** The individual R² values for VO₂-power and EE-power relationships at both Bₖₜ and Aₖₜ were 0.93 or higher. The mean (±SD) VO₂-power slope (mL/O₂/min/Watt) and intercept (mL/O₂/min) for Aₖₜ were significantly (p<0.01) steeper and lower than the mean Bₖₜ slope and intercept (Slope: Aₖₜ = 12.7±2.9 vs. Bₖₜ = 8.8±1.7; Intercept: Aₖₜ = -146.2±617.1 vs. Bₖₜ = 656.2±186.8). The mean EE-power slope (kcal/min/Watt) and intercept (kcal/min) for Aₖₜ were significantly (p<0.01) steeper and lower than the mean Bₖₜ slope and intercept (Slope: Aₖₜ = 0.067±0.015 vs. Bₖₜ = 0.045±0.009; Intercept: Aₖₜ = -1.624±3.248 vs. Bₖₜ = 2.934±0.917). No sex effect was observed on the changes in slope or intercept for either VO₂-power or EE-power. **Conclusion:** The relationship between power and either VO₂ or energy expenditure during a graded bicycle test suggests an increased oxidative energy requirement relative to power above the lactate threshold.
INTRODUCTION

Aerobic metabolism can be determined indirectly by measuring an individual’s oxygen consumption. Aerobic metabolism occurs primarily during endurance exercise and is the primary energy pathway for physical activities lasting longer than 90 seconds. As such oxygen consumption has been directly linked to the energy requirements for many physical activities. In the laboratory it is common to measure oxygen consumption during incremental exercise on a stationary bicycle or a treadmill. For cycling, oxygen consumption has been shown to increase as work rate (power) is increased. Traditionally, this relationship has been described as being linear (4, 6, 10). Based upon this assumption, predictive relationships have been developed for a variety of parameters such as energy expenditure (13), VO$_{2\text{Peak}}$ (5) and oxygen demands at supramaximal intensities (6, 14). However, some have questioned the linearity of this relationship (2, 5, 8, 9, 11, 12). Some researchers have found that the VO$_2$-power relationship remains linear for all submaximal workloads (4, 6, 10), while others have found either a disproportionate decrease (2,8,9) or increase (5,11,12) from linearity occurring at higher workloads approaching VO$_{2\text{Peak}}$. It is commonly accepted that oxygen consumption and power are linearly related at light to moderate workloads, however some have found an alteration in the relationship as one transitions from moderate to heavy workloads (2, 5, 8, 9, 11, 12). The alteration from linearity at higher workloads has been associated with a dramatic increase in blood lactate that is a parameter known as lactic threshold (LT) and hydrogen ion concentrations (5,11). Although LT has been documented as a threshold beyond which the relationship between VO2 and power are altered, causation and the
mechanisms underlying disproportionate increases or decreases in VO$_2$ are not fully known.

In 1924 a linear relationship between VO$_2$ and work was first observed (4) and has since been confirmed by subsequent investigations (6,10). More recently Medbo et al. (6) concluded that a linear relationship exists using multiple 10-minute stages of increasing treadmill speeds. In some studies non-linear effects showing higher than expected VO$_2$ values were found (6, 11), however to fit the linear model disproportionately high oxygen uptakes were attributed to hyperventilation (>20 ml unstated units/kg) and excluded from the data set (6). In these studies it was determined that the oxygen demand during high-intensity exercise could be underestimated for subjects showing the disproportionately high oxygen uptakes since severe hyperventilation is clearly present near exhaustion (7). It is also argued that using indirect calorimetry and finding a linear VO$_2$-power relationship is a valid representation of total energy expenditure because it has been found that any energy from anaerobic ATP formation is negligible even when blood and muscle lactate concentrations of several milimolars are present (6).

Conversely, there is evidence that supports a nonlinear VO$_2$-power relationship with a decrease in slope at higher exercise intensities (2,8,9). Bickham et al. (2) found a linear VO$_2$-speed response in endurance-trained runners below lactic threshold (B$_{LT}$) but found a significant decrease in the VO$_2$-speed slope and increase in the y-intercept above lactic threshold (A$_{LT}$). It has been proposed that the decrease in the slope of the VO$_2$-speed regression could be attributed to an increase in anaerobic contribution A$_{LT}$ (2,8), increased overall efficiency (2,9), and fast twitch fiber recruitment at higher intensities
Researchers speculate that an increased overall mechanical efficiency may occur at higher submaximal intensities that can be potentially attributed to training at high cadences and speeds (2). It is suggested that the increase overall mechanical efficiency is due to fast twitch fiber recruitment at the higher intensities combined with a higher contractile efficiency for these fast twitch fibers at the faster pedal rate or speed (9). A disproportionate increase in anaerobic contribution to energy expenditure $A_{LT}$ will result in a decreased slope at higher exercise intensities when using indirect calorimetry. Because indirect calorimetry measures aerobic energy expenditure, a decreasing $VO_2$-work slope will be seen if the aerobic portion of total energy expenditure is becoming disproportionately less with increasing energy demand. The reduced slope at higher workloads, and thus, the deviation from linearity, in these studies suggest that a linear regression of $VO_2$–speed data $B_{LT}$ may potentially overestimate actual $VO_2Peak$.

Countering findings that show a decreased slope at higher intensities, several studies have suggested that there is a disproportionate increase in $VO_2$ $A_{LT}$ resulting in a linear relationship that is steeper at higher workloads (1,3,5,11,12). Zoladz et al. (11) discovered a disproportionate increase in $VO_2$ at higher workloads independent of pedaling rate. Factors that may play a role in the increased $VO_2$ at high exercise intensities include increases in lactate and/or hydrogen ion concentrations, the presence of a slow component during exercise, and muscle fiber type recruitment (5, 11, 12). It was found that the magnitudes of the change in $VO_2$ from the expected initial linear relationship at lower workloads followed the degree of lactate accumulation (11). Jones et al. discusses the possibility that the catabolism of lactate and its role as an energy substrate during glyconeogenesis may partially explain the additional energy cost of high
intensity exercises. Zoladz et al. (11) has suggested that the decrease in pH associated with the increase in muscle lactate concentrations may play an important role in shifting the oxyhemoglobin dissociation curve to the right to facilitate the exchange of oxygen at the level of the tissue. The increase in lactate and/or hydrogen ion concentrations may lead to a disproportionate increase in VO₂ by accelerating the rate of mitochondrial respiration by increasing free creatine concentrations (5).

During continuous constant load protocols at workloads A_LT there is evidence that VO₂ does not plateau or reach a steady state but continues to rise until VO₂peak is reached, which is known as the slow component (5,11). During an incremental treadmill test with 7 minute stages, Jones et al. (5) analyzed ventilation between 3.0 and 3.75 minutes and again between 6.0 and 6.75 minutes to assess if VO₂ attains a steady state within 3 minutes. A significant difference in the change in VO₂ was found at running speeds above but not below LT. This alludes to the possibility that stages A_LT may have a disproportionately higher VO₂ due to slow component contribution. The slow component may be caused by an increase in additional accessory muscles such as those involved in stabilization, posture and respiration (11). An increase in core body temperature and recruitment of type II muscle fibers have also been linked to the contribution of the slow component (5,12). Current thinking suggests that the energetics of contraction of the different muscle fiber types may be the main driver of the slow component (5). Increases in core body temperature increase the metabolic activity of the body and therefore require a larger O₂ demand. When increasing exercise intensity, the hierarchical recruitment of different fiber types may contribute to the increase in VO₂ (11). The mitochondria of type II muscle fibers have a lower P to O ratio than type I fibers, which is a measure of the
efficiency of coupling phosphorylation to oxidation (5). This would suggest that type II fibers would have a greater VO\textsubscript{2} for any given rate of ATP resynthesis and that type II fibers are less efficient than type I fibers. It has been shown that type II muscle fibers are active during the exercise intensity domain that is associated with the slow component, and that both gross and net efficiency have been shown to be positively correlated with the percentage of type I fibers (5).

My research examined the changes in VO\textsubscript{2}-power and energy expenditure (EE)-power relationships below and above LT. It is hypothesized that a positive deviation from linearity will be observed \(A_{LT}\) primarily due to cumulative physiological effects such as an increase in core and muscle temperature, lactate and hydrogen ion concentrations, and slow component effects. This area of research has implications in both research and applied settings as currently linear relationships are still typically used. If there is a difference in the VO\textsubscript{2}-power and EE-power relationships \(B_{LT}\) and \(A_{LT}\), the physiological measure to be predicted must be considered carefully. If deviations from linearity exist, predicted physiological measures near maximal or at supramaximal intensities may deviate significantly from actual values. Using linear models to make predictions where a non-linear relationship exist would result in over or underestimations of the actual value.

**METHODS**

*Subjects*

Thirty healthy and physically active (15 male, 15 female) (mean ± SD; age 23.4 ± 4.6 years, weight 69.5 ± 10.5 kg, VO\textsubscript{2peak} 46.6 ± 8.9 ml/kg/min) volunteered for the
study. Only 18 subjects (13 males, 5 females) had a sufficient number of data points $B_{LT}$ and $A_{LT}$ to adequately describe the linear regressions. This was due to the use of an existing data set obtained from a study that was not originally designed to examine relationships $B_{LT}$ and $A_{LT}$. Subject characteristics are reported in Table 1. Subjects filled out a Physically Activity Readiness Questionnaire (PAR-Q) to ensure that they were healthy and free of medical contraindications and were classified as physically active by meeting the American College of Sports Medicine (ACSM) and the American Heart Association’s (AMA) updated physical activity recommendations. Participants in the study were required to exercise at least three times per week for at least 45 minutes per session for a total of at least 150 minutes per week. All subjects gave a written informed consent, which was obtained by the lead investigator prior to participation. This project was approved by the Internal Review Board of the University of Colorado at Boulder (Protocol Number: 11-0342).

**Table 1. Subject Characteristics.** Norm, normalized; LT, lactate threshold; Mech Eff, mechanical efficiency; Econ, economy. * Significant difference between M and F, $p<0.05$.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>BMI (kg/m²)</th>
<th>VO₂peak (l/min)</th>
<th>Norm VO₂peak (ml/kg⁻¹min⁻¹)</th>
<th>LT Power (W)</th>
<th>LT (%VO₂peak)</th>
<th>Econ at LT (W⁻¹min⁻¹)</th>
<th>Lab HRpeak (bpm)</th>
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<td>71%</td>
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<td>68%</td>
<td>76.7</td>
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<td>65%</td>
<td>78.7</td>
<td>171</td>
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<td>34.89</td>
<td>148</td>
<td>70%</td>
<td>55.5</td>
<td>176</td>
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</tbody>
</table>

**Female**

| Mean | SD  | 7.4 | 0.41 | 8.04 | 6.7 | 4% | 9.2 | 10.7 |

**Male**

| Mean | SD  | 21.7 | 2.6 | 0.69 | 8.91 | 27.1 | 4% | 9.6 |

* Significant difference between M and F.
Test Methods

This study was part of a larger investigation that aimed to compare actual energy expenditure to predicted energy expenditures from heart rate-power relationships during bicycling and non-bicycling exercise tasks under steady state and non-steady state conditions. Only data obtained from the first session of the larger study consisting of a graded exercise test (GXT) was used for the current protocol.

All testing was done on either a Lode bicycle ergometer or the subject’s own bicycle equipped with a CycleOps power meter mounted on a CompuTrainer® electronically braked bicycle trainer. Energy expenditure and VO\textsubscript{2} were measured using open circuit indirect calorimetry requiring subjects to be fitted with a head harness to properly secure a rubber mouthpiece in their mouth and a plastic nose clip to prevent airflow in and out of the nose during exercise. The mouthpiece was attached to a respiratory valve connected to hosing that leads to a gas analyzer and a pneumotach. A Parvo metabolic cart (Parvo Medics TrueOne® 2400) analyzed the expired gases (reported as 15 second averages) during the GXT. The metabolic cart was manually calibrated by performing gas and volume calibrations before each exercise test to ensure accurate results. To gather blood lactate data for all subjects, those with low finger blood flow wore a nitrile glove with a hand-warmer inserted under their palm to increase blood flow to their fingers for lactate sampling (explained below). Because subjects were unable to speak while oxygen consumption is measured, subjects pointed to a Borg’s Rate of Perceived Exertion scale (RPE) to evaluate their perceived exertion. Subjects were given strong verbal encouragement to go as long as possible during the GXT to elicit their best performance.
Graded Exercise Test

Prior to the GXT, subjects warmed up for 2-5 min at a lower power output than the initial stage. The subject began the test at a workload between 50 and 105 watts for women and 60 and 100 watts for men. This was done to allow the subject to complete approximately 6 to 8 stages. With more stages completed, there was a greater chance that there would be a sufficient number of data points $B_{LT}$ and $A_{LT}$ to adequately describe the linear relationships $B_{LT}$ and $A_{LT}$. Subjects were instructed to choose a comfortable cadence between 60 and 110 rpm that they would maintain throughout the test (7). Each stage lasted 4 minutes and the workload was increased by 30 watts with each new stage. Between the 3rd and 4th minutes of each stage, the subjects RPE was recorded and a blood lactate sample was taken. Once the subject reached volitional fatigue and could not maintain their cadence or stopped on their own volition, the test ended.

Blood lactate

Capillary blood samples were obtained prior to exercise, over the last 60 seconds of each workload during the GXT, and two minutes after the end of the test for blood lactate assessment. Blood was be drawn into a heparinized capillary tube from blood drops on the pricked finger and 25 µl of whole blood was mixed with 50 µl of a buffer solution, lysing agent (Octylphenoxethanol), and glycolytic inhibitor (NaF anhydrous) to prevent further metabolism from occurring. The blood-buffer solution was vortexed and analyzed in duplicate with a YSI 2300 Stat Plus lactate analyzer that was calibrated every 5 samples. Individual subjects’ blood lactate concentrations for each workload were plotted to determine LT (15). LT is the point of inflection (breakpoint) where the lactate
concentration begins to increase exponentially during intense exercise and the LT was defined as 1mmol/l above the LT breakpoint. LT was used to separate the high intensity workloads from the lower intensity workloads, more specifically if a change from linearity were to occur we would expect to see it $A_{LT}$ not $B_{LT}$.

Statistics

Linear regressions and correlations were developed using Microsoft Office Excel. Paired samples t-test and independent t-tests were used where appropriate using either Microsoft Office Excel or IBM SPSS Statistic’s version 20 and 21 software. Slopes and y-intercepts for the linear regressions of VO$_2$-power and EE-power were compared using paired t-test at a significance level of 0.05.

RESULTS

The individuals $R^2$ values for VO$_2$-power relationship at both $B_{LT}$ and $A_{LT}$ were 0.93 or higher. The mean (±SD) VO$_2$-power slope (mlO$_2$/min/Watt) and intercept (mlO$_2$/min) for $A_{LT}$ were significantly (p<0.01) steeper and lower than the mean $B_{LT}$ slope and intercept (Slope: $A_{LT}$ = 12.7±2.9, $B_{LT}$ = 8.8±1.7; Intercept: $A_{LT}$ = -146.2±617.1, $B_{LT}$ = 656.2±186.8) (Figure 1, Figure2). Additionally, by utilizing the respiratory quotient the subjects’ metabolic substrate utilization could be accounted for as a possible reason for a change in the VO$_2$-power relationship. A regression of energy expenditure (EE)-power was made and the same trends were observed. The mean EE-power slope (kcal/min/Watt) and intercept (kcal/min) for $A_{LT}$ were significantly (p<0.01) steeper and lower than the mean $B_{LT}$ slope and intercept (Slope: $A_{LT}$ = 0.067±0.015, $B_{LT}$ =
0.045±0.009, Intercept: A_{LT} = -1.624±3.248, B_{LT} = 2.934±0.917 (Figure 3, Figure 4). No sex effect was observed on the changes in slope or intercept for either VO\textsubscript{2}-power or EE-power.

**Figure 1. The mean slope and y-intercept for VO\textsubscript{2}-power.** The slope and y-intercept B_{LT} is significantly different from A_{LT}.

*Significantly steeper slope (P<0.000) and significantly decreased y-intercept (p<0.000) compared to B_{LT}.

**Figure 2. The mean ±SD for slope and y-intercept changes for VO\textsubscript{2}-power relationships.** A. The slope change B_{LT} and A_{LT}; B. The y-intercept change B_{LT} and A_{LT}. 

\[ y = 0.0088x + 0.6562 \]
Figure 3. **The mean slope and y-intercept for EE-power.** The slope and y-intercept \( B_{LT} \) is significantly different from \( A_{LT} \).

\[
y = 0.0447x + 2.9343
\]

\[
y = 0.0668x - 1.6238
\]

Figure 4. **The mean \(\pm\)SD for slope and y-intercept changes for EE-power relationships.** A. The slope change \( B_{LT} \) and \( A_{LT} \); B. The y-intercept change \( B_{LT} \) and \( A_{LT} \).

* Significantly different than \( B_{LP} \), \( p<0.01 \)

* Significantly different than \( B_{TD} \), \( p<0.01 \)
DISCUSSION

A significant increase in the slope of the VO$_2$-power and EE-power regressions $A_{LT}$ was demonstrated. The average increase in the slope of the VO$_2$-power regression $A_{LT}$ was 44.3% when compared to $B_{LT}$ resulting in a significantly different predicted VO$_{2\text{Peak}}$ between these regressions. The average increase in the slope of the EE-power regression $A_{LT}$ was 44.9% when compared to $B_{LT}$. This agrees with the data that shows a significant decrease in the $y$-intercept of the VO$_2$-power and EE-power regressions $A_{LT}$ was demonstrated. Using the power at VO$_{2\text{Peak}}$, the VO$_2$ predicted using the $A_{LT}$ regression was not significantly different than the actual VO$_{2\text{Peak}}$. However, VO$_2$ predicted using the $B_{LT}$ regression was significantly different from VO$_{2\text{Peak}}$, deviated by -7.7%. This data supports the hypothesis that a positive deviation from linearity occurs above $A_{LT}$ and can provide a better understanding of what may contribute to an increased VO$_2$ $A_{LT}$ and the possible physiological mechanisms responsible for the observed trend.

These findings are consistent with previous reports from Jones et al. (5) and Zoladz et al. (1995) (11) who reported that the deviations in VO$_2$ from the expected initial linear relationship at lower workloads followed the degree of blood lactate accumulation. Nevertheless, the finding of the present study and those of earlier studies (5,11,12) are in contradiction to the more traditional views that the VO$_2$-power relationship remains linear for all submaximal workloads up to VO$_{2\text{max}}$ (4,6,10). It has been suggested that the non-linearity of the VO$_2$-power relationship has not been recognized more often due to the type of protocol used and that any isolated data points which deviate from linearity may be dismissed either as experimental noise or the consequence of a disproportionate increase in ventilatory, cardiac or postural costs (11).
In many multi-stage tests it is possible that very few data points are recorded at high workloads $A_{LT}$ preventing the identification of non-linearity due to the inability of subjects to maintain exercise past 2-3 stages $A_{LT}$. Future considerations to account for a lack of data points $A_{LT}$ include using smaller incremental workload increases as well as first determining lactic threshold and VO$_{2Peak}$ and then having the subject at exercise intensities that lie within those two parameters.

To address some of the differences between protocols and to better understand reasons for disproportional VO$_2$ uptake, a within group pilot study was performed in order to examine some of the differences between using a continuous (CONT) versus discontinuous (DISCONT) GXT protocol. For the pilot study, 4 endurance-trained subjects performed the CONT GXT protocol beginning at 90 W. To maintain consistency with the original study, the workload increased by 30 W every 4 minutes until volitional exhaustion. The following two days after CONT, the subjects returned at the same time during the day and performed the same power outputs achieved during the CONT for 4 minutes, but each stage was separated by 10 minutes of seated rest. The power outputs were paired together and randomized across the two days. The pilot data supported the original findings providing evidence that there is a disproportionate increase in VO$_2$ $A_{LT}$ during the CONT, while data from the DISCONT shows a linear VO$_2$-power relationship (Figure 3, 4, 5). These finding show that the VO$_2$-power relationship may be protocol dependent and may allude to possible reasons for the non-linear relationship during a CONT.
Figure 3. The mean VO$_2$-power relationship for CONT and DISCONT. Significant differences in VO$_2$ are found at 240 watts and 270 watts.

* Significant difference between CON and DISCON (P<0.05)

Figure 4. The mean ±SD slope changes for VO$_2$-power relationships for CONT and DISCONT. Pre, A$_{LT}$; Post, B$_{LT}$. The slope change B$_{LT}$ and A$_{LT}$ For both CONT and DISCONT are significantly different.

*Significantly different than Pre-CONT; **Sign different than Post-CONT
Figure 5. The mean ±SD y-intercept changes for VO$_2$-power relationships for CONT and DISCONT. Pre, A$_{LT}$; Post, B$_{LT}$. The slope change B$_{LT}$ and A$_{LT}$ for both CONT and DISCONT are significantly different.

*Significantly different than Pre-CONT; ** Sign different than Post-CONT

The reasons for a disproportionate increase in VO$_2$ A$_{LT}$ is not clear, however, it is suggested that the change in the slope and intercept may be related to the same factors that attribute to the slow component (2,5,11,12). An increase in VO$_2$ contributing to the slow component could be partly due to the increased cardio and respiratory work as a result of an increase in overall energy demand. Data from the pilot work shows that there was significant difference for the average VE (l/min) between CONT and DISCONT at the higher workloads. Using data reported by Dempsey et al. (16) and Kitamura et al. (17) it was calculated that the additional O$_2$ cost of high pulmonary ventilation and heart rates would account for 10-12% of the total additional VO$_2$ at higher workloads in the pilot study. It was determined that 12% of the increase in oxygen consumption between
CONT and DISCONT we see at 240 watts can be attributed to the increase in energy demand of the respiratory and cardiac systems.

Additionally, the energetics of contraction and the recruitment of type II muscle fibers provide a likely explanation for the slow component and disproportionate increase in VO$_2$ A$_{LT}$ (5, 11). The mitochondria of type II muscle fibers have an 18% lower P:O ratio than type I fibers demonstrating that type II muscle fibers are less efficient at producing ATP then type I fibers (5). This suggests that type II fibers would have a greater VO$_2$ for any given rate of ATP resynthesis. A deceased efficiency of working muscle at higher workloads would then require more overall fibers to be recruited which in turn increases VO$_2$ demand.

In support of a disproportionate increase in O$_2$ uptake, proposed mechanisms for the decrease in efficiency include the accumulations of lactate and/or hydrogen ion concentrations and temperature (2, 5, 11, 12). The correlation between lactate and/or hydrogen ion concentrations and the disproportional increase VO$_2$ A$_{LT}$ may hold some answers in regard to efficiency. The catabolism of lactate and its role as an energy substrate during glyconeogenesis and the decrease in pH associated with the increase in muscle lactate concentrations may partially explain the additional energy cost of high intensity exercises (5,11). The increase in lactate and/or hydrogen ion concentrations may accelerate the rate of mitochondrial respiration causing a disproportionate increase in VO$_2$ (5). An increase in core and local muscle temperature may also have affected muscle efficiency and VO$_2$ A$_{LT}$. Core and muscle temperature were not measured in this present study, however, there is evidence to suggest that muscle temperature can affect muscle fiber recruitment patterns as well as the power-velocity relationship (5). An
increase in core temperature can increase the body’s metabolism leading to an overall increase in VO₂, known as the Q10 effect on enzyme activity.

The progressive increase in VO₂ seen over longer duration constant workload exercise A_LT known as the slow component was not evident in the present study. Although the slow component was not evident, a progressive increase in VO₂ during prolonged exercise at intensities A_LT is well documented and widely accepted (5, 11, 12). With that said, it is acknowledged that the length of each stage may not have been long enough to see a significant O₂ drift. The slow component could cause the total VO₂ to be greater than the predicted VO₂ from the extrapolation of the VO₂-power relationship for steady state exercise B_LT.

Based on our finding and the data provided from our more recent pilot work, the energy demands and thus VO₂ appear to be at least in part protocol dependent. A disproportionate increase in VO₂ A_LT is present during CONT but not DISCONT. This may be contributed to, but is not exclusive to, differences in body temperature, hydrogen ion concentration, and the reliance on oxidative and non-oxidative pathways. The continuous protocol may result in an overall increased core and/or muscle temperature as well as an increase in hydrogen ion concentration compared to the discontinuous protocol, effectively decrease the efficiency of working muscle. Furthermore, the discontinuous protocol may rely more on non-oxidative energy sources for acute 4-minute bouts than the prolonged exercise session of the continuous protocol through the recruitment of type II muscle fibers.

In conclusion, there was a disproportionate increase in VO₂ with increasing power at workloads A_LT. Since there is a difference in the VO₂-power and EE-power slopes B_LT.
and $A_{LT}$, the physiological measure to be predicted must be considered carefully. Using one relationship over the other can result in significant differences in $VO_{2\text{Peak}}$ or supramaximal $VO_{2}$. With that said, the protocol chosen may play a major role in the different $VO_{2}$-power and EE-power trends, and thus consideration of the continuous and discontinuous protocol must also be considered.

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