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# "May the Force be with you": The Role of Strength Training in Metabolic Efficiency and Exercise Tolerance

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*"May the Force be with you":*

*The Role of Strength Training in Metabolic Efficiency and Exercise Tolerance*

Federico Fontana

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*This thesis is dedicated to the people that let me be part of their life,*
*making mine a unique experience.*

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## ABSTRACT

Traditionally, exercise intensity is expressed as a percentage of maximal oxygen uptake ($VO_{2max}$). It is known that as exercise intensity increases in any individual, unique thresholds exist that demarcate boundaries associated with specific physiological and metabolic profiles. The concept of threshold intensities is complex and has generated a large amount of debate within the scientific community. Much of the controversy extends from differences in ideas in determining a specific intensity of exercise below which exercise is sustainable for long durations and above which is unsustainable for prolonged periods.

This thesis is organized into five main chapters.

The 1st study aimed at clarifying the physiological rational behind the concept of threshold-based exercise intensity. We focused on comparing the most common threshold indexes used to partitions intensity of exercise into ranges (*i.e.* moderate, heavy, very heavy and severe domains)). Those are: Maximal Lactate Steady State (MLSS), Critical Power (CP), Respiratory Compensation Point (RCP) and the deoxyhemoglobin breakpoint ($[HHb]_{BP}$). Whether commonalities between these "thresholds" exist, the notion of "critical metabolic rate" is presented as the highest metabolic rate at which exercise is well tolerated for long durations. The 2nd and 3rd studies focused on the determination of alternative methods for exercise intensity threshold identification using novel statistical approaches and/or different measurement techniques. Identification of exercise intensity domains has important implications/applications for research interventions, however the most common indexes of threshold intensity that are nowadays used are often cumbersome, invasive, time consuming and pose a burden to participants and researchers involved. For these reasons, alternative methods that are not only valid but also time effective and non-invasive are very attractive. Finally, in the 4th and the 5th study the body response to exercise above this threshold intensity phenomenon that demarks sustainable versus unsustainable physical activity is investigated. It is nowadays recognised that the exercise intensity at which this progressive loss of metabolic stability become

manifest (called "excess $VO_2$" and "$VO_2$ slow component" in the two exercise paradigms respectively) occurs at intensity of exercise above the "critical metabolic rate" (study #1), a landmark that demarcates the lower boundary of the "heavy" intensity domain. Attention is therefore given to the possible role of an intervention affecting this loss of efficiency in the heavy-intensity domain of exercise and therefore affects the genesis of the "excess" $VO_2$ and/or the $VO_2$ slow component.

Collectively, the studies contained within this thesis have contributed to better understand the "threshold" phenomenon and the physiological bases behind it. Additionally, new methods of threshold determination have been developed giving practical alternative tests for its determination in different contexts and populations. Finally, this thesis has also provided useful information regarding the body metabolic response in the heavy intensity domain (*i.e.* above "threshold") and the role of strength training as a possible determinant in affect such a body response to high intensity training.

# SUMMARY

This thesis is organized into five main chapters.

The 1st study aimed at clarifying the physiological rational behind the concept of threshold-based exercise intensity. Numerous physiological exercise-intensity 'thresholds' exist in the literature (each with unique nomenclature and methodology for determination) creating ambiguity regarding their physiological-bases and relevance. Each of these "paradigms" uses different methods of measurement and may be preferred based on consistencies with previous work, available instruments, and the test population, lending to confusion regarding which one represents the 'ceiling' of tolerable endurance exercise and whether they are physiologically equivalent. This first work focused on comparing, in twelve healthy young man, the most common threshold indexes used to partitions intensity of exercise into ranges (*i.e.* moderate, heavy, very heavy and severe domains)). Those are: Maximal Lactate Steady State (MLSS), Critical Power (CP), Respiratory Compensation Point (RCP) and the deoxyhemoglobin breakpoint ($[HHb]_{BP}$). Irrespective of the controversial physiological demarcation of these threshold intensities it is possible that each of the above mentioned "thresholds" might be the result of similar underlying physiological and metabolic processes reflecting a common level of aerobic metabolism beyond which there is a progressive loss of homeostasis. Whether commonalities between these "thresholds" exist, the notion of "critical metabolic rate" is presented as the highest metabolic rate at which exercise is well tolerated for long durations.

The 2nd and 3rd studies focused on the determination of alternative methods for exercise intensity threshold identification using novel statistical approaches and/or different measurement techniques. Identification of exercise intensity domains has important implications/applications for research interventions, however the most common indexes of threshold intensity that are nowadays used (*i.e.*, MLSS, CP, RCP) are often cumbersome, invasive, time consuming and pose a burden to participants and researchers involved. For these reasons, alternative methods that

are not only valid but also time effective and non-invasive are very attractive. The relevance of the present studies resides in providing objectives, reliable, and easily detectable demarcation indexes of an exercise intensity associated to a common "threshold" phenomena.

The study #2 investigated, in 118 healthy males (with age ranging from 20 to 79 years and fitness level spanning from 10 to 100 percentile of each individual's age group) whether Near Infrared Spectroscopy (NIRS) technology can be utilized for the determination of threshold intensities during a ramp incremental exercise. This technology provides the means to non-invasively collect high-resolution data and can be used in both laboratory and field settings.

In study #3 we tested in 14 healthy males, the ability to identify a critical intensity of exercise during cycling (*i.e.* "threshold") using a common marker on exercise intensity: lactate accumulation during a sub-maximal constant work rate exercise. In cycling, an individual critical intensity is traditionally calculated from the relationship between mean external power output and time-to-exhaustion during a series of exhausting and time consuming trials. For the purpose of this threshold determination in athletes as well as in non-athletic groups, the development of a quick and sub-maximal testing protocol rather than an exhausting task is paramount.

Finally, in the 4$^{th}$ and the 5$^{th}$ study the body response to exercise above this threshold intensity phenomenon that demarks sustainable versus unsustainable physical activity is investigated. It is widely accepted that the body metabolic response to high intensity exercise highlights a progressive loss of muscle homeostasis as exercise proceeds during both a ramp incremental exercise and a constant work rate exercise. It is nowadays recognised that the exercise intensity at which this progressive loss of metabolic stability become manifest (called "excess $VO_2$" and "$VO_2$ slow component" in the two exercise paradigms respectively) occurs at intensity of exercise above the "critical metabolic rate" (study #1), a landmark that demarcates the lower boundary of the "heavy" intensity domain. Beyond this boundary $VO_2$ will no longer stabilize (in the case of a step transition exercise) or keep increasing with the same slope (in the case of

a ramp incremental exercise) and prolonged exercise will cause it to project until $VO_{2max}$ is reached and exercise intolerance occurs. A progressive increase in fatigue of type I fibers, that is associated with the increase the ATP cost of contraction and of the $O_2$ cost of ATP resynthesize and/or a progressive increase in the recruitment of intrinsically less efficient type 2 fibers have been proposed as putative causes of the loss of efficiency and then task failure. Attention is therefore given to the possible role of an intervention affecting muscle recruitment pattern in reducing this loss of efficiency in the heavy-intensity domain of exercise and therefore affects the genesis of the "excess" $VO_2$ and/or the $VO_2$ slow component.

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## Definitions of Selected Terms

| | |
|---|---|
| $[La^-]$: | Blood Lactate Concentration |
| Δ: | Delta; Change |
| 1-β: | Power |
| 1RM: | One repetition Maximum |
| ANOVA: | Analysis of Variance |
| b: | Bias |
| $b_0$: | Intercept |
| $b_1$: | Slope |
| BF: | Body Fat |
| BMI: | Body Mass Index |
| BP: | Break Point |
| CI: | Confidence Interval |
| CI: | Critical Intensity |
| CP: | Critical Power |
| CV: | Coefficient of Variation |
| d: | Effect size |
| deoxyHb: | Deoxyhemoglobin |
| EMG: | Electromyography |
| F: | F-ratio |
| GET: | Gas Exchange Threshold |
| $[H^+]$: | Hydrogen Ion Concentration |
| HHb: | Haemoglobin |
| HR: | Heart rate |
| iEMG: | integrated EMG |
| IPF: | Isometric Peak Force |
| IPRDF: | Isometric Peak Rate of Force Development |
| LT: | Lactate Threshold |
| MA: | Major Axis Regression |
| MLSS: | Maximum Lactate Steady State |
| MPF: | Mean Power Frequency |
| $MS_m$: | Mean Squared Model |

| | |
|---|---|
| $MS_r$: | Mean Squared Residual |
| $MS_t$: | Mean Squared Total |
| MVC: | Maximal Voluntary Contraction |
| NIRS: | Near Infrared Spectroscopy |
| OBLA: | Onset of Blood Lactate Accumulation |
| p: | Probability |
| PCr: | Phosphocreatine |
| PO: | Power Output |
| $PO_{peak}$: | Peak Power Output |
| $R^2$: | Coefficient of determination |
| RCP: | Respiratory Compensation Point |
| RFD: | Rate of force development |
| RI: | Ramp Incremental Exercise |
| RMS: | Root Mean Square |
| $r_p$: | Pearson correlation coefficient |
| RPM: | Revolution per Minute |
| $r_s$: | Spearman correlation coefficient |
| SD: | Standard Deviation |
| SE: | Standard Error |
| SEE: | Standard Error of the Estimate |
| t: | time |
| $t_e$: | time to exhaustion |
| VL: | Vastus Lateralis |
| $VO_2$: | Oxygen Consumption |
| $VO_{2peak}$: | Maximal Oxygen Consumption |
| $VO_{2SC}$: | Slow Component of Oxygen Consumption |
| $VT_1$: | First Ventilatory Threshold |
| $VT_2$: | Second Ventilatory Threshold |
| z: | Z-score |
| $\alpha$: | Type I error rate |
| $\beta$: | Type II error rate |

# Chapter 1

# Introduction

## Background

For years, the concept of threshold-based exercise intensity has been used to assess and stratify cardiorespiratory fitness and health, for exercise prescription, and to quantify the outcomes of specific interventions (10). Yet, numerous physiological 'thresholds' exist in the literature (each with unique nomenclature and methodology for determination) creating ambiguity regarding their physiological-bases and relevance. One of the most known is the "lactate threshold" (LT). The LT is the metabolic rate at which blood lactate begins to increase above baseline values (12).

To date, a popular model for prescribing exercise is the 'intensity domain' model (outlined by Whipp et al. (16)) which partitions intensity into ranges (or clusters) of power outputs that elicit common pulmonary $O_2$ uptake ($VO_2$) response characteristics based on an indirect estimation of the LT through gas exchange measurements (i.e.: *i) moderate*: encompasses all work rates that are below the LT; *ii) heavy*: comprises those work rates lying between LT and the asymptote of the power duration relationship for high intensity exercise, that is the critical power (CP); *iii) very heavy*: comprises those work rates lying between the CP and the $VO_{2max}$; and *iv) severe*: corresponding to a work rate that is sufficiently high so as to limit the tolerable duration of exercise to <140s and in which fatigue intervenes before $VO_{2max}$ can be achieved). In this schema, the 'threshold' separating *heavy* from *very heavy* exercise (i.e., sustainable from unsustainable constant-power exercise) is CP (8). Traditionally, an individual's critical power is calculated from the relationship between mean external power output and time-to-exhaustion; this relationship is experimentally determined through a series (typically two to five) of constant-intensity exercise trials performed at different power outputs, each of them prolonged until exhaustion (9). Such a time-consuming and exhaustive effort requires high motivation from participants that might be difficult to obtain and/or present health risks that are unacceptable in

groups such as children and/or older adults. Moreover, in athletes, who are often concerned with possible impairment to their performance, highly demanding and/or time consuming protocols might reduce their willingness to be tested, especially close to important competitions (4). Given the constraints associated with this traditional evaluation, testing procedures that are not only valid, but also versatile, cost-effective and quick are warranted.

One alternative index of a threshold intensity that is commonly used is maximal lactate steady state (MLSS), which represents the highest exercise intensity at which an elevated blood lactate concentration can be stabilized and sustained for a prolonged period of exercise (1, 2). MLSS is considered the "gold standard" for measurements of exercise tolerance. However, the practical application of this test is cumbersome as it requires invasive blood measurements and is time consuming, thus posing a burden to participants and researchers involved in the test (1). As such, the use of ventilatory parameters has become a prevalent methodology to estimate the highest exercise intensity that is tolerable and sustainable for prolonged durations (15, 16).

Using ventilatory and gas exchange parameters collected during an incremental exercise test, two threshold can be identified: *i)* the first ventilatory threshold ($VT_1$), or gas exchange threshold (GET), which reflects the exercise intensity corresponding to the initial appearance of appreciable lactate concentration in the blood; *ii)* the second ventilatory threshold ($VT_2$), or respiratory compensation point (RCP), which is related to the ventilatory response to a metabolic acidosis (15). Although the use of ventilatory parameters provides a time-efficient and non-invasive estimation of landmarks of exercise intensity, several limitations to this approach have been identified (5). For instance, lower accuracy compared to more invasive methods, subjectivity in the determination, and potential calculation errors in subjects with irregular breathing pattern have been proposed (5).

Recent investigations have proposed the utilization of near infrared spectroscopy (NIRS) technology for the determination of threshold intensities during exercise (1, 5, 10). This technology provides the means to non-invasively collect high-resolution data and can be used in both laboratory and field settings. Specifically,

it has been shown that a deflection point in the NIRS-derived deoxygenated hemoglobin (*deoxy*Hb) signal during incremental cycle exercise occurred at the same $VO_2$ as that associated with the MLSS, indicating that MLSS could be accurately determined using quantitative measures of *deoxy*Hb (5).

During ramp incremental cycle exercise to exhaustion, there is a linear increase in $VO_2$ relative to the mechanical power output, with a functional gain ($\Delta_{VO2}/\Delta_{PO}$) that varies between 8 and 12 $ml \cdot min^{-1} \cdot W^{-1}$ (3). A homogeneous linear relationship is often assumed across the whole exercise intensity spectrum. However, when the exercise exceeds the above-mentioned critical intensity, the rise in $VO_2$ as a function of work rate displays an increased slope that justifies the description of $VO_2$/PO relationship as a double linear as opposed to a single-linear function (3, 11). The development of this so-called "excess $VO_2$" in the heavy-intensity domain of incremental exercise entails a progressive loss of efficiency (17). The more pronounced the excess $VO_2$ is, the earlier $VO_{2peak}$ will be reached and task failure will ensue, in turn causing a reduction/impairment of exercise tolerance.

The excess $VO_2$ appearing in the heavy-intensity domain of an incremental exercise has been considered related to the slow component of oxygen consumption ($VO_{2SC}$) occurring during constant-work rate exercise above the lactate threshold (6, 7). The $VO_{2SC}$ represents an increase in oxygen consumption during constant work rate exercise that projects above the expected $VO_2$ steady state for a given workload (14). This $VO_{2SC}$ occurs when exercise is performed in the heavy (above LT or the GET) or even in the very-heavy/severe (above the RCP or CP) intensity domains. It has been suggested that similar physiological mechanisms, intrinsic to the working muscles, may underpin the loss of efficiency that characterises the heavy-intensity domain in both exercise paradigms (13).

A progressive increase in fatigue of type I fibers, that is associated with the increase the ATP cost of contraction and of the $O_2$ cost of ATP resynthesis and/or a progressive increase in the recruitment of intrinsically less efficient type 2 fibers have been proposed as putative causes of the loss of efficiency (7). For this common nature of both the genesis of the "excess" $VO_2$ and of the the $VO_{2SC}$, it may be conceived that an intervention, such as strength training, that might be

able to increase maximal muscle force, would reduce the recruitment of high-threshold motor units to sustain a given absolute power output. In turn, if the recruitment of higher order motor units plays a role in the genesis of the excess $VO_2$, then a strength training intervention should be able to affect it.

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**Significance of the Research**

Identification of exercise intensity domains has important implications and applications for research and applied interventions. As in study #1, substantiating the existence of a unique "metabolic boundary" (expressed in $VO_2$, rather than power output as in different constructs such as CP, MLSS, RCP and $[HHb]_{BP}$) partitioning heavy from very heavy exercise domains (or sustainable versus unsustainable effort) might be of valuable importance for researchers, sport scientist, clinicians and practitioners. Demonstrating that each constructs may be physiologically equivalent ("interchangeability" of each index) may provide with a number of options for determining this limit of tolerable endurance exercise that represents the highest VO2 at which the metabolic response to exercise can be stabilized.

Whether these different "threshold" may occur as a result of similar physiological response, the relevance of the study #2 and the study #3 resides in providing alternative, objective, reliable and time effective methods to demarcate the heavy-, very-heavy boundary of tolerable effort. Additionally, for the purpose of such a critical intensity of exercise determination in athlete as well in non-athletic populations such as older or unfit individuals, the development of quicker and easier testing protocol is paramount. Lastly, the diffusion of new technology (*i.e.* NIRS) and/or alternative methods for threshold determination, that are both time and cost effective, may increase the applicability of the scientific method on a larger scale, facilitating repeated measures over time while reducing the health risks associated with exhaustive trials.

While the determination of such a "metabolic boundary" started to be easier with alternative methods for its determination, the study of the body response to high intensity exercise (*i.e.* above the "metabolic boundary") becomes more feasible. In particular, the study #4 and the study #5 focused on two well-known phenomena that characterized the metabolic response in the heavy-, very-heavy exercise domains: the "excess" $VO_2$ and the $VO_2$ slow component. The magnitude of both body responses can account for ~25% of the total increase in $VO_2$ during exercise and therefore they importantly impact the exercise tolerance.

Additionally, the occurrence of both phenomena have the following practical implications: *i)* undermine the fundamental concept of steady state; *ii)* may result in an upward curvature of the $VO_2$-PO relation during incremental exercise; *iii)* represent a progressive loss of skeletal muscle contractile efficiency and exercise efficiency; *iv)* confound calculation of the $VO_2$ deficit; *v)* they render the description of exercise intensity as a percentage of $VO_{2max}$ inappropriate; *vi)* they reduces exercise tolerance. For the above reasons, the study of the "excess" $VO_2$ and of the $VO_{2SC}$ and of possible intervention that that could reduce both might be useful for: *i)* basic physiology, providing insight into the mechanistic bases of $VO_{2SC}$; *ii)* sport sciences, informing strategies for improving exercise tolerance and performance in athletes; *iii)* in unfit individuals such as older and obese adults, guiding training interventions to increase exercise tolerance, daily mobility and quality of life.

**Purposes and Research Questions**

The research questions asked in this thesis have been separated in five studies, as listed below:

**Study one (Chapter Two)**

Is there a relationship between the metabolic/physiologic responses and the VO2 values associated to MLSS, CP, RCP and $[HHb]_{BP}$ suggesting that they may share a common underlying mechanistic link? It is possible that each of these used exercise intensity "thresholds" may be the result of similar underlying physiological and metabolic processes reflecting a common level of aerobic metabolism beyond which there is a progressive loss of homeostasis?

**Study two (Chapter Three)**

Can NIRS be used as an alternative technology to ventilatory and gas exchange-based measurements, for the determination of threshold intensities during exercise? In particular, deoxyhemoglobin signal from the *vastus lateralis* muscle during incremental cycle exercise can be used as an alternative method to estimate the anaerobic threshold?

**Study three (Chapter Four)**

In light of the fact that traditionally, critical intensity in cycling, is determined with an exhaustive and time consuming series of tests, can this threshold be determined using blood lactate accumulation in a sub-maximal cycle ergometer trial?

**Study four (Chapter five)**

The body response at an intensity of exercise above the anaerobic threshold during a ramp incremental exercise can be improved by a training intervention with the aim to improve the muscle contractile efficiency?

**Study five (Chapter six)**

The body response at an intensity of exercise above the anaerobic threshold during a constant work rate exercise can be improved by a training intervention with the aim to improve the muscle contractile efficiency?

**Research Hypothesis**

The research hypothesis tested in this thesis, are as follows:

**Study one**

Observational study designed to test in twelve young healthy male the hypothesis that the $VO_2$ associated with the most common indexes of a threshold intensity (*i.e.*, MLSS, CP, RCP and $[HHb]_{BP}$) would be equivalent and that each may be used to represent the boundary between *heavy* and *very heavy* exercise domains.

**Study two**

This observational study was made to test, in a large and heterogeneous sample of healthy individuals, whether the deflection point in the NIRS-derived deoxyhemoglobin signal from the *vastus lateralis* muscle during incremental cycle exercise can be used as an alternative method to estimate the Respiratory Compensation Point (RCP). We hypothesized that the deflexion point in deoxyhemoglobin signal would occur at the same $VO_2$ as that of the RCP.

**Study three**

In the present study we developed and tested a single 3 min non-exhaustive cycle ergometer test, as a practical, economical and widely applicable alternative to direct determination of critical intensity in cycling. The study: *i)* assessed the relationship between blood lactate accumulation and critical intensity; and *ii)* tested the performance of the predictive equation based on the above relationship. We hypothesized that the new sub-maximal 3-min test would accurately and precisely predict critical intensity during cycling.

**Study four**

The present study tested the hypothesis that strength training, by increasing maximal force and reducing the recruitment of high-threshold motor units to sustain a given absolute exercise in the heavy-intensity domain, would reduce the excess $VO_2$ in young males.

**Study five**

The aim of this study was to evaluate the possible effect of strength training on the $VO_2$ slow component ($VO_{2SC}$) in healthy young individuals. We tested the hypothesis that a 5-week strength training intervention would attenuate the development of the $VO_{2SC}$ and increase exercise tolerance, likely due to a reduction in the recruitment of high-threshold, type II motor units during constant work rate cycling exercise in the heavy intensity domain.

**List of Publications**

The chapters of this thesis have been previously published in, or submitted to international peer-reviewed journals. These publications are outlined below.

**Chapter Two**

Daniel A. Keir, **Federico Y. Fontana**, Taylor C. Robertson, Juan M. Murias, Donald H. Paterson, John M. Kowalchuk, Silvia Pogliaghi. Exercise Intensity Threshold: Identifying the Boundaries of Sustainable Performance. Medicine and Science in Sport and Exercise. *Accepted December, 2014.*

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**Chapter Three**

**Federico Y. Fontana**, Daniel A. Keir, Cecilia Bellotti, Gabriela F. De Roia, Juan M. Murias, Silvia Pogliaghi. Determination of RCP in healthy adults: can NIRS help?. Journal of Science and Medicine in Sport. *Accepted July, 2014.*

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**Chapter Four**

**Federico Y. Fontana**, Alessandro L. Colosio, Daniel A. Keir, Juan M. Murias, Silvia Pogliaghi. Identification of critical intensity from a single lactate measure during a 3-min, submaximal cycle-ergometer test. Journal of Sport Sciences. *Accepted November, 2016.*

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**Chapter Five**

**Federico Y. Fontana,** Andrea Zignoli, Giorgia Spigolon, Alessandro L. Colosio, Juan M. Murias, Silvia Pogliaghi. "Excess $VO_2$": how strength training affects metabolic efficiency. Journal of Science and Medicine in Sport. *Submitted January, 2017.*

**Chapter Six**

**Federico Y. Fontana,** Andrea Zignoli, Giorgia Spigolon, Alessandro L. Colosio, Juan M. Murias, Silvia Pogliaghi. "$VO_2$ slow component": how strength training affects exercise tolerance and metabolic stability. Journal of Science and Medicine in Sport. *Submitted January, 2017.*

**Additional publications arising from Ph.D research**

**Federico Y Fontana,** Alessandro L. Colosio, Gabriela F De Roia, Giorgio Da Lozzo, Silvia Pogliaghi. Anthropometrics of Italian Senior Male Rugby Union Players: From Elite to Second Division. International journal of sports physiology and performance. *Accepted May, 2015.*
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Daniel A. Keir, **Federico Y. Fontana,** Taylor C. Robertson, Juan M. Murias, Donald H. Paterson, John M. Kowalchuk, Silvia Pogliaghi. Considerations for identiyfing the boundaries of sustainable performance: Response to Craig et al.. Medicine and Science in Sports and Exercise. *Accepted September, 2015.*
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# Chapter 2

## Exercise intensity thresholds: Identifying the boundaries of sustainable performance


**Abstract**

Critical power (CP), respiratory compensation point (RCP), maximal lactate steady-state (MLSS), and deoxyhemoglobin ([HHb]) breakpoint ($[HHb]_{BP}$) are alternative functional indices that are thought to demarcate the highest exercise intensity that can be tolerated for long durations. **Purpose:** We tested the hypothesis that CP, RCP, MLSS, and $[HHb]_{BP}$ occur at the same metabolic intensity by examining the pulmonary oxygen uptake ($\dot{V}O_{2P}$) as well as power output (PO) associated with each "threshold". **Methods:** Twelve healthy men (mean±SD age: 27±3 years) performed the following tests on a cycle ergometer: *i)* four to five exhaustive tests for determination of CP; *ii)* two to three, 30-minute constant-power trials for MLSS determination; and *iii)* a ramp incremental exercise test from which the $\dot{V}O_{2P}$ and PO at RCP and $[HHb]_{BP}$ were determined. During each trial, breath-by-breath $\dot{V}O_{2P}$ and ventilatory variables were measured with a metabolic cart and flow-meter turbine; near-infrared spectroscopy-derived [HHb] was monitored using a frequency domain multi-distance system, and arterialized-capillary blood lactate was sampled at regular intervals. **Results:** There were no differences ($p>0.05$) amongst the $\dot{V}O_{2P}$ values associated with CP, RCP, MLSS, and $[HHb]_{BP}$ (CP: 3.29±0.48; RCP: 3.34±0.45; MLSS: 3.27±0.44; $[HHb]_{BP}$: 3.41 ± 0.46 $L \cdot min^{-1}$); however, the PO associated with RCP (262±48 W) and $[HHb]_{BP}$ (273±41 W) were greater ($p<0.05$) than both CP (226±45 W) and MLSS (223±39 W) which, themselves, were not different ($p>0.05$) **Conclusions:** Although the standard methods for determination of CP, RCP, MLSS, and $[HHb]_{BP}$ are different, these indices occur at the same $\dot{V}O_{2P}$ suggesting that: i) they may manifest as a result of similar physiological phenomenon; ii) each

provides a valid delineation between tolerable and intolerable constant-power exercise.

*Key words*: Critical power, Maximal Lactate Steady State, Respiratory Compensation Point, NIRS breakpoint, exercise tolerance

## Introduction

For years, the concept of threshold-based exercise intensity has been used to assess and stratify cardiorespiratory fitness and health, for exercise prescription, and to quantify the outcomes of specific interventions (28). Yet, numerous physiological 'thresholds' exist in the literature (each with unique nomenclature and methodology for determination) creating ambiguity regarding their physiological-bases and relevance. To date, a popular model for prescribing exercise is the 'intensity domain' model (outlined by Whipp et al. (38)) which partitions intensity into ranges (or clusters) of power outputs that elicit common pulmonary $O_2$ uptake ($\dot{V}O_{2P}$) response characteristics (i.e., *moderate*-, *heavy*-, *very heavy*-, and *severe*-domains, although alternative classifications exist (17)). In this schema, the 'threshold' separating *heavy* from *very heavy* exercise (i.e., sustainable from unsustainable constant-power exercise) is critical power (CP) (32). However, other 'thresholds' exist that also have been considered to represent this important physiological 'boundary'. Among the most common are maximal lactate steady state (MLSS), respiratory compensation point (RCP), and more recently, the deoxyhemoglobin breakpoint ($[HHb]_{BP}$) (15, 31). Each of these paradigms uses different methods of measurement and may be preferred based on consistencies with previous work, available instruments, and the test population, lending to confusion regarding which one represents (or should represent) the 'ceiling' of tolerable endurance exercise and whether they are physiologically equivalent.

Previous studies have attempted to examine the association between these indices of intensity by comparing the POs associated with each 'threshold' (5, 13, 31). The attractiveness of this approach resides in the simplicity of obtaining a PO associated with CP and MLSS while avoiding the necessity of: a) measuring gas exchange during prolonged constant-load exercise (at both CP and MLSS), and b)

assigning a $\dot{V}O_{2p}$ value during exercise where a $\dot{V}O_{2p}$ slow component is manifest. Using this design, the measurement of gas exchange and ventilatory variables is only required for the determination of RCP. However, it has previously been demonstrated that the PO associated with RCP can vary (while the $\dot{V}O_{2p}$ associated with RCP does not) depending on the selection of incremental exercise protocol (33). Furthermore, since the change in $\dot{V}O_{2p}$ ($L \cdot min^{-1}$) for a unit change in PO (W) is 0.01 (i.e., 100 times smaller) and $\dot{V}O_{2p}$ is also associated with an intrinsic measurement error between 2.5 and 5%, relatively small changes in metabolic rate (i.e., $\dot{V}O_{2p}$) may be interpreted as large changes in exercise intensity (i.e., PO). Additionally, Broxterman et al. (7) showed that the intra-subject variability between RCP and critical speed (a surrogate of CP) was greatest when the parameters were expressed in terms of speed compared to absolute $\dot{V}O_{2p}$. Therefore, it is possible that the methodological approaches previously used to compare CP, MLSS, and RCP, may have precluded the ability to detect their agreement.

Interestingly, it was recently demonstrated in a series of studies (using near infrared-spectroscopy [NIRS]) that the $\dot{V}O_{2p}$ at which deoxygenated hemoglobin ([HHb]) begins to 'plateau' during incremental exercise (i.e., $[HHb]_{BP}$), is strongly associated with both the $\dot{V}O_{2p}$ at RCP (15, 25) and at MLSS (3). Together, these studies suggest the existence of a link between MLSS, RCP and $[HHb]_{BP}$, yet correspondence between all three indices of exercise intensity has not previously been evaluated in a single group of subjects.

It is possible that each of the above mentioned "thresholds" may be the result of similar underlying physiological and metabolic processes reflecting a common level of aerobic metabolism beyond which there is a progressive loss of homeostasis. For example, the physiological characterization of exercising slightly above CP includes accumulation of fatigue-inducing metabolites (20), precipitous increases in intramuscular and arterial hydrogen ion concentration ($[H^+]$) (9, 29) and disproportionate increases in muscle blood flow and motor unit recruitment (11). It could be argued that the physiological consequences of exercising at CP includes the criteria for MLSS (i.e., the highest metabolic rate at

which [$La^-$] can achieve a steady-state (4)), RCP (i.e., the highest metabolic rate at which ventilatory compensation is able to maintain an elevated but stable metabolic acidosis (37)) and $[HHb]_{BP}$ (i.e., the metabolic rate at which there is a reduction in the $O_2$ delivery to $O_2$ utilization relationship); however before the mechanisms underlying these indices of exercise intensity can be examined, it first must be determined whether the intensity at which each occurs is similar within a single group of subjects. Therefore, this observational study was designed to test the hypothesis that the $\dot{V}O_{2P}$ (rather than PO) associated with CP, RCP, MLSS, and $[HHb]_{BP}$ would be equivalent and that each may be used to represent the boundary between *heavy* and *very heavy* exercise domains.

**Methods**

Subjects:

Twelve healthy young men (mean ± SD values: age, 25 ± 2 yrs; body mass, 86 ± 16 kg; height, 179 ± 7 cm) volunteered and gave written informed consent to participate in the study. All procedures were approved by The Department of Neurological and Movement Sciences' Ethical Committee for Research on Human Subjects. Subjects were non-smokers who were free of any musculoskeletal, respiratory, cardiovascular, and metabolic conditions and who were not taking any medications that might influence cardiorespiratory or metabolic responses to exercise.

All participants completed the following cycle ergometer tests within a maximum of 3 weeks: *i)* a preliminary *maximal ramp-incremental* (RI) exercise test for maximal $\dot{V}O_{2P}$ and peak power output ($PO_{peak}$) determination; *ii)* four to five exhaustive tests (*time-to-exhaustion trials*) and two to three, 30-minute *constant-power trials* at a fixed cadence for the determination of CP, PO at MLSS and $\dot{V}O_{2P}$ associated with CP and MLSS; *iii)* an exit RI test from which the $\dot{V}O_{2P}$ and PO associated with RCP and $[HHb]_{BP}$ were determined. All tests were conducted in an environmentally controlled laboratory on a minimum of eight occasions, each at a similar time of day, two to three hours after a standardized meal (composed of 500 mL of water and 2 – 3 $g \cdot kg^{-1}$ body mass of low glycemic index carbohydrates). Participants were instructed to abstain from vigorous physical

activity in the 24 h preceding each test and to avoid caffeine consumption on the day of testing.

Exercise Protocols

All exercise tests were preceded by 4 minutes of baseline 20 W cycling at a self-selected pedal cadence (range: 70 – 100 rpm). The freely-chosen cadence of each subject was recorded during the preliminary RI test and this cadence was maintained during all subsequent tests by means of visual feedback and verbal encouragement from the experimenters. Failure to maintain the indicated cadence to within 5 rpm (for longer than 5 sec) during testing despite strong verbal encouragement was considered as the criterion for exhaustion.

*Ramp-incremental (RI) Test*

Each participant performed two RI tests to volitional exhaustion: one before experimental testing (preliminary test) and one at the end of experimental testing (exit test, to ensure that there was no training effect of the experimental protocol). The RI tests consisted of 20 W cycling for 4 minutes followed by a 25 $W \cdot min^{-1}$ increase in PO (5 W every 12 sec) for determination of peak $\dot{V}O_{2p}$ ($\dot{V}O_{2peak}$), gas exchange threshold (GET), RCP, maximal HR ($HR_{max}$) and peak PO ($PO_{peak}$).

*Time to exhaustion trials*

For the determination of CP, each participant performed four to five constant-power trials to the limit of intolerance which were designed to generate a distribution of time-to-exhaustion ($t_{exhaustion}$) trials between ~1 and 20 minutes in duration (as recommended by Morton, (24)). The first three constant-power trials were performed at 80%, 95%, and 115% of $PO_{peak}$ (as determined from the preliminary RI test) in random order. Thereafter, a fourth and fifth trial were performed at a PO designed to generate an even distribution of exhaustion times within the target range (i.e., ~1 – 20 min). From these trials, a PO – $t_{exhaustion}$ relationship was obtained for each subject.

*Constant-power tests*

On successive appointments, participants performed two to three, 30-minute constant-power tests for the determination of the $\dot{V}O_{2p}$ at CP and for the determination of PO and $\dot{V}O_{2p}$ at MLSS. The first test was completed at CP. [$La^-$

] was measured at rest and at the fifth, 10$^{th}$, 15$^{th}$, 20$^{th}$, 25$^{th}$ and 30$^{th}$ minutes during exercise. The intensity of the successive test(s) was dependent on the change in [$La^-$] between the 10$^{th}$ and 30$^{th}$ minute of the previous test: if [$La^-$] increased by >1.00 mM, the successive test was performed at a PO of CP -10W; if [$La^-$] increase by < 1.00 mM, the successive test was performed at a PO of CP +10W. Thereafter, the PO was increased/reduced by 10W until the highest PO compatible with a stable [$La^-$] (i.e., increase < 1.00 mM between the 10$^{th}$ and 30$^{th}$ minutes) was identified. An example of the entire exercise protocol (excluding the preliminary RI) can be seen in Figure 1 (see caption for details).

Equipment and Measurements:

All exercise tests were performed on an electromagnetically braked cycle-ergometer (Sport Excalibur, Lode, Groningen, NL). Breath-by-breath pulmonary gas exchange and ventilation were continuously measured using a metabolic cart (Quark $B^2$, Cosmed, Italy) as previously described (12). The gas analyzers were calibrated before each experiment using a gas mixture of known concentration and the turbine flow-meter was calibrated using a 3-L syringe (Hans Rudolph Inc., USA). Heart rate (HR) was collected using radiotelemetry (SP0180 Polar Transmitter, Polar Electro Inc., Kempele, Finland) and calculated over the duration of each breath.

During all testing muscle oxygenation and deoxygenation ([HHb]) were evaluated using a quantitative NIRS system (Oxiplex TS$^{TM}$, ISS, Champaign, USA). After shaving, cleaning and drying of the skin area, the NIRS probe was longitudinally positioned on the belly of the *vastus lateralis* muscle ~15 cm above the patella and attached to the skin with a bi-adhesive tape. The probe was secured with elastic bandages around the thigh. The apparatus was calibrated on each testing day after a warm-up of at least 30 minutes as per manufacturer recommendations. Data were stored online at an output frequency of 25 Hz, but were reduced to 1-s bins for all subsequent analyses within the present study.

During all constant-load tests, blood lactate ([$La^-$], mM) was assessed at selected intervals by means of an electro-enzymatic method (Biosen C-line, EKF Diagnostics, Barleben, Germany) on arterialized-capillary blood samples (20 µL)

taken from the heated earlobe. The analyzer was calibrated with a 12 mM standard before and at regular intervals during analyses.

Data Analyses

Breath-by-breath $\dot{V}O_{2p}$ data were edited on an individual basis: aberrant data that lay 3 SD from the local mean (22) were removed, trials were linearly interpolated on a second-by-second basis, time-aligned such that time "zero" represented the onset of exercise (i.e., onset of constant-load or RI exercise), and averaged into 5-s and 30-s time bins.

Both the GET and RCP were independently determined by three blinded expert reviewers. The average of the three values was used for analysis as long as all estimates were within 200 $mL \cdot min^{-1}$. In instances where one of the reviewers' estimates was not within 200 $mL \cdot min^{-1}$, an average of the two in closest agreement was used. GET was determined by visual inspection as the $\dot{V}O_{2p}$ at which $CO_2$ output ($\dot{V}CO_{2p}$) began to increase out of proportion in relation to $\dot{V}O_{2p}$, with a systematic rise in the minute ventilation ($\dot{V}_E$)-to-$\dot{V}O_{2p}$ relationship and end-tidal $PO_2$ whereas the ventilatory equivalent of $\dot{V}CO_{2p}$ ($\dot{V}_E/\dot{V}CO_{2p}$) and end-tidal $PCO_2$ were stable (2). RCP was determined as the point where end-tidal $PCO_2$ began to fall after a period of isocapnic buffering (37). This point was confirmed by examining $\dot{V}_E/\dot{V}CO_{2p}$ plotted against $\dot{V}O_{2p}$ and by identifying the second breakpoint in the $\dot{V}_E$-to-$\dot{V}O_{2p}$ relationship.

$\dot{V}O_{2peak}$ was defined as the highest 20-s $\dot{V}O_{2p}$ computed from a rolling average and $PO_{peak}$ was defined as the PO achieved at termination of the RI test. The achievement of $\dot{V}O_{2\,max}$ was further confirmed by examining the $\dot{V}O_{2p}$ responses during several of the time-to-exhaustion trials.

The $[HHb]_{BP}$ was identified by fitting the individual values of [HHb] corresponding to the incremental portion of the exercise as a function of time. A piece-wise 'double-linear' model was used to characterize the increase in [HHb] as follows (36):

$f$ = if ($x < BP$, $g(x)$, $h(x)$)

$g(x) = i_1 + (s_1 \cdot x)$

$i_2 = i_1 + (s_1 \cdot BP)$

$h(x) = i_2 + (s_2 \cdot (x - BP))$

fit *f* to y,

where *f* is the double-linear function, x is time and y is [HHb], *BP* is the time coordinate corresponding to the interception of the two regression lines (i.e., the $[HHb]_{BP}$), $i_1$ and $i_2$ are the intercepts of the first and second linear function respectively and $s_1$ and $s_2$ are the slopes. Model parameter estimates for each individual were determined by linear least-square regression analysis. Thereafter, in order to determine the $\dot{V}O_{2p}$ and HR at which the $[HHb]_{BP}$ occurred, $\dot{V}O_{2p}$ and HR data from the RI test were left-shifted by the individual MRT (for details see Fontana et al. (15)); $\dot{V}O_{2p}$ and HR at $[HHb]_{BP}$ were calculated as 10-sec averages.

CP was determined by fitting a three-parameter hyperbolic model (24) to each subject's PO – $t_{exhaustion}$ relationship using non-linear least squares regression analysis:

$t = W'P{-}CP + W'CP{-}Pmax$

where *t* is time to exhaustion, in sec; *W'* is the anaerobic work capacity, in joules; CP is the critical power, in watts; $P_{max}$ is the maximal 'instantaneous' power. A weighted least squares procedure was used so that weights $w_i$ of each data point were proportional to the square of $y_i$ (i.e. $\sim t_i^2$) for the *i*th data point to account for the increase in variance accompanying increasing exercise times to exhaustion. The "goodness of fit" for the hyperbolic model was determined by computing the 95% confidence interval ($CI_{95}$) for CP. In order to improve the confidence in the CP parameter estimate, model convergence was established with the $P_{max}$ parameter first allowed to vary. Subsequently, the model was iterated again with a fixed value for this parameter.

$\dot{V}O_{2p}$, HR and ventilatory responses from the constant-power tests were averaged into 30-sec time bins so that responses across time could be examined. One-sample *Z*-tests were used to identify the time point at which the change in $\dot{V}O_{2p}$ between one minute intervals (starting at 6 min) and the last minute of exercise (i.e., the 30$^{th}$ min) was no longer different from "zero" (i.e. the time at which $\dot{V}O_{2p}$ reached a "steady-state"). Thereafter, the $\dot{V}O_{2p}$ and HR corresponding to

CP and MLSS for each subject was calculated as a one-minute mean at the time point when steady-state was reached. All data editing, processing, and modeling were performed using OriginLab Version 8.5 (OriginLab, Northampton, USA).

Statistical Analyses

Data are presented as means ± SD throughout. One-way repeated measures analysis of variance (ANOVA) was used to determine statistical significance for the dependent variables. Tukey's *post hoc* analyses were used when significant differences were found for the main effects of the dependent variables. Bland-Altman plots were used to assess the limits of agreement between the $\dot{V}O_{2p}$ at CP, MLSS, RCP and $[HHb]_{BP}$ and one sample *Z*-tests were used to determine if the average difference between values (i.e., the bias) was significantly different from zero. Two-tailed pairwise *t*-tests were used to compare differences between the values obtained from the preliminary and exit RI tests. All statistical analyses were performed using SigmaPlot Version 11.0, (Systat Software Inc., San Jose, USA). Statistical significance was accepted at an alpha level less than 0.05.

**Results**

*Ramp incremental exercise*

No differences were observed for any of the variables between the preliminary and exit RI test therefore, a possible training effect related to the testing protocol was excluded.

The $\dot{V}O_{2peak}$ achieved in the exit RI test (4.13 ± 0.52 L·min$^{-1}$) was not different ($p>0.05$) from the peak $\dot{V}O_{2p}$ identified during the shortest time-to-exhaustion trials (4.00 ± 0.60 L·min$^{-1}$) confirming that $\dot{V}O_{2peak}$ achieved during the RI test was representative of $\dot{V}O_{2\,max}$. The group mean values for $\dot{V}O_{2\,max}$, $PO_{peak}$, maximal HR ($HR_{max}$) and GET from the RI test were 4.13 ± 0.52 L·min$^{-1}$ (49.3 ± 8.7 mL·kg$^{-1}$·min$^{-1}$), 366 ± 48 W, 186 ± 12 bpm (96 ± 6% of age predicted value), and 2.54 ± 0.36 L·min$^{-1}$, respectively. The $\dot{V}O_{2p}$, PO and HR values associated with RCP and $[HHb]_{BP}$ are reported in Table 1.

*Time-to-exhaustion and constant-power trials*

The range of relative POs used in the time-to-exhaustion trials was ~60-115% $PO_{peak}$ resulting in $t_{exhaustion}$ ranging from ~1–24 min. The group mean

values of the CP and W' parameters derived from the three-parameter hyperbolic model were 226 ± 45 W and 31.1 ± 10.9 kJ. The $CI_{95}$ for CP was 15 ± 6 W. During constant-power trials at CP, all subjects displayed a delayed steady-state $\dot{V}O_{2p}$ which occurred at a maximum of ~13 minutes after exercise onset (difference between 13$^{th}$ [and thereafter, i.e., 14$^{th}$, 15$^{th}$, etc.] and 30$^{th}$ minute were not different from "zero" ($p>0.05$). Three of the twelve subjects satisfied the criteria for MLSS (i.e., $[La^-]_b$ increase <1.00 mM between the 10$^{th}$ and 30$^{th}$ minute) during the constant intensity trials performed at the estimated CP; MLSS occurred at a PO 10W above CP in two subjects, 10W below CP in five subjects (for example, see subject in Fig. 1), 20W above CP in one subject and 20W below CP in one subject, resulting in a mean difference between the PO at CP and MLSS equal to 2 ± 12 W. At the PO corresponding to MLSS, a plateau in $\dot{V}O_{2p}$ was observed in all subjects by the 13$^{th}$ minute of exercise. The group mean value of $[La^-]_b$ at MLSS was 6.34 ± 1.41 mM.

*Comparison of CP, RCP, MLSS, and $[HHb]_{BP}$*

The $\dot{V}O_{2p}$, PO, and HR values associated with CP, RCP, MLSS, and $[HHb]_{BP}$ are displayed in Table 1. There were no significant differences between the $\dot{V}O_{2p}$ and HR values associated with each of the indices of threshold intensity ($p>0.05$). However, the PO at RCP and $[HHb]_{BP}$ was greater than the PO at MLSS and CP ($p<0.05$).

Figure 2 shows Bland-Altman plots depicting the agreement between individual $\dot{V}O_{2p}$ ($L \cdot min^{-1}$) values at CP and MLSS (top panel), between CP and both RCP and $[HHb]_{BP}$ (left panels) and between MLSS and both RCP and $[HHb]_{BP}$ (right panels). The mean difference (bias) between CP and MLSS, RCP and $[HHb]_{BP}$ was not different from "zero" ($p>0.05$) with narrow 95% limits of agreement (range: ±0.55 to ±0.63 $L \cdot min^{-1}$). The mean bias between MLSS and RCP also was not different from "zero" ($p>0.05$), however, the bias between MLSS and $[HHb]_{BP}$ (0.13 $L \cdot min^{-1}$) was greater than zero ($p<0.05$).

Figure 3 displays a summary of the group mean $\dot{V}O_{2p}$ response during constant-power trials at MLSS and CP and selected variables from which RCP and $[HHb]_{BP}$ were determined plotted as a function of $\%\dot{V}O_{2\,max}$.

**Discussion**

The present study tested the hypothesis that the CP, MLSS, RCP and the $[HHb]_{BP}$ occur at the same metabolic rate (i.e., $\dot{V}O_{2p}$). The main finding was that the $\dot{V}O_{2p}$ values associated with CP, MLSS, RCP, and $[HHb]_{BP}$ were not different suggesting that each functional index of exercise intensity could provide a method by which to identify the boundary between *heavy* and *very heavy* exercise domains. This is the first study to directly demonstrate a commonality between these 'thresholds' in a single group of subjects substantiating the notion of a 'critical metabolic rate' (1, 35) as the highest metabolic rate at which exercise is well-tolerated for long durations. Results demonstrate that there is a relationship between the metabolic/physiological responses and the $\dot{V}O_{2p}$ values associated to each of these thresholds suggesting that they may share a common underlying mechanistic link.

To our knowledge, only one other study has attempted to directly compare CP, MLSS, and RCP (13) and did so on the basis of PO. Dekerele et al. (13) reported that CP and RCP occurred at a greater PO compared to that of MLSS, concluding that these "intensities" are distinct indices of aerobic function. However, it has been shown that in many cases PO can be disassociated from metabolic rate (i.e., $\dot{V}O_{2p}$) which may affect the interpretation of these data. For example, depending on the rate of increase during RI exercise, the PO at RCP will differ despite occurring at the same $\dot{V}O_{2p}$ (33). Additionally, Barker et al. (1) demonstrated that different combinations of pedaling cadences yield different POs at CP, but do not change the $\dot{V}O_{2p}$ associated with CP. In the present study, when PO is used for comparison between 'thresholds', the conclusion would be that RCP, but not CP, occurs at a greater intensity than MLSS.

However, when CP, MLSS, and RCP are described in terms of $\dot{V}O_{2p}$ (rather than PO), it is apparent that 'metabolic rate' is coincident among indexes. Many studies have consistently reported group mean values between ~75 and 80% $\dot{V}O_{2\,max}$ for MLSS (3, 13, 31) and for $[HHb]_{BP}$, RCP, or both (3, 13, 15, 25, 36), indirectly suggesting a possible coincidence between indices. On the contrary, the range of values for CP in the literature are broader (~75 to

85% $\dot{V}O_{2\,max}$ ) and its correspondence with MLSS is equivocal. For example, studies have reported both a continued increase in $[La^-]_b$ (13, 18, 19, 23, 26, 31, 35) and an elevated but stable $[La^-]_b$ during prolonged exercise at CP (1, 29, 30). Interestingly, those studies not observing a steady-state in $[La^-]_b$ during constant-power exercise at CP also reported a mean CP equal to ~85% $\dot{V}O_{2\,max}$ whereas, those that did, reported a mean CP of ~79% $\dot{V}O_{2\,max}$ (which is similar to that of the present study, 80% $\dot{V}O_{2\,max}$ ). Given that there are several methodological approaches that can be used to estimate CP and that these approaches can influence CP estimation (e.g., mathematical model (8, 16), duration of predictive trials (6), etc.), it is not surprising that accuracy issues could impair this index's ability to detect a very narrow level of muscle metabolic activation beyond which "physiological steady state" cannot be achieved. The present study cannot discriminate as to whether the similar $\dot{V}O_{2p}$ associated to CP, MLSS, RCP and $[HHb]_{BP}$ are mechanistically versus coincidentally linked. Yet given the high degree of variability and the technical challenges associated with estimation of each of these indices of threshold intensity, the demonstration of a robust association of $\dot{V}O_{2p}$ amongst all four intensity-based 'thresholds' suggests that they may be related and may manifest as a result of similar underlying mechanisms.

Recently, the point at which the rate of increase in [HHb] is reduced during RI exercise (i.e., $[HHb]_{BP}$) has been associated with both the RCP (15, 25) and MLSS (3). The results of the present study confirm that the $\dot{V}O_{2p}$ at $[HHb]_{BP}$ is not different from that of RCP. Although the bias was significantly different from 'zero' compared to the MLSS, it should be noted that the absolute value of the bias was very small (130 $mL \cdot min^{-1}$) and practically equivalent to the minimum detectable change (in our laboratory between 100-170 $mL \cdot min^{-1}$ at a $\dot{V}O_{2p}$ of 2.1 to 3.5 $L \cdot min^{-1}$) expected of breath-by-breath $\dot{V}O_{2p}$ measurement for steady-state exercise (21)). Additionally, this is the first study to associate CP with the $[HHb]_{BP}$. These data suggest that there is a link between the $[HHb]_{BP}$ and the metabolic boundary demarcating *heavy* from *very heavy* exercise. The coincidence may be related to an arterial acidosis expected at this metabolic

intensity as both interstitial lactate concentration and reductions in pH have been considered to contribute to reductions in vascular tone (10, 27). The increased arterial $[H^+]$ which is associated (in part) by an increased $[La^-]_b$, could contribute to an increase in local microvascular vasodilation causing an increase in muscle perfusion and $O_2$ availability reducing the requirement for local $O_2$ extraction (although no observable increase in total hemoglobin at this intensity partially contraindicates this mechanism). Alternatively, the "plateau" observed in [HHb] may reflect an increase in the recruitment of Type II glycolytic fibres (relative to Type I oxidative fibres) (14) and indeed a progressive recruitment of higher-order motor units replacing those that drop out due to fatigue during RI exercise has been proposed in humans (34) and demonstrated in rats exercising above versus below critical speed (11). An increased rate of glycolytic ATP-resynthesis in Type I oxidative fibres and consequent reduction in the rate of oxidative-ATP resynthesis could also reduce the rate of increase in $O_2$ utilization relative to the rate of $O_2$ delivery. Additional studies are necessary before a mechanistic link between $[HHb]_{BP}$ and CP, RCP, and MLSS can be elucidated.

Identification of exercise intensity domains has important implications/applications for research interventions, however identification of these indices of aerobic function within an individual is cumbersome as numerous exercise tests are traditionally required for accurate identification of the *heavy* – *very heavy* domains. To avoid this issue, many studies have arbitrarily chosen "delta50" (Δ50, or 50% of the difference in $\dot{V}O_{2p}$ between GET and $\dot{V}O_{2\,max}$) to represent "*heavy*-intensity" exercise. Interestingly, in the present study, Δ50 corresponded to a $\dot{V}O_{2p}$ of 3.33 ± 0.42 L·min$^{-1}$ (81 ± 10% $\dot{V}O_{2\,max}$) which was not different from any of the "thresholds" examined. These findings suggest that within a given sample of individuals, selection of an intensity corresponding to Δ50 would likely elicit $\dot{V}O_{2p}$ responses from both the *heavy* and *very heavy* intensity domains and therefore, depending on the goals of a prospective study, results could be influenced by individual differences in metabolic and gas exchange responses.

**Conclusion**

The current study has demonstrated that the concepts of CP, MLSS, RCP, and $[HHb]_{BP}$ may be unified by determining the $\dot{V}O_{2P}$ (rather than the PO) associated with each functional index of exercise intensity substantiating the existence of a "metabolic boundary" partitioning *heavy* from *very heavy* exercise domains. These data suggest that the CP, MLSS, RCP, and $[HHb]_{BP}$ constructs may be physiologically equivalent and, providing optimal design and appropriate determination, each could theoretically represent the highest $\dot{V}O_{2P}$ at which $[La]_b$ (and $\dot{V}O_{2P}$) can be stabilized, and thus the "boundary" of sustainable versus unsustainable constant-power exercise. This is of valuable practical importance as the interchangeability of each index may provide exercise physiologists, sport scientists and clinicians with a number of options for determining the limits of tolerable endurance exercise depending upon the test population, the available resources and the desired intensity target for exercise prescription.

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**Conflicts of Interest**

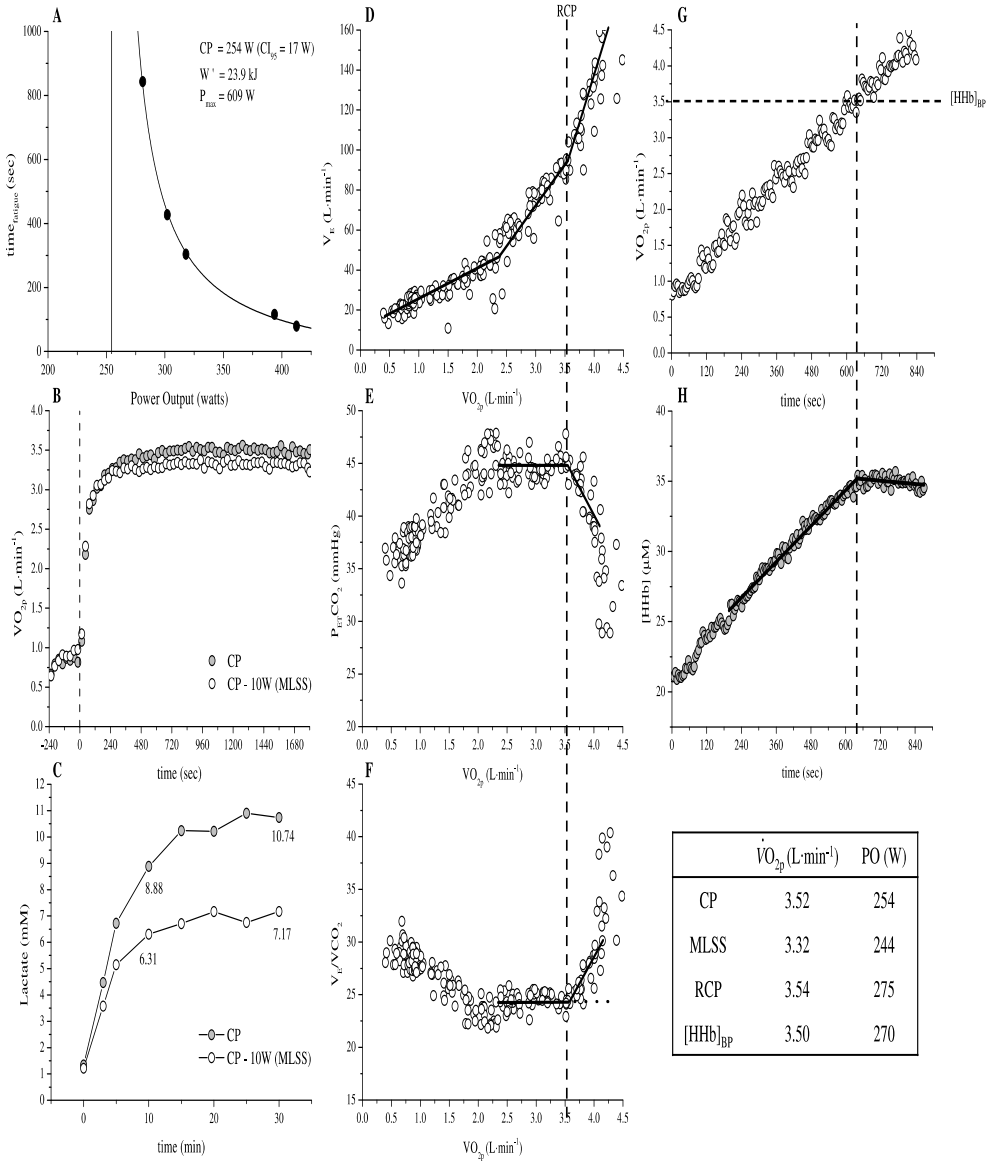
The authors declare no conflicts of interest. Results of the present study do not constitute endorsement by ACSM.

**Table 1.** Group mean ± SD for the $\dot{V}O_{2P}$, PO, and HR values associated with critical power (CP), maximal lactate steady-state (MLSS), respiratory compensation point (RCP), and the deoxyhemoglobin breakpoint ($[HHb]_{BP}$).

| | CP | MLSS | RCP | $[HHb]_{BP}$ |
|---|---|---|---|---|
| $\dot{V}O_{2P}$ ($L \cdot min^{-1}$) | 3.29 ± 0.48 | 3.27 ± 0.44 | 3.34 ± 0.45 | 3.41 ± 0.46 |
| PO (W)* | 226 ± 45 | 223 ± 39 | 262 ± 48[a,b] | 273 ± 41[a,b] |
| HR (bpm) | 162 ± 10 | 161 ± 10 | 158 ± 9 | 160 ± 8 |

* denotes main effect of threshold detection method, [a] denotes significant difference from CP; [b] denotes significant difference from MLSS; $p<0.05$.

## Figures




| | $\dot{V}O_{2p}$ (L·min$^{-1}$) | PO (W) |
|---|---|---|
| CP | 3.52 | 254 |
| MLSS | 3.32 | 244 |
| RCP | 3.54 | 275 |
| $[HHb]_{BP}$ | 3.50 | 270 |

**Figure 1.** The experimental protocol is depicted in a representative subject: Panel **A**, shows the three-parameter hyperbolic plot from which critical power (CP) was derived. The parameter estimates and hyperbolic model fit are embedded; Panels

**B** and **C** show the pulmonary $O_2$ uptake ($\dot{V}O_{2p}$) (**B**) and blood lactate (**C**) responses to two 30-minutes exercises conducted respectively at a power output corresponding to the estimated CP (*grey circles*) and at the maximal lactate steady state (MLSS) (in this case 10 W below CP, *white circles*). The $\dot{V}O_{2p}$ values associated with MLSS and CP were determined from these exercises; Panels **D-F** show the ventilation ($\dot{V}_E$, **D**), end-tidal $CO_2$ ($P_{ET}CO_2$, **E**) and ventilatory equivalent for $CO_2$ ($\dot{V}_E/\dot{V}CO_2$, **F**) responses (plotted as a function of $\dot{V}O_{2p}$) from the ramp incremental (RI) used for the estimation of the respiratory compensation point (RCP) (see *dashed* vertical line). Panels **G** and **H** show the $\dot{V}O_{2p}$ (**G**) and deoxyhemoglobin ([HHb], **H**) responses (plotted as a function of time) from the RI test. The deoxyhemoglobin breakpoint ($[HHb]_{BP}$) was determined by fitting a double-linear to the [HHb] data (*black line*) and the $\dot{V}O_{2p}$ at $[HHb]_{BP}$ (identified by the *dashed line*) was determined by aligning the time at $[HHb]_{BP}$ with the left-shifted $\dot{V}O_{2p}$ data (see text for details). A table showing the $\dot{V}O_{2p}$ and PO associated with CP, MLSS, RCP, and $[HHb]_{BP}$ for this representative subject is embedded (bottom left).

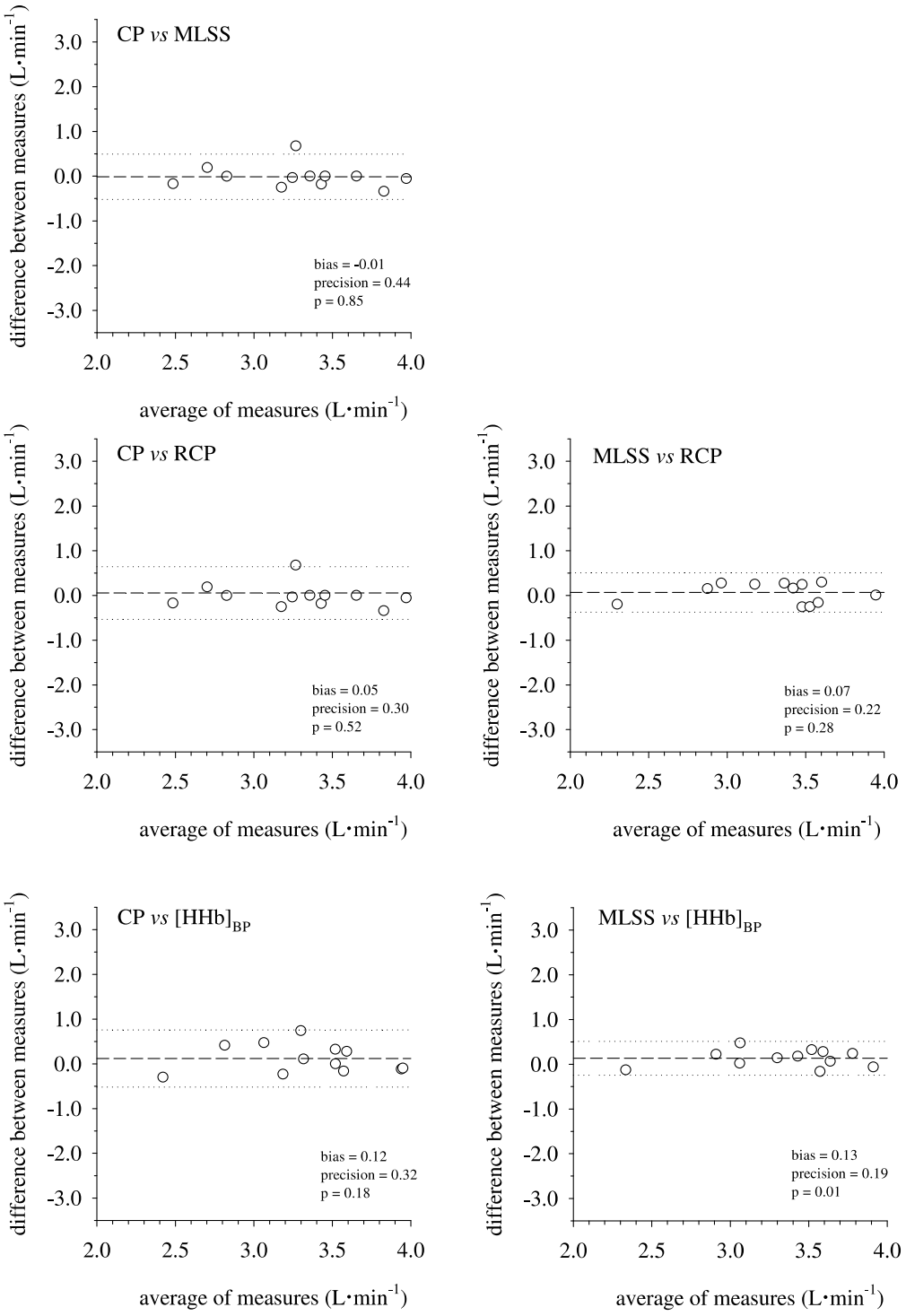
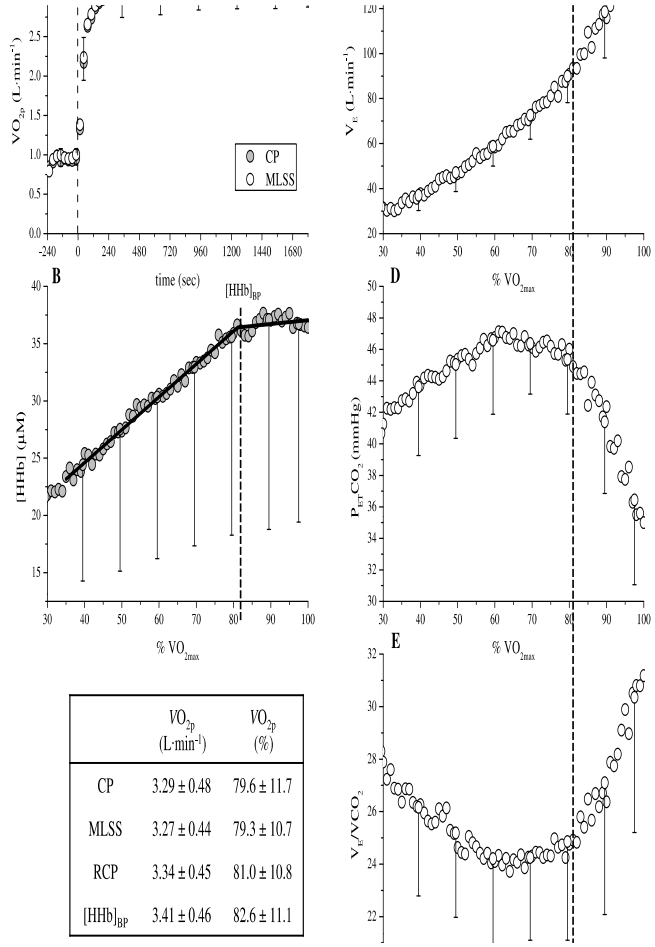



**Figure 2.** Bland-Altman plots displaying agreement between individual measures of $\dot{V}O_{2p}$ at CP and MLSS (top panel); between CP and both RCP and $[HHb]_{BP}$ (left panels) and between MLSS and both RCP and $[HHb]_{BP}$ (right panels). The differences between measures (*y*-axis) are plotted as a function of the mean of the

two measures (*x*-axis) in absolute values ($L \cdot min^{-1}$). The horizontal dashed line represents the mean difference (i.e., bias) and paired horizontal dotted lines represent the limits of agreement (mean ± 2SD).




| | $\dot{V}O_{2p}$ (L·min$^{-1}$) | $\dot{V}O_{2p}$ (%) |
|---|---|---|
| CP | 3.29 ± 0.48 | 79.6 ± 11.7 |
| MLSS | 3.27 ± 0.44 | 79.3 ± 10.7 |
| RCP | 3.34 ± 0.45 | 81.0 ± 10.8 |
| $[HHb]_{BP}$ | 3.41 ± 0.46 | 82.6 ± 11.1 |

**Figure 3.** Group mean (with standard deviation bars) data displaying the pulmonary $O_2$ uptake ( $\dot{V}O_{2p}$ ) profiles (**A**) during 30-minutes constant load exercises performed at an intensity corresponding to maximal lactate steady state (MLSS, *white* circles) and critical power (CP, *grey* circles). Panels **B-E** display group mean values of deoxyhemoglobin (**B**), ventilation ($V_E$, **C**), end-tidal $CO_2$ ($P_{ET}CO_2$, **D**) and ventilatory equivalent for $CO_2$ ($V_E/VCO_2$, **E**) responses (plotted as a function of $\%\dot{V}O_{2\,max}$) from the ramp incremental (RI). The dashed vertical lines show respectively the relative $\dot{V}O_{2p}$ ($\%\dot{V}O_{2\,max}$) for the group mean deoxyhemoglobin breakpoint ($[HHb]_{BP}$) and the relative $\dot{V}O_{2p}$ ($\%\dot{V}O_{2\,max}$) for the group mean respiratory compensation point (RCP). The embedded table summarizes the group mean values for CP, MLSS, RCP, and $[HHb]_{BP}$ in terms of absolute (L·min$^{-1}$) and relative (%) $\dot{V}O_{2p}$ .

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# Chapter 3

## Determination of RCP in healthy adults: can NIRS help?


**Abstract**

**Purpose:** We tested the hypothesis that the respiratory compensation point (RCP) can be accurately determined in healthy subjects during incremental cycling exercise using non-invasive near-infrared spectroscopy (NIRS)-derived measures of deoxygenated hemoglobin (*deoxy*Hb) **Methods:** 118 healthy men (average age 47 ± 19 yrs, range 20-79 yrs) performed an incremental cycling test to exhaustion. Breath-by-breath pulmonary oxygen uptake ($\dot{V}O_2$) and other ventilatory and gas exchange variables were measured and used to determine RCP. Vastus lateralis *deoxy*Hb was monitored using a time-resolved NIRS device and *deoxy*Hb data were modelled with a piece-wise double-linear function from which the *deoxy*Hb deflection point (*deoxy*$Hb_{DP}$) was determined. The absolute ($L \cdot min^{-1}$) and relative (% maximal $\dot{V}O_2$ [$\dot{V}O_{2max}$]) $\dot{V}O_2$ values associated with the RCP and *deoxy*$Hb_{DP}$ were determined for each individual and compared. **Results:** *Deoxy*Hb increased as a function of exercise intensity up to a point (*deoxy*$Hb_{DP}$) after which the signal displayed a "near-plateau". The *deoxy*$Hb_{DP}$ corresponded to a $\dot{V}O_2$ of 2.25 ± 0.69 $L \cdot min^{-1}$ (74 ± 12 % $\dot{V}O_{2max}$) which was not significantly different from the $\dot{V}O_2$ at RCP (2.28 ± 0.70 $L \cdot min^{-1}$ and 74 ± 10 % $\dot{V}O_{2max}$, $p<0.05$). Both indices were highly correlated ($r^2 = 0.86$) and Bland Altman analyses confirmed a non-significant bias for $\dot{V}O_2$ (-0.024 $L \cdot min^{-1}$) concomitant with a small imprecision of 0.26 $L \cdot min^{-1}$. **Conclusions:** During incremental cycling exercise, the $\dot{V}O_2$ associated with the onset of a plateau in NIRS-derived *deoxy*Hb occurs in coincidence with the $\dot{V}O_2$ at RCP. These data suggest that RCP parameter can be accurately estimated, non-invasively, using NIRS-derived *deoxy*Hb in alternative to the use of ventilatory-based techniques.

*Key words:* functional evaluation, exercise prescription, non-invasive techniques, anaerobic metabolism.

## Introduction

The concept of exercise intensity provides a framework for research and training design in the field of exercise physiology (13). Traditionally, exercise intensity is expressed as a percentage of maximal oxygen uptake ($\dot{V}O_{2max}$) (1). This method is convenient in that a heterogeneous pool of subjects can be examined while working at an 'identical' relative intensity. However, it is known that as exercise intensity increases in any individual, unique thresholds exist that demarcate boundaries associated with specific physiological and metabolic profiles (19). The rationale for delimiting these threshold intensities is controversial as disagreements exist in the scientific community in terms of both a theoretical basis for their existence as well as an appropriate methodology for their determination (24). Despite these disagreements, the concept of intensity-dependent thresholds has been used for years in athletes, healthy sedentary population, and patients to assess cardiovascular or pulmonary health, to stratify individuals based on fitness status, to determine and monitor exercise intensities, and to quantify the outcomes of specific interventions (1,19,24). One index of a threshold intensity that is commonly used is maximal lactate steady state (MLSS), which represents the highest exercise intensity at which an elevated blood lactate concentration can be stabilized and sustained for a prolonged period of exercise (4). MLSS is considered the "gold standard" for measurements of exercise tolerance. However, the practical application of this test is cumbersome as it requires invasive blood measurements and is time consuming, thus posing a burden to participants and researchers involved in the test. As such, the use of ventilatory parameters has become a prevalent methodology to estimate the highest exercise intensity that is tolerable and sustainable for prolonged durations (13).

Using ventilatory and gas exchange parameters collected during an incremental exercise test, two threshold can be identified: *i)* the first ventilatory threshold ($VT_1$), or gas exchange threshold (GET), which reflects the exercise intensity

corresponding to the initial appearance of appreciable lactate concentration in the blood; *ii)* the second ventilatory threshold ($VT_2$), or respiratory compensation point (RCP), which is related to the ventilatory response to a metabolic acidosis (29). Although the use of ventilatory parameters provides a time-efficient and non-invasive estimation of landmarks of exercise intensity, several limitations to this approach have been identified. For instance, lower accuracy compared to more invasive methods, subjectivity in the determination, and potential calculation errors in subjects with irregular breathing pattern have been proposed (5,28).

Recent investigations have proposed the utilization of near infrared spectroscopy (NIRS) technology for the determination of threshold intensities during exercise (10,16,26). This technology provides the means to non-invasively collect high resolution data and can be used in both laboratory and field settings. Specifically, it has been shown that a deflection point in the NIRS-derived deoxygenated hemoglobin (*deoxy*Hb) signal during incremental cycle exercise occurred at the same $\dot{V}O_2$ as that associated with the MLSS (3), indicating that MLSS could be accurately determined using quantitative measures of *deoxy*Hb. Additionally, a recent study showed, in a small population of healthy young men and women, that the deflection point in the *deoxy*Hb signal during ramp incremental cycle exercise was associated to the RCP but not the GET (17). The authors suggested that even though the RCP and MLSS are typically determined during different experimental conditions, these two events might share a mechanistic basis. However, further experiments with larger sample size and age dispersion and adequate stastistical evaluations are warranted to establish the validity of this NIRS measure as a proxy for estimation of a threshold intensity associated to the RCP.

Thus, the goal of this study was to test, in a large and heterogeneous sample of healthy individuals, whether the deflection point in the NIRS-derived *deoxy*Hb signal from the vastus lateralis muscle during incremental cycle exercise can be used as an alternative method to estimate the RCP. We hypothesized that the deflexion point in the *deoxy*Hb would occur at the same $\dot{V}O_2$ as that of the RCP.

**Methods**

Subjects: 118 healthy males [47±19 yrs (range 20-79 yrs), 40±12 $mL \cdot kg^{-1} \cdot min^{-1}$ $\dot{V}O_{2max}$ (range 17-68 $mL \cdot kg^{-1} \cdot min^{-1}$)] were recruited by local advertisement in the metropolitan area of Verona. Inclusion criteria were age between 18 and 80 years and male gender. Exclusion criteria were: smoking; metabolic or cardiovascular conditions or the use of medications that might interfere with the physiological response to the exercise tests (diabetes, high blood pressure, chronic heart failure, etc.); double skin fold thickness on the lateral aspect of the thigh exceeding 20 mm. In conformity with the principles of the Declaration of Helsinki, the study was approved by the Ethical Committee of the Department and subjects provided written consent after being informed of the aims, procedures, and possible risk involved in study participation. A medical evaluation preceded the inclusion in the study.

Protocol: subjects performed an incremental exercise to exhaustion on a cycle ergometer (Excalibur Sport Device, Lode, The Netherlands) in comfortable standardized room conditions (22-25°C, 55-65% relative humidity), two hours after a light meal. The incremental exercise was preceded by a 3-min rest period. Thereafter, the workload was increased to 50 watt for 3 min and then by 10-30 watt every minute until voluntary exhaustion. The increase in workload above the initial warm-up was chosen based on age and anticipation of the individual aerobic fitness, with the aim of bringing subjects to exhaustion within 8-12 min (1). The accepted criteria for maximal effort were: a plateau in the $\dot{V}O_2$ response or, in absence of this, a respiratory exchange ratio >1.1, and a heart rate (HR) > 90% of the predicted maximum based on age (1).

Measures: The electromechanically braked cycle ergometer, was connected to and operated by a metabolic cart (Quark $b^2$, Cosmed, Rome, Italy) that allowed continuous breath-by-breath measures of pulmonary ventilation and gas exchanges at the mouth. Before each test, the gas analyzers and turbine flow meter were calibrated according to the manufacturer guidelines using a gas mixture of known concentration ($FO_2$: 0.16; $FCO_2$: 0.05; $N_2$ as balance) and a 3.0-litre calibrated syringe. The cycle ergometer seat was adjusted to obtain complete knee extension during pedaling.

During the incremental test muscle oxygen extraction was evaluated by means of a quantitative NIRS system (Oxiplex TS$^{TM}$, ISS, USA). After shaving, cleaning and drying of the skin area, the NIRS lightweight plastic probe was longitudinally positioned on the belly of the *vastus lateralis* muscle ~15 centimeters above the patella and attached to the skin with a biadhesive tape. The probe was secured with elastic bandages around the thigh. The apparatus was calibrated on each testing day after a warm-up of at least 30 minutes as per manufacturer recommendations.

NIRS provides a continuous measurement (sampling frequency 120 Hz) of absolute concentrations (µM) of oxyhemoglobin (*oxy*Hb), *deoxy*Hb and *total*Hb. The physical principles behind these measurements have been detailed elsewhere (12).

Calculations: The ventilatory thresholds (GET and RCP) were determined based on the breath-by-breath fractional concentrations of end-tidal $O_2$ and $CO_2$ and on the ventilatory equivalents for $O_2$ and $CO_2$ ($\dot{V}_E/\dot{V}O_{2p}$ and $\dot{V}_E/\dot{V}CO_{2p}$) from the incremental exercise (27). Furthermore, maximal parameters during the incremental cycling test ($W_{max}$, $\dot{V}O_{2max}$, $HR_{max}$, $R_{max}$ and *deoxy*$Hb_{max}$) were calculated as the average of the highest 10 s before exhaustion.

Individual *deoxy*Hb data from the incremental exercise were averaged at 1 s and plotted as a function of time. The NIRS-derived *deoxy*Hb deflection point (*deoxy*$Hb_{DP}$) was identified by fitting the individual values of *deoxy*Hb corresponding to the incremental portion of the exercise (i.e. excluding the initial warm-up phase) as a function of time. The following piece-wise double-linear regression was fitted that minimized the residual sum of squares (Sigmaplot 11.0, Systat Software Inc, Chicago, IL, USA) (3) (Figure 1):

$f = if\ (x > DP, g(x), h(x))$

$g(x) = i_1 + (s_1 \bullet x)$

$i_2 = i_1 + (s_1 \bullet DP)$

$h(x) = i_2 + (s_2 \bullet (x-DP))$

fit f to y,

where f is the double-linear function, x is time and y is *deoxy*Hb, DP is the time coordinate corresponding to the interception of the two regression lines, $i_1$ and $i_2$

are the intercepts of the first and second linear function respectively and $s_1$ and $s_2$ are the slopes.

Based on the cardio-respiratory data from the incremental exercise, the $\dot{V}O_2$ at the time-point corresponding to DP (after a left-ward shifting of the data by the individual mean response time, to account for the $\dot{V}O_2$ kinetics (7)) were calculated as a 10-seconds average.

**Statistics**

Average and standard deviations (SD) were calculated for all parameters. A paired t-test was used to compare the average values of $\dot{V}O_2$ at RCP and at *deoxy*$Hb_{DP}$. The association between individual values of $\dot{V}O_2$ at RCP and at the *deoxy*$Hb_{DP}$ was tested by linear regressions and Pearson's product moment correlation while agreement between measures was tested by Bland-Altman analysis and one-tailed z test (6). A significance level of $p<0.05$ was set for all comparisons.

**Results**

The average anthropometric characteristics (age, weight, stature, BMI, $\dot{V}O_{2max}$) of the 118 subjects included in the study are reported in Table 1. All subjects completed the incremental cycle exercise, reaching exhaustion at a $HR_{max}$ of 168±18 (96±6% of age-predicted value (25)) and a respiratory exchange ratio ($R_{max}$) of 1.17±0.11, both indicative of a maximal effort. A plateau in $\dot{V}O_{2max}$ was detectable in about 50% of the subjects. The group values of GET and RCP are summarised in Table 2.

Figure 1 depicts a typical profile for the *deoxy*Hb signal during the incremental test and the model fit with the *deoxy*$Hb_{DP}$. In all subjects, *deoxy*Hb increased very little in the warm-up phase of the test. Thereafter, in the incremental portion of the exercise, *deoxy*Hb increased linearly as a function of time and workload up to a deflection point, where the rate of increase was greatly reduced, often resembling a plateau. The average value of *deoxy*Hb corresponding to the deflection point was 35±12.4 µM. The values of $\dot{V}O_2$ measured at *deoxy*$Hb_{DP}$ were not significantly different from (Table 2) and highly correlated with ($r^2$= 0.86; Figure 2) the $\dot{V}O_2$ associated with the RCP. The Bland-Altman analysis (Figure 3)

showed a non-significant bias (-24.4 ml·min$^{-1}$, equal to 1.07% of the signal) between the $\dot{V}O_2$ associated with the RCP and *deoxy*$Hb_{DP}$ and a small imprecision (SD) (263 mL·min$^{-1}$, equal to 11.5% of the signal). The values of $\dot{V}O_2$ measured both at RCP and *deoxy*$Hb_{DP}$ were significantly higher compared to GET (Table 2).

**Discussion**

The present study tested a large and heterogeneous group of men to determine if the occurrence of the *deoxy*$Hb_{DP}$ coincided with the RCP during incremental cycle exercise to exhaustion. The main finding was that $\dot{V}O_2$ values observed at the *deoxy*$Hb_{DP}$ were highly correlated with and not significantly different from those observed at the RCP, suggesting that NIRS-derived measurements can be used to accurately estimate the exercise intensity associated with the RCP as an alternative to ventilatory and gas exchange-based measurements.

In agreement with other studies, our data reported a time course of *deoxy*Hb (3,17,23) as well as responses of the GET and RCP (11,18,19) similar to those typically observed during either ramp or step incremental cycle exercise. Importantly, the present study showed that the $\dot{V}O_2$ at the *deoxy*$Hb_{DP}$ (which was easily detectable in all subjects) was not different from and highly correlated with ($r = 0.93$) that observed at the RCP. Furthermore, the bias between the measures was not significantly different from 0 and its value was smaller than the minimum detectable difference (1.7% of the signal) (9) Previous studies had reported a correspondence of NIRS-derived events with ventilatory and lactate thresholds (10,26) and with the MLSS (3). More specifically, an association between the $\dot{V}O_2$ at the *deoxy*$Hb_{DP}$ and the RCP had been previously reported (17). The present study provides necessary support by demonstrating this phenomenon in a much larger and representative sample.

The concept of threshold intensities derived from incremental exercise testing is complex and has generated a large amount of debate within the scientific community. Much of the controversy extends from differences in ideas regarding the criteria for determining a specific intensity of exercise below which exercise is sustainable for long durations (presumably limited by stored energy) and above

which is unsustainable for prolonged periods (presumably due to disturbances in systemic homeostasis). It is widely accepted that the exercise intensity at which a $\dot{V}O_2$ "slow component" becomes manifest occurs at intensities above the lactate threshold, a landmark that demarcates the lower boundary of the "heavy" intensity domain. Within this domain, it may take ~10-15 minutes for gas exchange to stabilize (delayed steady-state) and it does so in spite of an increased (but stable) metabolic acidosis. The upper boundary of this domain occurs at some threshold beyond which $\dot{V}O_2$ will no longer stabilize and prolonged exercise will cause $\dot{V}O_2$ to project until $\dot{V}O_{2max}$ is reached and/or exercise intolerance occurs. In this "very heavy" intensity domain, $\dot{V}O_2$ no longer reaches a steady-state, blood lactate [$La^-$] and hydrogen ions [$H^+$] progressively increase, and muscle phosphocreatine (PCr) stores are rapidly depleted. It is tenable that the metabolic status associated with the "heavy"-"very heavy" intensity boundary as outlined by Wassermann et al. (27) is in agreement with the occurrence of the RCP providing support for the importance of the development of alternative methodology (that are both time and cost effective) for its detection.

Irrespective of the controversial physiological demarcation of these threshold intensities, from a practical stand-point, the RCP is commonly used as landmark intensity for training design and for performance prediction in trained endurance athletes (2,9). The relevance of the present study resides in providing an objective, reliable, and easily detectable demarcation of an exercise intensity associated to the RCP through the use of NIRS and the *deoxy*Hb signal. Considering the recent development of low cost (20), portable, and easy to wear NIRS devices (12,15), and taking into account that none of the more invasive or gas exchange derived measures completely satisfies the requirements of accuracy, precision and overall economy of testing for determination of exercise threshold intensities, the use of the NIRS-derived methodology becomes appealing.

Notwithstanding the coincidence between *deoxy*$Hb_{DP}$ and the RCP, the possible physiological mechanism underpinning the relationship between these variables during an incremental trial remains to be elucidated. The time course of *deoxy*Hb in the vastus lateralis muscle during incremental exercise has been interpreted to

reflect the balance between microvascular blood flow and muscle $O_2$ utilization and has been described and interpreted in detail elsewhere (3,17). Briefly, as work rate progressively increases during incremental exercise, a steep slope in the *deoxy*Hb signal is observed which may reflect a slower adjustment of microvascular blood flow compared to muscle $O_2$ utilization and a shift from predominantly slow-twitch muscle fibers to include more fast-twitch muscle fibers. The reduced slope/plateau in *deoxy*Hb in the presence of an increase in muscle $\dot{V}O_2$ that characterizes the "high-intensity" portion of the incremental exercise would imply an increased $O_2$ provision, likely related to the metabolic vasodilation (14) and sympathetically-mediated redistribution of blood flow from less metabolically active tissues towards the active muscle (21). This increase in blood flow would be required to support further increments in $\dot{V}O_2$ when $O_2$ extraction in the vastus lateralis muscle might have reached its upper limit during dynamic exercise. Another factor to consider is that closer to the end of the incremental test, pulmonary $\dot{V}O_2$ may increase due to the contribution of other muscles (respiratory, trunk stabilizers) while the ATP production within the working muscles may partly come from non-oxidative metabolic pathways. Thus, the plateau observed in the *deoxy*Hb signal might indicate a progressively larger contribution from non-oxidative sources to ATP resynthesis, and this might be progressively more preponderant towards the end of exercise.

Whereas the present study established that the $\dot{V}O_2$ at the *deoxy*$Hb_{DP}$ was virtually the same as that observed at the RCP, a recent study from our laboratory demonstrated that the $\dot{V}O_2$ at the *deoxy*$Hb_{DP}$ and MLSS were also occurring at the same absolute and relative $\dot{V}O_2$ as that determined in the current investigation (3). It seems worth mentioning that, in the subset of data presented in the above mentioned study, at least under specific experimental conditions (incremental exercise to exhaustion), the RCP and the MLSS were detected at a similar exercise intensity. In agreement with our findings, a recent publication suggested that the RCP and the MLSS might share similar mechanistic basis (30). In contrast to this idea, Dekerele et al. (8) reported that the power output associated to the RCP occurred well beyond the work rate corresponding to MLSS.

However, it should be noted that methodological issues related to the determination of the RCP and the MLSS might play a role in the lack of agreement observed in these two variable. For instance, the RCP is based on the relative profiles of $V_E$, $P_{ETCO2}$ and $\dot{V}CO_2$ during incremental exercise and the MLSS is dependent on sub-maximal power output and measurements of blood [$La^-$]. While theoretically, these 'thresholds' may occur as a result of similar physiological responses, conceptually, they are quite different and it is likely that the parameters and methodological approaches used in their detection may account for the differences reported in the literature. Furthermore, Dekerele et al. (8) did not evaluate the absolute $\dot{V}O_2$ values associated with both the MLSS and RCP. Therefore, this comparison warrants further investigation since the work rate associated with RCP can be greatly varied depending on incremental exercise protocol (22).

One of the strong aspects of the research design in the present study was the inclusion of a large and heterogeneous sample of men (with age ranging from 20 to 79 years and fitness level spanning from 10 to 100 percentile of each individual's age group (1)). The characteristics of this sample results in a high external validity when trying to make inferences with respect to this population. One limitation of the design was that women were not included in the sample. This decision was based on the fact that NIRS technology does not allow for accurate quantification of the signal when a "thick" layer of adipose tissue is located in the region of NIRS inspection (i.e., when skinfold thickness measures on the lateral aspect of the thigh are larger than ~20 mm). Considering that this is a common characteristic observed in women of all ages (and more markedly in sedentary, older women), our analysis in women would have been limited only to those that do not share this attribute. Nevertheless, Murias et al. (17), demonstrated that *deoxy*$Hb_{DP}$ could be detected during ramp incremental cycle exercise in a small group of women (n=10). Furthermore, the $\dot{V}O_2$ associated with the *deoxy*$Hb_{DP}$ was in close agreement to that of the RCP in that same group of women. Therefore it is expected that the phenomenon observed in the present study would likely also extend to a large heterogeneous group of women.

**Conclusion**

Our study demonstrated in a large and heterogeneous sample of healthy men that the $\dot{V}O_2$ associated with the *deoxy*$Hb_{DP}$ was highly correlated to that observed at the RCP. Compared to ventilatory-based techniques, *deoxy*$Hb_{DP}$ offers the advantage of being objective and independent from irregularities of breathing pattern that can heavily affect the former. Furthermore, its non-invasive nature, the ability to evaluate even small muscle masses and the high sampling frequency allow the characterization of the response to exercise even in subjects with a limited exercise capacity and/or motivation. Finally, the recently developed low cost (20) and/or portable/wearable devices (12,15) could allow the diffusion of this technology on a large scale, in different types of effort and on the field. In conclusion, our data suggest that the RCP, a variable commonly used to prescribe exercise training intensities and to determine exercise performance, can be easily, objectively and accurately detected using non-invasive NIRS-derived measures.

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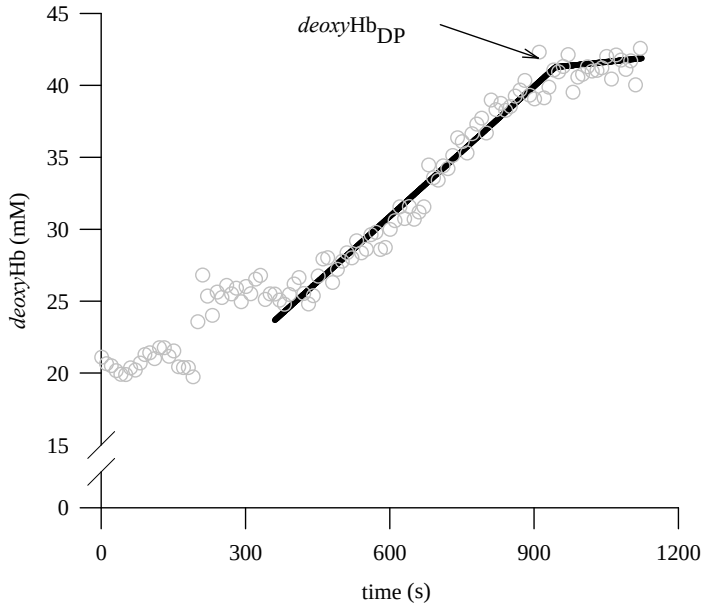


**Figure 1**: Concentration of deoxygenated hemoglobin (*deoxy*Hb, mM) plotted as a function of time (s) from the beginning of the incremental test up to exhaustion in a typical subject. The black line is the result of the double linear function fitting that was performed on the data of the incremental portion of the exercise (i.e., from the end of the warm-up phase up to exhaustion). The change in slope of the *deoxy*Hb signal as a function of time corresponds to the *deoxy*$Hb_{DP}$

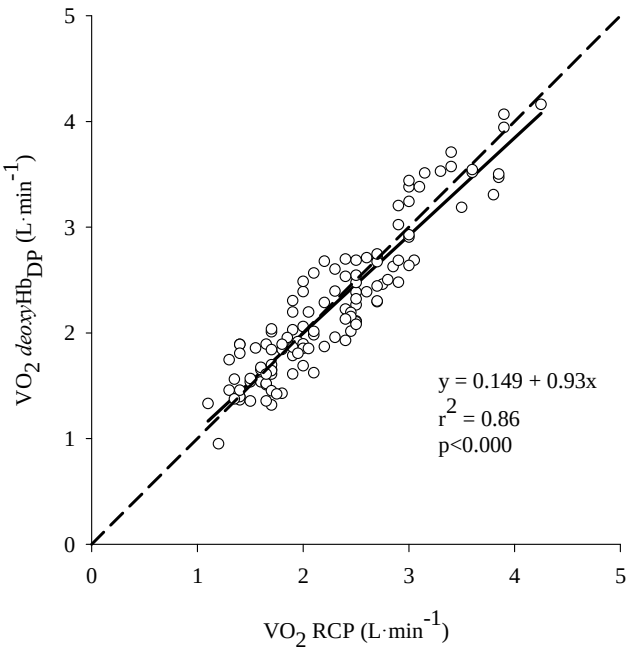


**Figure 2**: Individual values of $\dot{V}O_2$ measured at *deoxy*$Hb_{DP}$ during the incremental cycling exercise are plotted as a function of $\dot{V}O_2$ measured at RCP. The identity (dashed) and the regression (solid) line are displayed along with the regression equation parameters.

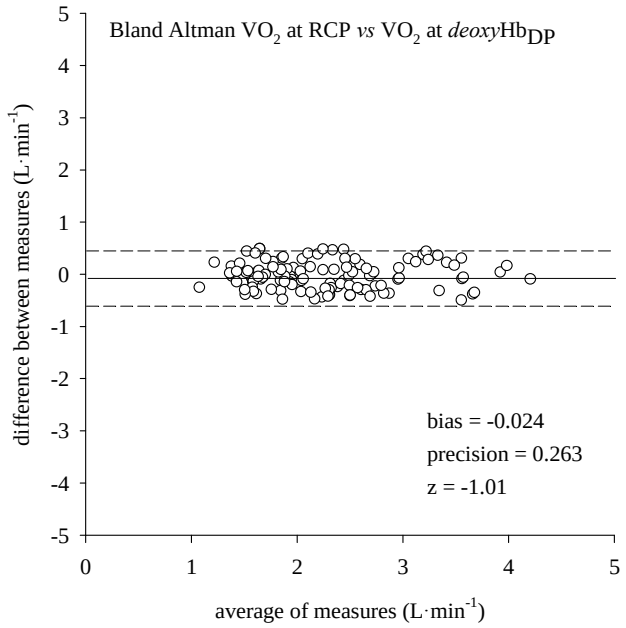


**Figure 3**: Individual differences between the RCP and *deoxy*$Hb_{DP}$ values are plotted as a function of the average of the two measures. The dotted line corresponds to the average difference between measures (i.e. bias) while the dashed lines correspond to the upper and lower limits of agreement (precision) that are displayed in the text box

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# Chapter 4

## Identification of critical intensity from a single lactate measure during a three-minute, submaximal cycle-ergometer test


### Abstract

**Purpose:** We tested the hypothesis that critical intensity in cycling can be determined from a single delta blood lactate in the third minute of a submaximal cycle ergometer trial. Fourteen healthy young men performed four to six constant-power-output trials on a cycle ergometer to the limit of tolerance. **Methods:** Critical intensity was calculated via a linear model and subsequently validated. Lactate was measured at baseline and at 3 min from exercise onset. Delta lactate was the difference between these measures. Based on individual trials, we obtained the delta lactate–% validated critical intensity relationship and thereafter an estimate of critical intensity was computed. Validated and estimated critical intensity were compared by effects sizes, paired-sample *t*-test and Bland-Altman analysis. **Results:** Delta lactate was a linear function of the intensity of exercise, expressed as % validated critical intensity ($R^2$=0.89). Estimated critical intensity was not different from ($d$ = 0.03, $p$ = 0.98) and highly correlated with ($R^2$=0.88) validated critical intensity. The bias between measures was 0.03 W ($\neq$ 0) with a precision of 7 W. **Conclusions:** The results suggest that critical intensity in cycling can be accurately and precisely determined from delta lactate during a sub-maximal trial and so provides a practical and valid alternative to direct determination.



*Keywords: Critical Intensity, Critical Power, Functional Evaluation, Exercise Tolerance, Lactate*


### Introduction

Among indices of exercise capability, "critical power" (11,17) or more correctly termed critical intensity (31,32), provides a simple, sensitive and clear marker for

functional evaluation of exercise capability/tolerance. It can be used both to prescribe and evaluate the effectiveness of training programs (5,17), predict endurance performance (12) in high-standard (22) and amateur athletes (17), and in disease populations (29).

In cycling, an individual's critical intensity is traditionally calculated from the relationship between mean external power output and time-to-exhaustion; this relationship is experimentally determined through a series (typically two to five) of constant-intensity exercise trials performed at different power outputs, each of them prolonged until exhaustion (20). Such a time-consuming and exhaustive effort requires high motivation from participants that might be difficult to obtain and/or present health risks that are unacceptable in groups such as children and/or older adults. Moreover, in athletes, who are often concerned with possible impairment to their performance, highly demanding and/or time consuming protocols might reduce their willingness to be tested, especially close to important competitions (25). Given the constraints associated with this traditional evaluation, testing procedures that are not only valid, but also versatile, cost-effective and quick are warranted.

In attempts to reduce the number of testing sessions required to determine critical intensity, other authors have used different predictive models based on single "all-out" tests. While substantially reducing the overall testing time, these protocols require either a preliminary incremental exercise before the actual single "all-out" test to exhaustion (3,9,34), or a standard external load (*i.e.* 4.5% of the subject's body weight) (3). Nevertheless, all the alternative approaches proposed (3,6,21) still demand an effort to exhaustion from the participants. In addition, the alternative approaches to a gold-standard technique should be supported with measures of agreement between results from the new and criterion test (13).

For the purpose of critical intensity determination in athletes as well as in non-athletic groups such as older or unfit individuals, the development of a quick and sub-maximal testing protocol rather than an exhausting task is important. Based on the very well established relationship between lactate production and relative exercise intensity (1), earlier studies have proposed the utilization of blood lactate accumulation at a given time and absolute submaximal intensity of exercise as a

predictor of the external power output (15,16,26) or running speed at onset of blood lactate accumulation (OBLA) (30). While OBLA, as a marker of the heavy-severe intensity boundary, and critical intensity share a common physiological basis (18), prediction of critical intensity from delta lactate during constant power output sub-maximal exercise has not been investigated.

In light of the above, we developed and tested a single 3 min non-exhaustive cycle ergometer test, as a practical, economical and widely applicable alternative to direct determination of critical intensity. The study: *i)* assessed the relationship between delta lactate and critical intensity; and *ii)* tested the performance of the predictive equation based on the above relationship. We hypothesized that the new sub-maximal 3-min test would accurately and precisely predict critical intensity during cycling.

**Methods**

*Participants*

Fourteen healthy, recreationally active men (31 ± 7 years age (range 23 - 44 years), 79 ± 9 kg body mass, 1.76 ± 0.06 m stature, 49 ± 7 ml· $kg^{-1}$·$min^{-1}$ $\dot{V}O_{2peak}$ and 349 ± 9 W peak power output), were recruited and completed. The study was approved by Departmental Ethics Committee. Participants were non-smokers, free of any musculoskeletal, respiratory, cardiovascular and metabolic condition that could influence cardiopulmonary or metabolic responses to exercise. To minimise variability of glycogen stores and glucose oxidation, all participants were instructed to avoid consumption of caffeinated beverages for at least 8 h before each test and to abstain from vigorous physical activity in the 24 h preceding each testing session. Furthermore, participants followed a standard food intake prescription before all the testing sessions (18).

*Methods*

After medical clearance, all participants completed the following cycle-ergometer tests within a maximum of three weeks: *i)* a preliminary maximal ramp-incremental exercise test; *ii)* two to four exhaustive tests (time-to-exhaustion trials) for traditional (11,20) determination of critical intensity; *iii)* two to three, 30 min constant external power output trials for critical intensity validation (18).

All exercise tests were conducted in an environmentally controlled laboratory at a similar time of the day and were performed on an electromagnetically braked cycle-ergometer (Sport Excalibur, Lode, Groningen, NL).

Each participant performed a ramp incremental exercise test consisting of 20 W cycling for 4 min followed by a 25 $W \cdot min^{-1}$ increase until volitional exhaustion. Participants chose a self-selected cadence (allowed range: 60-100 rpm), and were instructed to maintain the chosen cadence throughout all the tests. Breath-by-breath pulmonary gas exchange and ventilation were continuously measured using a metabolic cart (Quark $B^2$, Cosmed, Italy) (9) and peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$) and peak power output were determined as previously described (18).

Thereafter participants performed two to four constant external power output trials to the limit of tolerance, designed to generate a distribution of times to exhaustion between ~2 and 20 min (18). A 4 min 20 W baseline preceded all exercise transitions. Participants were verbally encouraged to pedal for as long as possible at a constant cadence (*i.e.* the self-selected cadence they had chosen during the ramp incremental test). Time to exhaustion was the difference between the onset of the target power output and exhaustion, defined as failure to maintain the assigned cadence to within 5 rpm (for longer than 5 s) in spite of operator's encouragement. Based on power output and time to exhaustion the individual critical intensity was determined using a traditional linear model (4,11,20).

On successive appointments, participants performed two to three, 30 minute constant-power-output tests for the validation of critical intensity as previously detailed (18). This validated value was used for all successive comparisons.

During all constant-external-power-output trials, capillary blood samples (20 μl) were drawn from the ear lobe at baseline and at the 3$^{rd}$ and 6$^{th}$ min from exercise onset and, if applicable, at five-minute intervals from exercise onset thereafter. Samples were immediately analysed using an electro-enzymatic technique (Biosen C-Line, EKF Diagnostics, Barleben, Germany) and delta lactate was calculated as the difference between the value at the 3$^{rd}$ minute and the baseline value.

*Reliability of measures*

Between-days test-retest reliability of measures (assessed with the relative technical error of measurement (25)) was determined in a separate sample of 8 young healthy men (28 ± 2 years, body mass 77 ± 6 kg, stature 176 ± 6 cm) that performed three identical 3-min constant-mean external power output trials (preceded by a 4 min 20 W baseline). Lactate measures and the subsequent critical intensity estimation had technical errors of measurement of 1.9% and 0.8% respectively. This indicates low variability in the measured/estimated variables when one individual is tested many times. The relative technical error of measurement is considered acceptable when < 2% (25).

*Statistical analysis*

Data are presented as means ± SD throughout.

Based on individual trials >3 min in duration, exercise intensity relative to the validated value of critical intensity was plotted as a function of delta lactate (Figure 1). Thereafter the linear relationship between the exercise intensity relative to validated critical intensity and the delta lactate was modelled, after assumptions verification, by a Multilevel analysis (28). Data were hierarchically structured to model the relationship between correlated residuals, overcoming the problem of non-independent observations (8).

Based on this model the following relationship was obtained:

% validated critical intensity = $b_0 + b_1$ delta lactate + $\varepsilon$

The overall fit of the multilevel model was assessed using a chi-square likelihood ratio test (32; 12). The accuracy of the model across different samples was estimated by cross validation (Stein's formula) (8) and expressed as adjusted-$R^2$.

Thereafter an estimate of critical intensity was determined based on the above model as follows:

Estimated critical intensity = (test power output*100)/(% validated critical intensity)

where test power output is the absolute power output in watts used for the individual test.

After normality assumption verification (using Shapiro-Wilk test and Q-Q plot) estimated critical intensity, validated critical intensity and traditional critical intensity were square-root transformed resulting in the correction of distribution

shape and meeting of the assumption of normality. Estimated critical intensity and validated critical intensity were then compared by effect sizes (8) and paired-samples *t*-test, Bland-Altman analysis followed by one-sample *z* test (5) (Figures 2). The relationship between measures was evaluated via Pearson's correlation coefficient In addition, major-axis regression (19) was used to assess the relationship between estimated and validated critical intensity. This type of analysis assumes that both variables are affected by some errors. In doing so, what is minimized is the distance perpendicular to the regression line, not just the vertical distance (along the y axis) as in ordinary least squares regression.

The variability of the estimated critical intensity values, as measured based on trials conducted at different mean external power outputs, was evaluated by the standard error of measurement (within-subject standard deviation of the individual's predicted critical intensity values) (13).

Finally, validated critical intensity measures were compared with traditional linear estimates (*i.e.* based on a linear power-time relationship) by effects sizes and paired-samples *t*-test.

All statistical analyses were performed using STATA (Version 14, Texas, USA) and $\alpha = 0.05$; statistical significance was accepted when $p < \alpha$. Confidence intervals (CIs) and effect sizes (Cohen's *d*, ranked as trivial (0-0.19), small (0.20-0.49), medium (0.50-0.79) and large (0.80 and greater)), (7)) are also reported as objective and standardized measures of magnitude of effects and as alternative metrics of meaningfulness (33).

**Results**

The total number of tests above 3 min in duration that were included in development of the predictive model was 70 (5 ± 1 *per* participant). During these tests, the group mean value of delta lactate was 3.89 ± 1.76 mmol·L$^{-1}$ (range 1.74-9.91 mmol·L$^{-1}$) and the group mean power output was 242 ± 48 (range 167-374 W) equal to 69 ± 11 % (range 53-95%) of peak power output.

Delta lactate was linearly related to the intensity of exercise, expressed as % of validated values of critical intensity ($F(1,68) = 472.83$, $p < 0.001$, $R^2=0.89$) (Figure 1) with an adjusted $R^2 = 0.88$ and a mean error of 6.79 W:

% validated critical intensity = 76.79 + (10.079 * delta lactate)

The mean value of critical intensity estimated from the above regression was not different from ($t$ = 0.03, $p$ = 0.98, $d$ = 0.03, 95% CI [-10.30, 10.24], Table 1 and Figure 2(a)), and highly correlated with, (r = 0.94, $p$ < 0.001, 95% CI [0.91, 0.96], Figure 2(b)) validated critical intensity.

In addition, the slope of the major axis regression line was not different from one ($b_1$ = 1.02, $p$ = 0.99, 95% CI [0.94, 1.11]) and the intercept was not different from zero ($b_0$ = -5.86, $p$ = 0.45, 95% CI [-22.10, 10.03]). The Bland-Altman analysis showed no bias ($d$ = 0.01, z = 0.04) between the power output associated with estimated and validated critical intensity, with a precision of 7 W, that in this sample of participants corresponds to 3.3% of the mean critical intensity value (Figure 2(c)). The agreement between estimated and validated critical intensity was unaffected by the relative intensity of the test, across a large range (between 55 and 95% peak power output), as indicated by a slope and intercept not different from zero (Figure 2(d)).

Finally, the variability of the estimated critical intensity values when testing the same participant at different power outputs, showed a mean within-participant SD of ± 7 W.

The critical intensity estimated with the traditional power-time model, was not different (t = 0.61, $p$ = 0.54, $d$ = 0.023, 95% CI [-9.57, 11.03], Table 1), and highly correlated (r = 0.91, $P$ < 0.001, 95% CI [0.86, 0.95]), with the validated critical intensity. The Bland-Altman analysis showed no bias ($d$ = 0.07, z = 0.58) between the power output associated with traditional and validated critical intensity, with a precision of 11 W.

**Discussion**

The present study developed and tested the suitability of a single, 3 min non-exhaustive cycle-ergometer test as an alternative to traditional critical intensity determination. In agreement with our hypothesis: *i)* there was a linear relationship between delta lactate and % of validated critical intensity and *ii)* critical intensity during cycling was accurately and precisely estimated from the delta lactate measured at the 3$^{rd}$ minute of a single, sub-maximal and constant-intensity bout of

cycling exercise in healthy adults; *iii)* the results are generalizable outside our sample (*i.e.* indicating a good cross validity) as indicated by the high adjusted $R^2$ from the regression model and by the narrow confidence intervals around the mean estimated critical intensity value (Table 1; Figure 1 and 2(a)); *iv)* the predictive power of our model (explained as the mean difference between the true (validated critical intensity) and the estimated critical intensity values), remains valid (*i.e.* slope not ≠ 0) across a wide range of relative exercise intensities (*i.e.* from 55 to 95% peak power output, Figure 2(d)) and *v)* the within-subject variability in the estimated critical intensity values, when testing the participant at different power outputs, showed a mean value of 7W (corresponding to roughly 3% in the present sample).

The appropriateness of the term "critical power" for the demarcation of the edge between heavy and severe exercise intensity domains has been recently questioned, because of emphasis on the word "power" that assesses external output. In cycling, that output is adversely affected if pedalling rate is not controlled (31,32). Furthermore, a recent paper from our group demonstrated that internal load, as described by the absolute value of $\dot{V}O_2$, might be the common denominator of several indexes of the heavy to severe intensity boundary (18). Hence use of the term "critical intensity" has been proposed as an elegant and appropriate way to describe the intensity of exercise at which lactate accumulation steadily exceeds lactate removal (31,32), independently of the exercise form, and to reconcile the apparent discrepancy between different indexes of the heavy-to-severe-intensity boundary (18).

Monitoring of blood lactate is commonplace in sport physiology (1)), and is of high practical utility as a quantitative tool for customized exercise prescription and for evaluation/monitoring of training (10,24,27) and fatigue (2,14,25). After the transient phase, anaerobic glycolysis makes a marked contribution to ATP resynthesize only as exercise intensity exceeds 40-60% $\dot{V}O_{2max}$ (influenced by force development, fiber type involved in muscle action, enzyme activity, sympathetic drive and $O_2$ supply to the exercising muscles). Such contribution is reflected by lactate production that depends on exercise intensity and duration. The lactate produced by the active muscles can diffuse into the interstitium and be

taken up and used as substrate by adjacent fibers or diffuse into the bloodstream (with a variable diffusion gradient based on age, fitness, fibre type, blood flow, etc.) where it is removed. Lactate removal reflects the rate at which lactate is being metabolized (by oxidation, transamination or gluconeogenesis) in inactive muscles or internal organs, and lactate elimination through sweat. Whenever production exceeds removal, accumulation occurs (1). Some studies have suggested the possible utilization of delta blood lactate as a predictor of relative exercise intensity (15,16,26). While these findings are promising, the proposed protocols (requiring more than one test to exhaustion or a fixed, single and elevated absolute intensity) are probably suboptimal for their application on a large scale. Therefore, to the best of our knowledge, this is the first study to establish a link between delta lactate during sub-maximal exercise and critical intensity.

The physiological rationale behind the present testing approach is that delta lactate over a given time reflects the extent of the mismatch between lactate production, use and removal which in turn is a function of relative intensity (*i.e.* % of validated critical intensity). In addition, lactate accumulation also reflects the oxygen deficit contracted during the transient phase of the exercise, that in turn depends on the speed of the individual $\dot{V}O_2$ kinetics and on the absolute exercise intensity (1 mmol of lactate is produced for an aerobic equivalent of 2.8 $ml \cdot kg^{-1}$ of $\dot{V}O_2$ in excess of the capacity of the oxidative phosphorylation) (1). While the contribution of early lactate becomes progressively diluted for longer duration exercises, it could impact on the overall delta lactate in short-duration exercises such as the one used in the present study. The contribution of early lactate could explain at least part of the variability in critical intensity estimates in the current study for which the 3-min duration was chosen for its time-effectiveness. Yet, it is of note that the elaboration of a predictive model based on 6-min lactate values and on the difference between delta lactate at 3 and 6 min does not add accuracy or precision in this homogeneous sample of individuals. However, the potential confounding effect of early lactate on lactate accumulation at 3 min and on the successive critical intensity prediction requires verification in other samples (*i.e.* disease, aging, training, etc.).

As a demarcation index between heavy and severe exercise intensity domains (18), critical intensity represents a key determinant of endurance performance (17). The reference method adopted for its determination (11,20), however, requires repeated laboratory visits, trials to exhaustion and high motivation, which limit its applicability for both diagnostic and research purposes. Several alternative approaches have allowed fewer trials required for critical intensity estimation (3,6,21,30). Yet the requirement for an exhaustive effort has not been eliminated. To overcome this limitation we developed a sub-maximal 3-min test as a possible alternative for critical intensity estimation.

To recognize this new sub-maximal test as a valid alternative to the traditional model for estimating critical intensity, consideration should be given both to practical advantages and to statistical prediction qualities. The main practical advantages of this new 3-min approach over the traditional (11,20) and alternative methods for critical intensity estimation (3,6,21,30), are: *i)* critical intensity can be established in a single 3-min trial (increasing the practical applicability and time-effectiveness of the test); *ii)* exercise tests are performed at sub-maximal intensity and hence, do not require exhaustive efforts (reducing the potential health risk associated with higher intensities of exercise to exhaustion and decreasing the impact of motivation on the accuracy of the measure); *iii)* only two lactate samples (*i.e.* baseline lactate and the 3$^{rd}$ minute values are required) reducing the overall cost of the procedure); and *iv)* a wide range of exercise intensities (53-95% peak power output), as opposed to a single, specific exercise intensity, can be used to estimate critical intensity (allowing a versatile application and customisation of this test in different contexts and populations). Hence, a simple determination of the individual physical activity habits (as previously extensively described (23)) allows a sufficiently accurate anticipation of the individual aerobic fitness to "hit" such a very wide (53-95% peak power output) intensity target.

In addition, to validate readings from this new sub-maximal test, the statistical association between estimated and validated critical intensity (as assessed by major axis regression analysis) (Figure 2(b)), should: i) provide a linear function close to that of the line of identity ($b_1 \approx 1.00$ and $b_0$ not different from 0); ii) offer a predictor of the criterion with a minimal standard error of the estimate (SEE);

and iii) display a high degree of explanatory power (8). According to the above criteria the new 3-min test yields an accurate determination of an individual's critical intensity (*i.e.* $b_1$ = 1.02; $b_0$ = -5.86; SEE = 10 W (equal to 5% of the mean of the outcome measure); $R^2$ = 0.88). Furthermore, contemplating the mean bias and precision of the traditional model with respect to the validated model, our new 3-min sub-maximal test grants lower systematic error (*i.e.* accuracy; 0.03 vs. 0.73 W) and lower random error (*i.e.* precision; 7 vs. 11 W) while offering the same plausibility range around the mean critical intensity value (*i.e.* 95% CIs, Table 1).

Further studies are required to determine the extent to which results of the current study can be generalized to other groups in terms of participants' fitness, age and sex and whether exercise intensities below 55% of the individual peak power output would still provide accurate and precise estimates of critical intensity.

**Practical Implications**

1. Critical intensity provides functional evaluation of exercise capacity and a landmark for training design and prescription in different groups;
2. Our study demonstrated that in a group of healthy young to middle-age men, a single 3-min sub-maximal test detects the exercise intensity associated with the critical intensity;
3. Compared with traditional techniques, the new test offers the following advantages: 1) the time-effectiveness (only 3-min duration) and cost-effectiveness (only two lactate determinations) increase the applicability on a large scale and for repeated measures; 2) the sub-maximal nature reduces the health risks associated with higher exercise intensities/exhaustive trials and decreases the impact of motivation on the accuracy of the measure; and 3) the wide range of intensities that can be used allows a versatile application and customisation.

## Conclusions

In conclusion, the data from this study, conducted on a homogeneous group of young to middle age men, indicates that critical intensity can be accurately and precisely predicted based on delta blood lactate measured at the 3$^{rd}$ minute of a single sub-maximal, non-exhaustive exercise trial performed at a constant power output over a wide range of relative exercise intensities (55-95% peak power output). Based on these observations, this new 3-min sub-maximal test offers an economical, practical and valid alternative to traditional determination of critical intensity.

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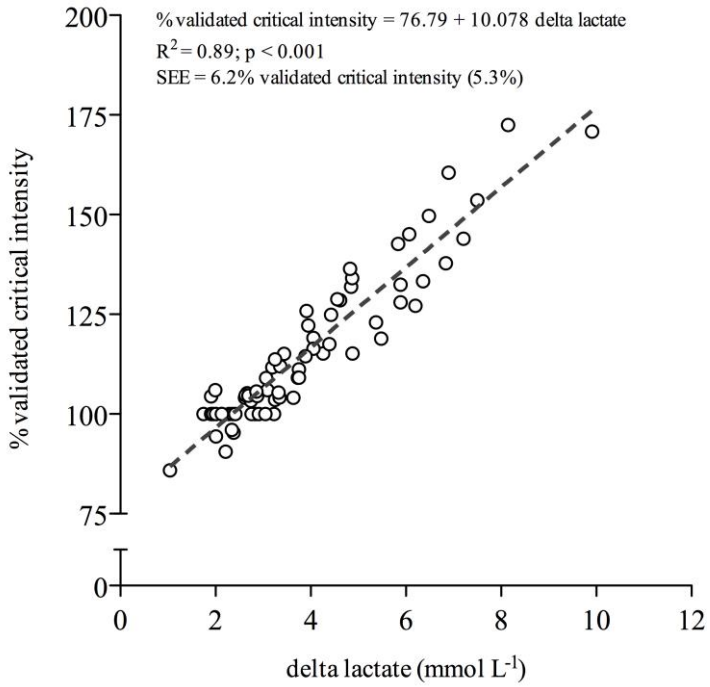


**Figure 1.** Individual values of mean external power output of the test (expressed as % of validated critical intensity) are plotted as a function of delta blood lactate (measured as the difference between the baseline and the 3$^{rd}$ minute values during a constant-external power exercise). The group regression line (dashed black) is displayed along with the regression equation parameters.

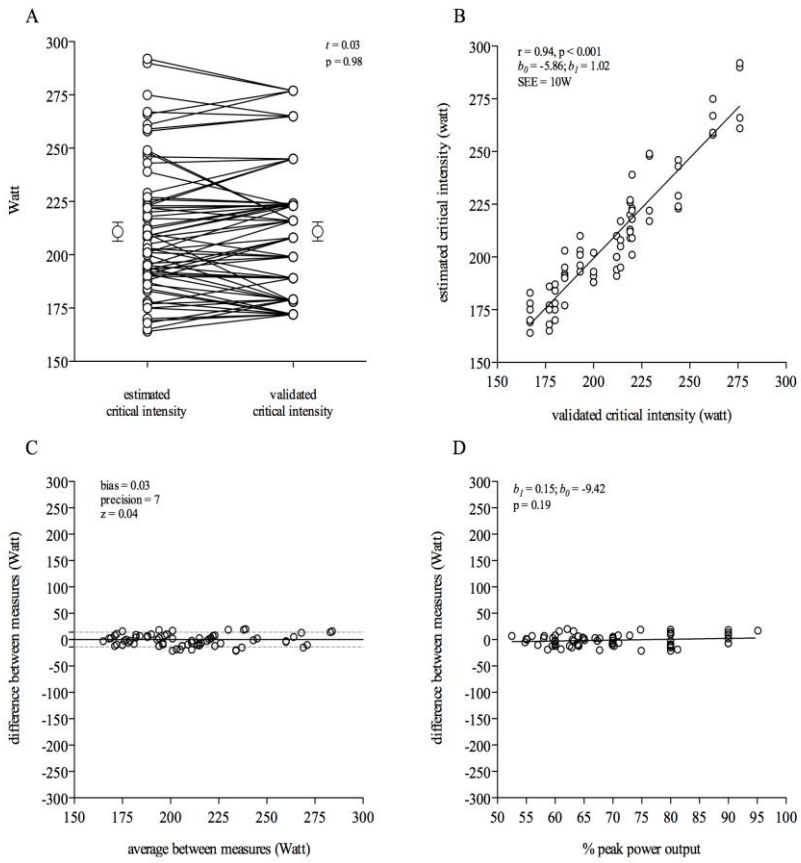


**Figure 2:** (a): Individual estimated and validated values of critical intensity are shown as small circles joined by black lines. Mean (lager circles) and 95% CIs are also displayed. (b): Individual estimated critical intensity values are plotted as a function of validated values. The major-axis regression line (solid black line) is displayed along with the Pearson's correlation coefficient ($r_s$), the slope and intercept of the relationship between the predictor and the outcome ($b_1$ *and* $b_0$) and the standard error of the estimate (SEE). (c): Individual differences between the validated and estimated critical intensity values are plotted as a function of the mean of the two measures. Bias (*i.e.* mean difference between measures; solid line) and precision (*i.e.* limits of agreement; dashed line) are displayed along with numerical values and the results of the one-tail z test on the bias. (d): Individual differences between validated and estimated critical intensity values are plotted as a function of the relative exercise intensities (% of peak power output) of the

individual trials. The regression line is displayed along with its parameters (*i.e.* slope ($b_1$), intercept ($b_0$) and p-*value*).

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# Chapter 5

## "Excess $VO_2$": how strength training affects metabolic efficiency


**Abstract**

During whole-body physical activity (e.g. cycling) exercise tolerance is compromised by the oxygen cost ($VO_2$) of energy production (i.e. *efficiency*) that become progressively higher (i.e. non-proportional) as power output (PO) increases. As a consequence of this efficiency reduction (i.e. higher $VO_2$/PO ratio or "excess" $VO_2$) exercise tolerance is compromised and exercise capacity reduced. To improve exercise intolerance endurance training is effective, however, the effects of a different intervention (i.e. strength training) has never been studied. **Purpose**: test the hypothesis that a strength intervention lowers the $VO_2$/PO ratio during a cycling incremental exercise. **Methods**: 16 healthy young subjects were randomly allocated into two groups ('*control'*: no training; '*intervention*: 5-week periodized strength training). Force was measured during both a 1RM field test on squat and deadlift and during a maximal isometric laboratory test on a force platform. $VO_2$/PO relationship was assessed during an incremental ramp (RI) test to exhaustion (in terms of oxygen consumption, electromyography and deoxyhemoglobin as a function of the power output) and modelled using a double-linear fit. After training (*Pre* vs. *Post*) and between groups (*Intervention* vs *Control*) measured parameters were compared using a two-way analysis of variance. **Results**: the ability to produce force increased significantly in the strength-training group for both 1RM and IS tests ($p < 0.05$), whereas no significant changes were observed in the control group ($p > 0.05$). During the RI exercise, a significant excess $VO_2$ ($ml \cdot min^{-1}$) was present before training in the intervention group (ΔCI around the mean difference [0.73, 5.15], $d = 1.5$, $p = 0.02$). This difference disappeared after strength training (ΔCI [-2.96, 2.97], $d = 0.01$, $p = 0.99$). In control participants, the excess $VO_2$ was present before in all participants (ΔCI [1.67, 5.53], $d = 2.4$, $p = 0.01$) and after the control

period (ΔCI [0.64, 4.16], $d$ = 1.7, $p$ = 0.01). **Conclusions:** In agreement with our hypothesis, strength training induced lowering of the $VO_2$/PO ratio, although further studies are warranted to identify a direct cause-effect relationship. This finding is relevant both in terms of performance during everyday life as well as during sporting activities.

## Introduction

During ramp incremental cycle exercise to exhaustion, there is a linear increase in $VO_2$ relative to the mechanical power output (PO), with a functional gain ($\Delta_{VO2}/\Delta_{PO}$) that varies between 8 and 12 $ml \cdot min^{-1} \cdot W^{-1}$ (11). A homogeneous linear relationship is often assumed across the whole exercise intensity spectrum. However, when the exercise exceeds a critical intensity, the rise in $VO_2$ as a function of work rate displays an increased slope (11,49) that justifies the description of $VO_2$/PO relationship as a double-linear as opposed to a single-linear function (11). The development of this so called "excess $VO_2$" (49) in the heavy-intensity domain of incremental exercise entails a progressive loss of efficiency. The more pronounced the excess $VO_2$ is, the earlier $VO_{2peak}$ will be reached and task failure will ensue, in turn causing a reduction/impairment of exercise tolerance (21).

The excess $VO_2$ appearing in the heavy-intensity domain of an incremental exercise has been considered related to the slow component of oxygen consumption occurring during constant-work rate exercise above the lactate threshold (21). For this reason, it has been suggested that similar physiological mechanisms, intrinsic to the working muscles (25), may underpin the loss of efficiency that characterises the heavy-intensity domain in both exercise paradigms (11,21). A progressive increase in fatigue of type I fibers, that is associated with the increase the ATP cost of contraction and of the $O_2$ cost of ATP resynthesis (12) and/or a progressive increase in the recruitment of intrinsically less efficient type 2 fibers (25) have been proposed as putative causes of the loss of efficiency; however, the relative role of fatigue and recruitment and their possible interaction remain to be fully elucidated (21).

Importantly, aerobic training interventions have been shown to contribute to a reduction or elimination of the loss of efficiency during ramp incremental (31) and constant work rate exercises (25). Additionally, the slow component of oxygen consumption tends to be relatively small in endurance trained athletes (25). A reduction of muscle fatigue, thanks to enhanced muscle blood flow (48), improved mitochondrial biogenesis (16) and muscle oxidative capacity (31) and reduced $O_2$ cost of ATP production/ATP cost of contraction (25,47), has been proposed to explain the improvement of muscle efficiency associated with endurance training.

On the contrary, a possible role of an intervention affecting muscle recruitment pattern in reducing the loss of efficiency in the heavy-intensity domain of exercise has never been evaluated. During an isometric exercise the increase in relative force production is obtained through the recruitment an increasing percentage of the muscle motor units, starting from type I and progressively including larger type II motor units (24). Similarly, a progressive recruitment of motor units has been observed in the *vastus lateralis* over a ramp incremental exercise; furthermore, a "threshold" phenomenon has been observed (*i.e.* an increased slope of the root mean square of the surface EMG as a function of power output), supporting the idea that higher-threshold motor units are recruited to sustain the increase in the mechanical power output above a specific relative exercise intensity (30,38,42). In this context, it may be conceived that an intervention, such as strength training, that was able to increase maximal muscle force, would reduce the recruitment of high-threshold motor units to sustain a given absolute power output (5). In turn, if the recruitment of higher order motor units plays a role in the genesis of the excess $VO_2$, then a strength training intervention should be able to affect it.

Thus, the present study tested the hypothesis that strength training, by increasing maximal force and reducing the recruitment of high-threshold motor units to sustain a given absolute exercise in the heavy-intensity domain, would reduce the excess $VO_2$ in young males.

**Methods**

*Participants*

16 healthy young males (mean ± SD: age 26 ± 3 years, height 1.76 ± 0.67 m; body mass 74 ± 11 kg) took part of this study. Participants were randomly assigned to perform either 5 weeks of strength training (intervention group) or to maintain their normal lifestyle (control group). Inclusion criteria were: healthy young (18-35 years old) male that had not been involved in any exercise training program for at least 6 months. Exclusion criteria were: being an athlete/well trained individual undergoing regular strength training, smoking, BMI>30, and medical conditions that are known to affect cardiovascular or metabolic response to exercise, that can interfere with the ability to perform exercise and/or potentially increase the risk of exercise-related injuries. Participants provided written informed consent to participate in the study that was conducted with permission of the Ethical Committee of the University of Verona and in accordance with the Declaration of Helsinki.

*Testing*

After medical clearance, within 3 days before and after the 5-week training period, all participants completed the following tests: *i)* a maximal ramp-incremental exercise test to the limit of tolerance on a cycle ergometer; *ii)* a one repetition maximum (1RM) test in the weight room (33); and *iii)* an isometric strength test on a force platform (8). All tests were conducted in an environmentally controlled laboratory (22-25°C, 55-65% relative humidity) at a similar time of the day. Participants were asked to avoid heavy exercise and caffeinated/alcoholic beverages the day before each test. A resting period of 24 h was imposed between each test.

Ramp incremental test: Each participant performed a ramp incremental test on an electromagnetically braked cycle ergometer (Sport Excalibur, Lode, Groningen, NL) consisting of a 4-min baseline cycling at 20 W, followed by a 25-$W \cdot min^{-1}$ increase in PO until volitional exhaustion. Participants were asked to cycle in the range of 70-90 rpm and the same self-selected cadence was used for both pre and post tests. The accepted criteria for maximal effort were: (*i)* a plateau in the $VO_2$

response; (*ii)* a respiratory exchange ratio ($R_{peak}$) >1.1; and (*iii)* a peak heart rate ($HR_{peak}$) > 90% of the predicted maximum based on age (19).

Breath-by-breath pulmonary gas exchange and ventilation were continuously measured using a metabolic cart (Quark $B^2$, Cosmed, Italy) (19).

Muscle oxygenation and deoxygenation ([HHb]) were evaluated using a quantitative NIRS system (Oxiplex TSTM, ISS, Champaign, USA). After shaving, cleaning and drying of the skin area, the NIRS probe was longitudinally positioned on the belly of the *vastus lateralis* (VL) muscle ~15 cm above the patella and attached to the skin with a bi-adhesive tape. The probe was secured with elastic bandages around the thigh. The apparatus was calibrated before each test after a warm-up of at least 30 minutes as per manufacturer recommendations. A comprehensive description of this method has been previously reported by Fontana et al. (2015) (19).

Surface electromyography (EMG) of the left *vastus lateralis* muscle was continuously recorded by means of a wireless system (Wave wireless EMG, Cometa, Milan, Italy). A pair of surface electrodes (Blue sensor, Ambu®, Ballerup, Denmark) was attached to the skin with a 3-cm inter-electrode distance. The electrodes were placed longitudinally with respect to the underlying muscle fibers arrangement and located according to the recommendations by Surface EMG for Non-Invasive Assessment of Muscles (SENIAM). Before electrode application, the skin was shaved and cleaned with alcohol in order to minimize impedance. The skin was marked using non-permanent ink in order to place the electrodes on the same site on the two tests (pre and post training) thus reducing the variability associated with day-to-day differences in EMG electrodes placement. The EMG transmitter connected to the electrodes was well secured with adhesive tape to avoid movement-induced artifacts and the EMG signal was checked prior each test. Raw EMG signals were pre-amplified (gain 375, bandwidth 10–500 Hz) and digitized at a sampling rate of 2 kHz (Wave wireless EMG, Cometa, Milan, Italy).

<u>1RM test:</u> after familiarization (see below), 1RM was determined directly for two lower-body exercises (Squat and Deadlift) as the maximum resistance that could be lifted once throughout the full range of motion (determined in the unweight

position) maintaining a correct execution form. Before attempting a 1RM, participants performed a standard warm up as per ACSM guidelines (3). Then, a series of 3-5 single repetitions with increasing loads was performed until failure to complete one movement with correct form over the full range of motion (3).

Isometric strength test: All isometric contractions were performed on a custom-built isometric rack that allowed the bar to be fixed at any height above the floor. The isometric rack was placed over a force plate (Advanced Mechanical Technologies, Newton, MA), which sampled at 600 hz. All participants performed a minimum of two familiarization-testing sessions one week before the initiation of the actual study to ensure that maximal isometric attempts were completed. A standardized warm-up based upon previous literature was utilized (23). The position for each isometric pull was established before each trial with the use of a goniometry to ensure a knee angle of 140±5° with the barbell placed at the mid-thigh position (i.e. the position that allows the highest force generation during a whole-body exercise (8)). Once the position was established, participants were strapped to the bar in order to avoid any movement. With each trial, participants were instructed to pull as hard and as fast as possible. Each participant performed four isometric mid-thigh pulls separate by a 3-minutes recovery between trials. The best attempt was used for further analysis.

*Data analysis*

Ramp incremental test: Gas exchange threshold (GET), respiratory compensation point (RCP), peak $VO_2$ ($VO_{2peak}$) and peak PO ($PO_{peak}$) were determined as previously described (26). Briefly, $VO_{2peak}$ was determined as the highest $VO_2$ obtained over a 30s interval and $PO_{peak}$ was defined as the highest mechanical power output achieved at termination of the RI exercise. GET and RCP were estimated by visual inspection from gas exchange variables by three blinded expert reviewers (44).

The slope of the relationship between the change in $VO_2$ for a given change in power output ($\Delta_{VO2}/\Delta_{PO}$) was modeled using a double linear fit (OriginPro 2016, Origin Lab Corp, Massachusetts, USA) by considering the first linear function ($\Delta_{VO2}/\Delta_{PO}$_slope$_1$) from the region of the start of the ramp up to the deflection point in the $VO_2$ signal (*i.e.*, the point at which the $VO_2$ response starts

to display a steeper slope). The second linear function ($\Delta_{VO2}/\Delta_{PO}$_slope$_2$) was considered from the deflection point up to the point at which $VO_2$ reached its peak or just before any visible plateau in $VO_2$ (38). The following piece-wise double-linear regression was fitted minimizing the residual sum of squares:

$f = \text{if } (x < BP, g(x), h(x))$

$g(x)=i_1 +(s_1 \cdot x)$

$i_2 = i_1 + (s_1 \cdot BP)$

$h(x)= i_2 + (s_2 \cdot (x-BP))$

fit *f* to *y*

where *f* is the double-linear function, x is power output and *y* is $VO_2$, BP is the power output coordinate corresponding to the interception of the two regression lines, $i_1$ and $i_2$ are the intercepts of the first and second linear function respectively and $s_1$ and $s_2$ are the slopes. Finally, based on the individual linear regression for the $\Delta_{VO2}/\Delta_{PO}$ relationship below the break point, the expected power output at the observed $VO_{2peak}$ was calculated and the peak PO "deficit" was calculated with respect to the experimentally observed $PO_{peak}$.

The NIRS-derived [HHb] deflection point ($[HHb]_{BP}$) and the plateau in the [HHb] signal ($[HHb]_{plateau}$; indicative of the upper limit in $VO_2$ extraction in the observed muscle during the incremental test (19)) were identified by fitting the individual values of [HHb] corresponding to the incremental portion of the RI exercise as a function of power output. A piece-wise 'double-linear' model was used to characterize this response as detailed in Bellotti, 2013 (9). Thereafter, $[HHb]_{BP}$ was determined as the power output coordinate corresponding to the interception of the two identified regression lines. $\Delta_{[HHb]}/\Delta_{PO}$ relationship were calculated as the %[HHb] change as a function of the power output (*i.e.*, [HHb]_slope).

The raw EMG signals were rectified and smoothed using a fourth-order band-pass Butterworth digital filter with a frequency range set between 20 and 500 Hz. A one-second average of the root mean square (RMS) was calculated from the raw signal and was used as an index of the total muscle activation (32,36). For each participant, a resting and a peak RMS value were identified respectively as the average of the last 60-seconds at 20W before ramp initiation

and the highest value reached during the incremental portion of the exercise (with resting value equal to 0 and peak value equal to 100). Based on these normalized values the $\%RMS \cdot W^{-1}$ slope was determined using a piece-wise 'double-linear' model as explained before regarding $VO_2$ and [HHb] data fitting. EMG signal was analyzed using custom-made programs written in MATLAB software (MathWorks Inc., Natick, MA).

Finally, the power output, the $VO_2$ (after left-shifting the $VO_2$ signal to account for the mean response time (27)) and the $\%VO_{2peak}$ corresponding to the break points in RMS and [HHb] signals during the ramp incremental exercises were also calculated.

Isometric strength test test: Based on the vertical force component (Fz) recorder by the force platform, two isometric variables were calculated. The Isometric Peak Force was determined as the highest force value (N) recorder during the isometric strength maximal attempts. Contractile rate of force development was defined as the slope of the force–time curve from the onset of contraction up to the Isometric Peak Force. Isometric Peak rate of force development (IPRFD) was determined as the highest RFD value ($N \cdot s^{-1}$) recorded from the onset of contraction up to the IPF in incrementing time periods of 10 ms. The onset of contraction was defined as the instant when the force signal exceeded the baseline by 7 N (5).

*Training program*

Before the beginning of the study, all the participants took part of a 2-week familiarization period, which consisted of 6 strength-training sessions on non-consecutive days and performed with no overload to avoid any possible adaptations that could interfere with the main sub-sequent intervention. During this period, each participant received close supervision and instruction on proper exercise technique and training principles.

Subjects in the intervention group trained in a weight room 3 times per week (90 min each session) on non-consecutive days, for 5 weeks and performed a total of 15 training sessions. All training session were supervised and instructed by a qualified strength coach with an instructor/participants ratio of 1/4. The training exercises (three fundamentals whole-body exercises and two complementary

exercises) were performed with Olympic barbell and plates (Eleiko, Sweden) in a power rack. As *per* ACSM guideline for novice lifters, the load modulation over time was conducted using a linear model that implies a decreasing of training volume while increasing intensity (3). Training characteristics are detailed in table 1.

*Statistics*

After assumptions verification (*i.e.*, outliers, normality, homogeneity of variance and covariance and sphericity, tested respectively using studentized residuals analysis, Q-Q plot, Levene's test, Box's test and Mauchly's test), a two-way mixed ANOVA (2x2; BW) was performed to assess whether differences existed between independent groups (between-subjects factor: intervention *vs.* control) over time (within-subjects factor: pre *vs.* post) in the measured statistics. In addition, a three-way mixed ANOVA (4x2x2; BBW) was performed to assess any statistical difference between RCP, $PO_{BP}$, $[HHb]_{BP}$ and $EMG_{BP}$ (break points) (measured in Watt, $VO_2$ and $\%VO_{2peak}$), pre-and-post training (time) within and between the two groups (groups). For both ANOVAs, the F-statistic for both higher and lower order effects, were interpreted using the Greenhouse-Geisser correction (17) and, whether significant, pairwise comparisons were performed to detect any intra and inter-factor differences. The adjusted α level for every pairwise comparison was calculated using Student-Newman-Keuls's method (40). The required sample size was calculated based on an expected effect size estimation (medium effect size) on the primary dependent variable of interest (the absolute change in the $VO_{2SC}$ amplitude), using G-power package (http://gpower.hhu.de) and ensureing 1-β >80%.

Data are presented as means ± SD. 95% Confidence intervals around mean differences (95% ΔCIs [lower limit, upper limit]) and effect sizes of those differences (Cohen's *d*, ranked as trivial (0-0.19), small (0.20-0.49), medium (0.50-0.79) and large (0.80 and grater) (14)) are also reported as objective and standardized measures of magnitude of effects and as alternative metrics of meaningfulness (45). In effect size calculation, the SD in the control group at baseline, was used to standardize the mean difference for each contrast (17).

Regarding regression analyses (*i.e.*, double linear fitting procedures) the goodness of fit was assessed using the residual sum of squares (representing the degree of inaccuracy in the fitting), the model sum of squares (representing the improvement in prediction resulting from using a double-linear model rather than a straight line) and the $R^2$ (interpreted as the proportion of improvement using a double-linear model).

All statistical analyses were performed using STATA (Version 14, Texas, USA) and α was set in advance at the 0.05 level; statistical significance was accepted when $p < \alpha$.

**Results**

For all participants adherence to the training program was 100%. Volume (number of repetitions) and average intensity (% 1RM) performed as a function of weeks are displayed in table 1.

Changes in the morphological (Body mass and BMI) and functional ($HR_{peak,}$ $VO_{2peak}$, $R_{peak,}$ $PO_{peak}$, GET and RCP) statistics as a function of group and time are represented in table 2.

A shown in table 3, the ability to produce force increased significantly in the strength training group for both 1RM and IS tests, whereas no significant changes were observed in the control group.

During the RI exercise, a significant excess $VO_2$ ($ml \cdot min^{-1}$) was present before training in the intervention group in 87% on the participants (7 out of 8), with $\Delta_{VO2}/\Delta_{PO}$_$slope_1$ being significantly higher than $\Delta_{VO2}/\Delta_{PO}$_$slope_2$ (ΔCI around the mean difference [0.73, 5.15], $d$ = 1.5, $p$ = 0.02). This difference in the slopes disappeared after strength training (ΔCI [-2.96, 2.97], $d$ = 0.01, $p$ = 0.99). In control participants, the excess $VO_2$ was present before in all participants (8 out of 8) (ΔCI [1.67, 5.53], $d$ = 2.4, p = 0.01) and after the control period (ΔCI [0.64, 4.16], $d$ = 1.7, $p$ = 0.01) (table 3 and figure 1). In addition, the BP did not change after training in both groups (table 3 and figure 1).

In the incremental portion of the exercise, [HHb] increased linearly as a function of workload up to a deflection point (detected in 100% of participants), where the rate of increase was reduced, often resembling a plateau (figure 1). The $[HHb]_{BP}$ (expressed in $mL \cdot min^{-1}$ or in watt), the [HHb]_slope and the plateau in

the [HHb] signal did not change significantly after strength training or after the control period (table 2 and table 3).

The RMS increased from initial values during the ramp incremental exercise up to a deflection point ($EMG_{BP}$) (figure 1). A significant difference in the $\Delta_{RMS}/\Delta_{W}$ was present before training in the intervention group in 87% on the participants (7 out of 8), with $\Delta_{RMS}/\Delta_{PO}$-$slope_2$ being significantly higher than $\Delta_{RMS}/\Delta_{PO}$-$slope_1$ (ΔCI [0.11, 0.22], $d$ = 2.9, $p$ = 0.01). This difference in the slopes 2 disappeared after strength training (ΔCI [-0.02, 0.14], $d$ = 0.70, $p$ = 0.13). In control participants, a difference in $\Delta_{RMS}/\Delta_{PO}$_slopes was present before in 75% on the participants (6 out of 8) (ΔCIs [0.06, 0.22], $d$ = 1.9, p = 0.01) and remained so after the control period (ΔCIs [0.07, 0.19], $d$ =2.3, $p$ =0.03) (table 2 and figure 1). Finally, the $EMG_{BP}$ did not change after training in both groups (table 3 and figure 1).

No significant differences existed between the RCP, the $VO_{2BP}$, the $[HHb]_{BP}$ and the $EMG_{BP}$ measured in watt, in $VO_2$ or in % $VO_{2peak}$, in both groups, pre-and-post training as shown by: *i)* non significant three-way interactions (breakpoints*time*group; all Fs < 0.30, *d*s < 0.03, *p*s > 0.91); *ii)* non significant two-way interactions (breakpoints*time, breakpoint*group, time*group; all Fs < 0.36, *d*s < 0.06, *p*s > 0.40) (table 3).

**Discussion**

The present study tested the hypothesis that strength training, by increasing maximal force and affecting the recruitment of high-threshold motor units to sustain a given exercise intensity, would reduce the excess $VO_2$ observed during a ramp incremental exercise in young males. The primary finding of this investigation was that a 5-week strength training program significantly improved the ability to produce force (*i.e.* 1RM, IPF and IPRFD) in young healthy individuals. In addition, in agreement with our hypothesis, these changes were associated with: *i)* a significant and large reduction in the magnitude of the excess $VO_2$ (measured as a 29% reduction in the $\Delta_{VO2}/\Delta_{PO}$-$slope_2$) accompanied by a change in the $PO_{peak}$ by an average of 16±10 watt (5±3%) and a 89% reduction of the $PO_{deficit}$; and *ii)* a temporal-associated large reduction in the intensity of muscle activation during a ramp incremental exercise (measured as a 25%

reduction in $\Delta_{RMS}/\Delta_{PO}$-slope$_2$). Furthermore, this training intervention resulted in no changes in the GET, RCP, $VO_{2peak}$ or any of the [HHb] indexes. This is the first study indicating a role of strength training in the reduction of the excess $VO_2$ observed during a ramp incremental cycling exercise.

Based on our baseline (i.e., pre-training) results of a steeper and progressive increase in the $\Delta_{RMS}/\Delta_{PO}$ relationship after the appearance of a threshold response ($EMG_{BP}$), a significant increase in the recruitment of motor units containing presumably less efficient type II fibers occurs at exercise intensities above approximately 75% of $VO_{2peak}$. This rapid and steeper increase in RMS observed as a function of power output during a ramp incremental exercise above the anaerobic threshold would represent the point at which an increased contribution from fast twitch motor units occurs to maintain the required energy supply for muscle contraction (29,38). This phenomenon may occur as a result of a change in the pattern of motor unit recruitment from predominantly slow twitch motor units to fast twitch motor units (38,42).

Additionally, in agreement with previous reports (11,49), the present study observed a disproportional rise in $VO_2$ in relation to power output at exercise intensities exceeding the anaerobic threshold (excess $VO_2$). This excess $VO_2$ could be caused by the same progressive recruitment of less-efficient type II fibers that occurs during a constant work rate exercises (11,21,25) denoting the so called slow component. A positive correlation between the mechanical muscle inefficiency during a step transition exercise and the percentage distribution of fibers types has been previously reported (7). In that study, participants with higher proportion of type II fibers have a greater inefficiency of muscle contraction than those with a lower proportion of type II fibers (7). This "recruitment hypothesis" is also supported by previous studies showing a close relationship between the type of active fibers in the *vastus lateralis* and the intensity of exercise during a ramp incremental exercises such that at about 40% $VO_{2peak}$ type I fibers are almost exclusively recruited, whereas at about 60% $VO_{2peak}$ both type I and type II fibers are activated. Additionally, above 75% $VO_{2peak}$ a predominance of type II fibers recruitment is observed (29,43).

Five weeks of strength training resulted in an attenuation of both the excess $VO_2$ and the increase in the RMS signal as a function of the mechanical power output, observed during a ramp incremental exercise, suggesting that the mechanism(s) responsible for this training-induced enhancement in the mechanical efficiency of muscle contraction might also be those involved in strength gain (18). Following strength training there are specific fiber-type changes that might be involved in the observed attenuation of the excess $VO_2$. A reduction in myosin heavy chain IIb and an increase in myosin heavy chain IIa (mirroring a change in fiber type composition, with a reduction in the percentage of type IIb fibers and an increase in the percentage of type IIa fibers (4)) have been observed during the early phase of strength training (39). These adaptations, along with a shift in metabolism to more aerobic fiber type recruitment during exercise, would reduce the metabolic cost related to muscle tension and enhance contractile efficiency (28). Furthermore, considering the relative short intervention period, it is conceivable that a training induced adaptation affected the neural mechanisms involved in the skeletal muscle contraction (20,34) including: muscle recruitment (15), motor units discharge rate (41), motoneurons excitability (22) and motoneurons inhibitory stimuli (e.g. Renshaw's cells activity (13,28). In fact, the following adaptations have been described following strength training: *i)* less muscle is required to lift a given absolute load (41); *ii)* a lower neural stimulus is required to evoke the same absolute response (1,2,28) and *iii)* a lower presynaptic inhibition on motoneurons (evoking a different firing pattern, higher in its maximal nature and lower in its sub-maximal nature) (13,28). Therefore, based on the above adaptations after strength training, it is plausible that the same force requirement may induce less and different muscle recruitment and a lower firing rate from motoneurons. In doing so, the fatigue effect on muscle caused by fibers recruitment and by the discharge rate on myofibrils, could be reduced improving the contraction efficiency of the muscle itself.

Finally, the kinetics of force development after strength training can also play a role in altering metabolic demand of the exercising muscle during cycling (6). Specifically, metabolic cost is greatest at the beginning of a muscle contraction compared with the maintenance phase of the contraction (6). As

documented in this study (table 3) strength training induced a change in the rate of force development. Possessing the ability to generate force more rapidly lengthens the portion of the contraction phase devoted to the maintenance of developed force, thus, potentially reducing the metabolic demand of the muscle, and enhancing intramuscular work efficiency during exercise (6).

From a health benefits perspective, the reduction in the oxygen excess associated with higher intensities of exercise is an important training adaptation that improves exercise tolerance and/or metabolic efficiency. A reduction in the magnitude of the excess $VO_2$ in response to endurance training has been previously reported (31). Several adaptive changes within the muscles after endurance training might contribute to the observed reduction in the excess $VO_2$. For example, improvements in muscle blood flow distribution (31)), increased muscle oxidative capacity (46) and mitochondrial biogenesis (49), as well as a change in the ATP production/utilization per unit of generated power output (47) are the putative mechanisms. Although this information is important, no previous study has investigated the effect of strength training in the reduction of the excess $VO_2$ during ramp incremental exercise in young healthy subjects. Unlike the results from endurance training studies, the findings from this 5-week strength training intervention indicate no changes in metabolic responses to cycling exercise, as no differences were observed in the upper limit of aerobic power ($VO_{2peak}$) or in the indexes that set the exercise intensities boundaries (*i.e.*, GET and RCP) (table 2). Similarly, the [HHb] kinetic response during exercise did not change after training, suggesting that the balance between microvascular blood flow and muscle $O_2$ utilization were not affected by this strength training intervention. Overall, these results indicate that changes in the patter of muscle fiber recruitment, rather than "metabolic" adaptations following strength training, were mainly responsible for the reduction in the oxygen excess observed at higher intensities of exercise.

In the present study, muscle oxygenation ([HHb]), muscle activation (EMG) and ventilator response (RCP) break points that have been previously investigated (19,27) were observed during the ramp incremental cycling exercise. These breaking points occurred at the same intensity of exercise (expressed as

$VO_2$, $\%VO_{2peak}$ or power output) irrespective of time (i.e., before and after the 5-week period) or group. Recently, Boone and colleagues (10) observed that, during the same exercise paradigm, these break points occurred in a specific temporal order. Specifically, it was observed that the BP in total hemoglobin preceded the BP in EMG that in turn preceded the BP at the RCP and in [HHb], and they proposed that a specific cascade of physiological events occurred during ramp incremental tests. Although promising it should be noted that the average differences in the observed BPs were very small ($\approx 2\%VO_{2peak}$ or $\approx 90$ ml $min^{-1}$) with respect to the results of our study ($\approx 5\%VO_{2peak}$ or $\approx 200$ ml $min^{-1}$). However, despite the bigger effect detected in the present study, we were not able to declare any statistical effect (likely due to the sample size), suggesting that all the above-mentioned indexes occurred at the same time. As stated previously (10), it is not clear whether is possible to establish a temporal physiological link between the responses arising from the NIRS, the $VO_2$ and the EMG signals, and it is likely that, even if a temporal difference can be established despite the normal variability in the signal, these different physiological thresholds have a common metabolic stimulus and that any sequence of events simply reflects temporal differences in the measurement technique associated to the same physiological response. Therefore, given these contrasting results, additional research is needed to clarify the interrelationship between these different break points.

It should be noted that the interpretation of some of the results and conclusions presented in the current study are based on measurement of muscle EMG activity. The technical difficulties of EMG measurement are well recognized, and the reproducibility of EMG remains a topic of discussion (35,37). Furthermore, the interpretation of EMG during cycling exercise poses some challenges as the dynamic nature of this movement could have induced some alterations in electrodes positioning and so affected the records of surface EMG. In addition, it has to be acknowledged that an additional limitation of the present study is that indirect measures were used to infer muscle recruitment pattern during exercise. Although the RMS responses during exercise suggest that progressive recruitment of muscle fibers is, to some extent, associated with the

onset of the excess $VO_2$, the involvement of other putative mechanisms that were not measured in this study cannot be ruled out.

**Conclusions**

In conclusion, data from this study suggest that the training-induced decrease in the magnitude of the non-linearity in the $\Delta_{VO2}/\Delta_{PO}$ relationship above the anaerobic threshold can be at least partly explained by the observed increase in the ability to produce force after strength training, which results in an improvement of muscle work efficiency and leads to an enhancement in metabolic stability during cycling incremental exercise.

**Table 1**: Daily training regimen characteristics and average daily volume (kg) and relative intensity (% 1RM) are displayed as a function of the training weeks.

| | **Week 1-2** rep x set@RI | | | **Week 3** rep x set@RI | | | **Week 4** rep x set@RI | | | **Week 5** rep x set@RI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | A | B | C | A | B | C | A | B | C |
| 1.**Squat** | 8x4@65% | 8x4@65% | 4x6@75% | 4x6@75% | 2x2@80%<br>2x2@85%<br>1x2@90%<br>1x3@95% | 4x4@80% | 3x4@90% | 7x3@65%<br>5x3@70% | 5x5@75% | 4x1@80%<br>3x2@83%<br>2x3@87%<br>3x2@83% | 3x2@80%<br>3x3@87%<br>3x2@83% | 2x2@85%<br>2x2@87%<br>1x4@95%<br>3x1@85% |
| **2.Bench** | 8x4@65% | 8x4@65% | 4x6@75% | 4x6@75% | 2x2@80%<br>2x2@85%<br>1x2@90%<br>1x3@95% | 4x4@80% | 3x4@90% | 7x3@65%<br>5x3@70% | 5x5@75% | 4x1@80%<br>3x2@83%<br>2x3@87%<br>3x2@83% | 3x2@80%<br>3x3@87%<br>3x2@83% | 2x2@85%<br>2x2@87%<br>1x4@95%<br>3x1@85% |
| **3.Deadlift** | 8x4@65% | 8x4@65% | 4x6@75% | 4x6@75% | 2x2@80%<br>2x2@85%<br>1x2@90%<br>1x3@95% | 4x4@80% | 3x4@90% | 7x3@65%<br>5x3@70% | 5x5@75% | 4x1@80%<br>3x2@83%<br>2x3@87%<br>3x2@83% | 3x2@80%<br>3x3@87%<br>3x2@83% | 2x2@85%<br>2x2@87%<br>1x4@95%<br>3x1@85% |
| **4.Pull ups** | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM |
| **5.Push Press** | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM |
| Total Volume | 148 | 148 | 124 | 124 | 91 | 100 | 88 | 160 | 127 | 118 | 115 | 97 |
| Average Intensity | 65% | 65% | 75% | 75% | 87.5% | 80% | 90% | 67.5% | 75% | 83% | 83% | 89% |

**Table 2.** Participants' morphological and physiological characteristics as a function of group and time.

Data are presented as mean ± SD.

| | Intervention group | | | | Control group | | | |
|---|---|---|---|---|---|---|---|---|
| | Pre | Post | $\Delta_{CIs}$ [LL, UL] | *p* | Pre | Post | $\Delta_{CIs}$ [LL, UL] | *p* |
| Age (yrs) | 24±2 | 24±2 | [-2, 2] | 1.00 | 28±2 | 28±2 | [-2, 2] | 1.00 |
| Height (cm) | 177±8 | 177±8 | [-9, 9] | 1.00 | 176±6 | 176±6 | [-6, 6] | 1.00 |
| Body mass (kg) | 72.7±15.2 | 73.6±13.9 | [-1.1, 2.8] | 0.35 | 76.0±5.6 | 75.9±5.7 | [-0.7, 0.8] | 0.87 |
| BMI ($kg/m^2$) | 23±3 | 23±3 | [-3, 3] | 0.89 | 24±3 | 24±3 | [-2.48, 3.08] | 0.88 |
| $VO_{2peak}$ ($L \cdot min^{-1}$) | 3.31±0.40 | 3.29±0.44 | [-0.43, 0.47] | 0.92 | 3.86±0.56$^{\#}$ | 3.71±0.68 | [-0.82, 0.52] | 0.63 |
| $PO_{peak}$ (watt) | 309±30 | 325±42 | [-5, 36] | 0.11 | 346±37 | 352±52 | [-13, 28] | 0.38 |
| $HR_{peak}$ (bpm) | 185±5 | 186±6 | [-6, 5] | 0.72 | 187±5 | 185±4 | [-7, 3] | 0.39 |
| $R_{peak}$ | 1.24±0.04 | 1.22±0.03 | [-0.06, 0.02] | 0.28 | 1.21±0.05 | 1.22±0.05 | [-0.04, 0.06] | 0.69 |
| GET (W) | 132±19 | 135±31.3 | [-17, 11] | 0.63 | 164±27$^{\#}$ | 167±30 | [-16, 43] | 0.32 |
| GET ($L \cdot min^{-1}$) | 1.90±0.27 | 1.84±0.28 | [-0.35, 0.23] | 0.66 | 2.31±0.48 | 2.27±0.49 | [-0.55, 0.47] | 0.87 |
| GET (% $VO_{2peak}$) | 57±6 | 56±8 | [-8, 7] | 0.78 | 59±10 | 60±11 | [-10, 12] | 0.85 |
| RCP (W) | 201±27 | 210±31 | [-7, 25] | 0.21 | 243±29$^{\#}$ | 251±54 | [-26, 39] | 0.63 |
| RCP ($L \cdot min^{-1}$) | 2.51±0.36 | 2.42±0.34 | [-0.46, 0.28] | 0.61 | 2.90±0.51 | 2.84±0.51 | [-0.61, 0.49] | 0.82 |
| RCP (% $VO_{2peak}$) | 78±11 | 74±10 | [-16, 7] | 0.46 | 75±12 | 76±11 | [-11, 13] | 0.86 |

Note: $VO_{2peak}$: peak oxygen consumption; $PO_{peak}$: peak power output; GET: gas exchange threshold; RCP: respiratory compensation point; $\Delta_{CIs}$: confidence interval around the mean difference. $^{*}$ indicate a significant difference with respect to the pre training condition ($p < 0.05$). $^{\#}$ underlies any difference between groups measured at baseline ($p < 0.05$).

**Table 3.** Force, $\Delta_{VO2}/\Delta_W$, [HHb] and $\Delta_{RMS}/\Delta_W$ variables are presented as a function of group and time.Data are presented as mean ± SD.

| | | Intervention group | | | | Control group | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pre | Post | $\Delta_{CIs}$ [LL, UL] | *p* | Pre | Post | $\Delta_{CIs}$ [LL, UL] | *p* |
| Force | 1RM Squat (kg) | 99±19 | 112±19* | [6, 20] | **0.01** | 104±17 | 103±16 | [-4, 3] | 0.82 |
| | 1RM Deadlift (kg) | 100±22 | 111±22* | [6, 15] | **0.01** | 110±9 | 110±12 | [-4, 4] | 0.86 |
| | IPF (N) | 1498±283 | 1749±272* | [7, 496] | **0.04** | 1627±239 | 1603±230 | [-116, 68] | 0.55 |
| | IPRFD ($N \cdot s^{-1}$) | 10165±3022 | 13564±2596* | [542, 6257] | **0.03** | 11196±2863 | 12350±2393 | [-1393, 1701] | 0.82 |
| $\Delta_{VO2}/\Delta_W$ | $Slope_1$ ($ml \cdot min^{-1} \cdot W^{-1}$) | 8.1±0.6 | 8.1±1.1 | [-1.1, 1.0] | 0.94 | 8.0±1.0 | 7.5±0.9 | [-1.6, 0.5] | 0.28 |
| | $Slope_2$ ($ml \cdot min^{-1} \cdot W^{-1}$) | 11.4±1.0 | 8.2±2.1* | [-6.3, -0.1] | **0.04** | 11.6±2.1 | 10.0±1.8 | [-4.1, 0.6] | 0.13 |
| | BP (W) | 210±37 | 211±49 | [-45, 47] | 0.96 | 247±40 | 251±49 | [-54, 45] | 0.83 |
| | BP ($L \cdot min^{-1}$) | 2.64±0.51 | 2.52±0.46 | [-0.64, 0.41] | 0.63 | 2.72±0.40 | 2.76±0.52 | [-0.46, 0.54] | 0.86 |
| | BP ($\%VO_{2peak}$) | 79±15 | 74±13 | [-20, 10] | 0.49 | 71±10 | 74±14 | [-10, 16] | 0.63 |
| [HHb] | Slope ($\% \cdot W^{-1}$) | 0.43±0.06 | 0.45±0.09 | [-0.06, 0.10] | 0.61 | 0.36±0.03 | 0.39±0.07 | [-0.03, 0.09] | 0.28 |
| | Plateau (mMol) | 33.4±12.1 | 36.3±12.8 | [-10.4, 16.2] | 0.65 | 31.2±14.0 | 33.1±8.5 | [-10.5, 14.3] | 0.74 |
| | BP (W) | 215±37 | 222±41 | [-34, 49] | 0.72 | 256±40 | 251±52 | [-54, 45] | 0.83 |
| | BP ($L \cdot min^{-1}$) | 2.69±0.34 | 2.51±0.39 | [-0.57, 0.22] | 0.34 | 2.83±0.45 | 2.72±0.43 | [-0.38, 0.29] | 0.76 |
| | BP ($\%VO_{2peak}$) | 81±10 | 76±12 | [-16, 6] | 0.38 | 73±12 | 73±11 | [-12, 12] | 0.99 |
| $\Delta_{RMS}/\Delta_W$ | $Slope_1$ ($\% \cdot W^{-1}$) | 0.28±0.06 | 0.27±0.07 | [-0.08, 0.06] | 0.76 | 0.27±0.08 | 0.27±0.05 | [-0.07, 0.07] | 0.99 |
| | $Slope_2$ ($\% \cdot W^{-1}$) | 0.44±0.05 | 0.33±0.08* | [-0.18, -0.04] | **0.01** | 0.41±0.07 | 0.40±0.06 | [-0.08, 0.06] | 0.76 |
| | BP (W) | 208±51 | 209±58 | [-57, 59] | 0.97 | 264±46 | 256±39 | [-53, 37] | 0.71 |
| | BP ($L \cdot min^{-1}$) | 2.67±0.47 | 2.55±0.57 | [-0.68, 0.44] | 0.65 | 2.82±0.47 | 2.80±0.39 | [-0.48, 0.44] | 0.93 |
| | BP ($\%VO_{2peak}$) | 81±14 | 77±16 | [-20, 12] | 0.60 | 73±12 | 75±11 | [-10, 14] | 0.72 |

Note: IPF: isometric peak force; IPRFD: isometric peak rate of force development; $\Delta_{VO2}/\Delta_W$: relationship between oxygen consumption and mechanical power output; [HHb]: deoxyhemoglobin; $\Delta_{RMS}/\Delta_W$: relationship between relative root mean square and mechanical power output; BP: break point; $\Delta_{CIs}$: confidence interval around the mean difference; *p* is the probability value associated with the observed mean difference; * indicate a significant difference with respect to the pre training condition ($p < 0.05$). # underlies any difference between groups measured at baseline ($p < 0.05$).

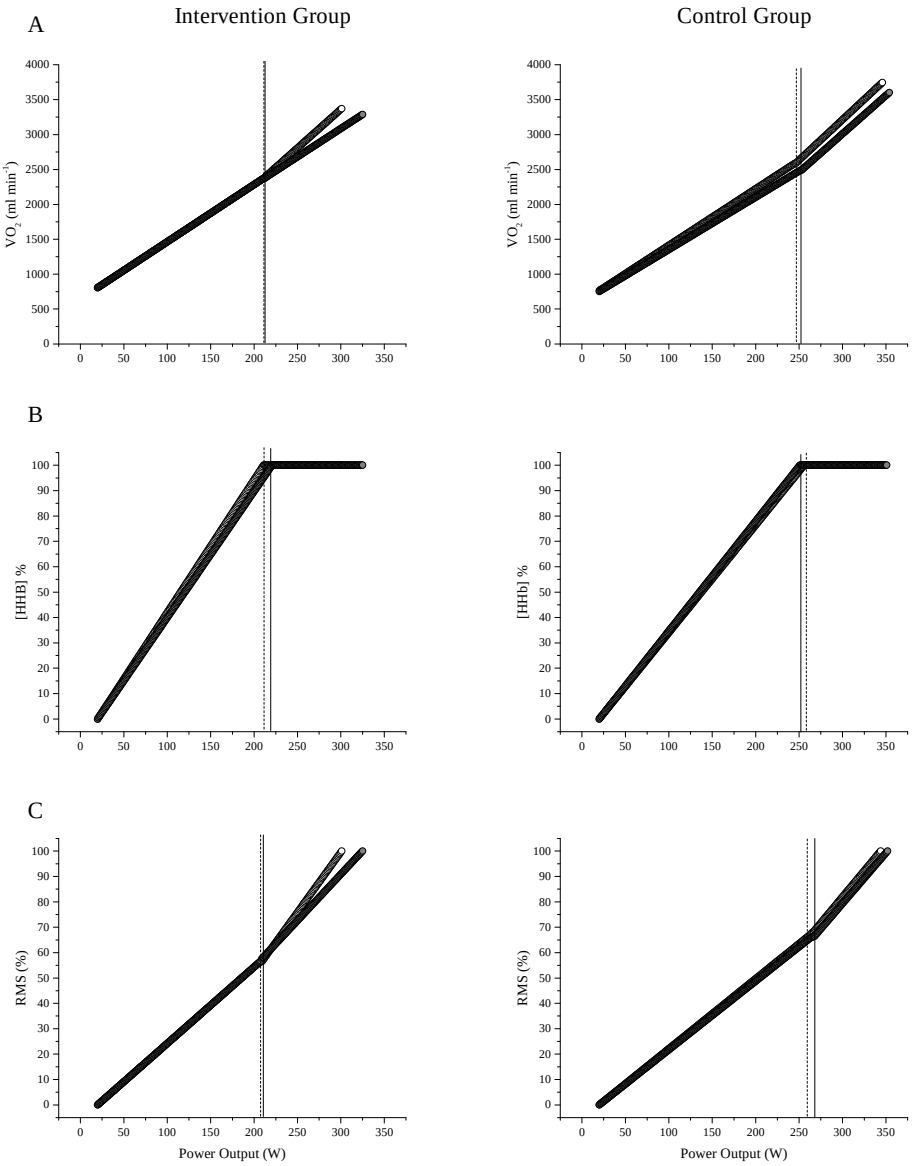


**Figure 1:** Groups' average data have been calculated based on the results of the fitting procedures and displayed for (A) $VO_2$ ($ml \cdot min^{-1}$), (B) %[HHb] and (C) %RMS as a function of power output (watt). Data are presented for the pre (white circles) and the post (grey circles) condition and for the intervention (left column) and the control group (right column). Vertical lines (dashed: pre training; continuous: post training) represent break points (BP) among each variable.

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# Chapter 6

## "$VO_2$ Slow Component": how strength training affects metabolic stability


### Abstract

The $VO_2$ slow component ($VO_{2SC}$) is a slowly developing increase in Oxygen consumption ($VO_2$) as a function of time, during a constant work rate exercise (CWR) performed above the heavy-intensity boundary. It represents a progressive loss of skeletal muscle contractile efficiency and has the most important practical implication in reducing exercise tolerance. For this reason, a possible intervention that could reduce it is of undoubted practical utility. Several such interventions exist, most of which involve endurance exercise training. However, the possible role of a different intervention (i.e. strength training) in attenuating the $VO_{2SC}$ has never been evaluated. **Purpose**: test the hypothesis that a strength intervention will reduce the magnitude of the $VO_{2SC}$ in young healthy men. **Methods**: 16-subjects were recruited to participate in a randomized controlled trail, splitted in two groups and involved in a 5-weeks strength training. Force was measured during both a 1RM field test on squat and deadlift and during a maximal isometric laboratory test (IS) on a force platform. Oxygen consumption was measured in tri-replicate during a CWR exercise performed at Δ50 (50% of the difference between the first ventilator threshold and the maximal oxygen consumption). $VO_2$ was modelled using a bi-exponential equation and the magnitude of the $VO_{2SC}$ quantified pre and post intervention. Time to exhaustion ($t_e$) was also measured. Additionally, electromyography, near infrared spectroscopy and the force applied on the pedals were continuously measured during the cycling

exercises. Groups were compared using a two-way (time, group) analysis of variance. **Results**: the ability to produce force increased significantly in the strength-training group for both 1RM and IS tests ($p < 0.05$), whereas no significant changes were observed in the control group ($p > 0.05$). Before training all subjects exhibited a significant slow component ($d = 3.05$, $p = 0.001$, 95% CI [0.37, 0.88]). After training a significant reduction of the $VO_{2SC}$ was observed in the intervention group (ΔCI [-606, -351], $d = -4.01$, $p = 0.001$) with no difference detected for the control group (ΔCI [-220, 145], $d = -0.21$, $p = 0.68$). The $VO_{2SC}$ reduction in the training group was associated with a 74±56% (8±5 min) increase in the time to reach exhaustion while no comparable gain was detected in the control group (3±17% or 0.5±2 min). **Conclusions:** In agreement with our hypothesis, strength training induced lowering of the $VO_2$ slow component, although further studies are warranted to identify a direct cause-effect relationship. This finding is relevant both in terms of performance during everyday life as well as during sporting activities.

**Introduction**

The $VO_2$ slow component ($VO_{2SC}$) represents an increase in oxygen consumption ($VO_2$) during constant work rate exercise that projects above the expected $VO_2$ steady-state for a given workload. This $VO_{2SC}$ occurs when exercise is performed in the heavy (above lactate threshold or the gas exchange threshold (GET)) (21) or even in the very-heavy/severe (above the respiratory compensation point (RCP) or critical power (CP)) (22) intensity domains. Depending on the intensity at which the exercise is performed, the magnitude of the slow component can account for ~25% of the total increase in $VO_2$ above the pre-exercise baseline (30), or even rise until $VO_{2peak}$ is reached and exercise tolerance is impaired (21).

Although the mechanisms underpinning the $VO_{2SC}$ are still debated (16), it is proposed that the majority of $VO_{2SC}$ (> 85%) originates from the working muscles (21). In fact, recent studies indicated that this extra $VO_2$ component is related to an increased energy demand in the muscles, caused by a time-dependent (*i.e.*, duration of exercise) and intensity-dependent increase in ATP and/or $O_2$ cost of force production (16). Other factors such as a disturbance in muscle metabolites concentration (e.g,., an increase in inorganic phosphate and $[H^+]$), cardio-respiratory work, and temperature increase have been recognized as minor contributors to the $VO_2$ slow component (21).

This reduced efficiency in the oxidative system to perform work could be caused by a progressive recruitment of less-efficient type II fibers (8), by fatigue (11) or by a complex interaction of both (6). However, the most prevalent hypothesis postulates that a progressive recruitment of less oxidatively efficient and more glycolytic type II muscle fibers is the main mechanism responsible for the $VO_{2SC}$ at exercise intensities above the lactate threshold (21). In relation to this, a strong positive correlation has been demonstrated between the percent of type II fibers within the working muscles, and the magnitude of $VO_{2SC}$ (6,20).

Regardless of the mechanisms that are responsible for the development of the $VO_2$ slow component, several interventions have been shown that it can be attenuated or eliminated (Jones et al. 2011), and most of these interventions involved endurance exercise training (21). Endurance training may reduce the $VO_{2SC}$ by enhancing muscle blood flow (and/or its distribution) as well as muscle oxidative capacity (9,26), which in turn might reduce fatigue in type I muscle fibers. Interestingly, despite the interest for performing exercise interventions that would help reduce the $VO_{2SC}$ and elucidate its mechanisms, the role of strength training interventions to the $VO_{2SC}$ has never been evaluated. This is surprising as strength training, with the aim to increase maximal force (19), should reduce the recruitment of high-threshold motor units to sustain mechanical power output during cycling exercise at a given exercise intensity (4). If the main mechanism responsible for the $VO_{2SC}$ is the progressive recruitment of high threshold glycolytic motor units, then a strength training intervention should reduce the $VO_{2SC}$ associated to higher intensities of exercise, and thus enhance exercise tolerance.

Therefore, the aim of this study was to evaluate the possible effect of strength training on the $VO_{2SC}$ in healthy young individuals. We tested the hypothesis that a 5-week strength training intervention would attenuate the development of the $VO_{2SC}$ and increase exercise tolerance, likely due to a reduction in the recruitment of high-threshold, type II motor units during constant work rate cycling exercise in the heavy intensity domain.

## Methods

### *Participants*

16 healthy young males (mean ± SD: age 26 ± 3 years, height 1.76 ± 0.67 m; body mass 74 ± 11 kg) took part of this study. Participants were randomly assigned to

perform either 5 weeks of strength training (intervention group; n= 8) or to maintain their normal lifestyle (control group; n= 8). Inclusion criteria were: healthy young (18-35 years old) male that had not been involved in any exercise training program for at least 6 months. Exclusion criteria were: being an athlete/well trained individual undergoing regular strength training, smoking, BMI >30, and medical conditions that are known to affect cardiovascular or metabolic response to exercise, that can interfere with the ability to perform exercise and/or potentially increase the risk of exercise-related injuries. Participants provided written informed consent to participate in the study that was conducted with permission of the Ethical Committee from the University of Verona and in accordance with the Declaration of Helsinki.

*Testing*

After medical clearance, within 8 days before and after the 5-week training period, all participants completed the following tests: *i)* a maximal ramp-incremental (RI) exercise test to the limit of tolerance on a cycle ergometer; *ii)* 2 repetitions of a 10-min constant work rate exercise at an intensity that represented a power output associated to 50% of the difference between the GET and $VO_{2peak}$ (Δ50). Additionally, participants performed 1 additional repetition of the Δ50 exercise at the same relative intensity until exhaustion; *iii)* a one repetition maximum (1RM) test in the weight room (34); and *iv)* an isometric, whole-body strength test on a force platform for the measurement of the maximal isometric force during a whole-body exercise (17). All tests were conducted in an environmentally controlled laboratory (22-25°C, 55-65% relative humidity) at a similar time of the day. Participants were asked to avoid heavy exercise and caffeinated/alcoholic beverages the day before each test. A resting period of 24h was imposed between each test.

Ramp incremental exercise: Each participant performed a RI test to exhaustion on an electromagnetically braked cycle ergometer (Sport Excalibur, Lode, Groningen, NL) consisting of 4 min baseline cycling at 20 W, followed by a 25-W·min$^{-1}$ increase in PO until volitional exhaustion (15). Participants were asked to cycle in the range of 70-80 rpm. The accepted criteria for maximal effort were: (*i)* a plateau in the $VO_2$ response; (*ii)* a respiratory exchange ratio ($R_{peak}$) >1.1; and (*iii)* a peak heart rate ($HR_{peak}$) > 90% of the predicted maximum based on age (REF).

Constant work rate exercises: For the quantification of the $VO_{2SC}$ each participant performed 3 constant work rate bouts exercise on an electromagnetically braked cycle ergometer (Sport Excalibur, Lode, Groningen, NL) consisting of 1 min baseline, 4 min cycling at 20 W followed by a 10 min cycling at an intensity of exercise relative to the individual Δ50. This heavy work rate was chosen because it has been demonstrated that a sizeable slow component can be reliably measured at this intensity, with a steady state being achieved within 10 min in the population studied (7,35). The constant work rate exercise duration was chosen to allow a sufficient evolution and duration of the $VO_{2SC.}$ One of the 3-Δ50 exercises (random order) was prolonged until reaching the individual limit of tolerance (*i.e.*, time to exhaustion ($t_e$)). The trial was terminated upon the participant being unable to maintain a pedal cadence of 60 rpm, despite strong verbal encouragement.

During all cycle ergometer exercises, breath-by-breath pulmonary gas exchange and ventilation were continuously measured using a metabolic cart (Quark $B^2$, Cosmed, Italy) (15). Muscle oxygenation and deoxygenation ([HHb]) were evaluated during the Δ50 exercise using a quantitative NIRS system (Oxiplex TSTM, ISS, Champaign, USA). After shaving, cleaning and drying of the skin area, the NIRS probe was longitudinally positioned on the belly of the *vastus lateralis* (VL) muscle

~15 cm above the patella and attached to the skin with a bi-adhesive tape. The probe was secured with elastic bandages around the thigh. The apparatus was calibrated on each testing day after a warm-up of at least 30 minutes as per manufacturer recommendations. A comprehensive description of this method has been reported by Murias et al. (28).

During Δ50 exercises, surface electromyography (EMG) activity of the left VL muscle was continuously recorded by means of a wireless system (Wave wireless EMG, Cometa, Milan, Italy) during one of the three laboratory visits (randomly chosen). A pair of surface Ag/AgCl electrodes (Blue sensor, Ambu®, Ballerup, Denmark) was attached to the skin with a 3 cm inter-electrode distance. The electrodes were placed longitudinally with respect to the underlying muscle fibers arrangement and located according to the recommendations by Surface EMG for non-invasive assessment of muscles (SENIAM) (33). Before electrode application, to minimize inter-electrode resistance, the skin was shaved and cleaned with alcohol in order to minimize impedance. The skin was marked using non-permanent ink in order to place the electrodes on the same site for the two tests (pre and post training) thus reducing the variability associated with day-to-day differences in EMG electrodes placement. The remote controller connected to the electrodes was well secured with adhesive tape to avoid movement-induced artifacts, and the EMG signal was checked prior each test. Raw EMG signals were pre-amplified (gain 375, bandwidth 10–500 Hz) and digitized at a sampling rate of 2 kHz (Wave wireless EMG, Cometa, Milan, Italy).

Crank torque was measured independently from the left and right crank arms by strain gauge transducers (Sport Excalibur, Lode, Groningen, NL; peak force 2000 N, <0.5 N resolution and measurement uncertainty of <3%). Instantaneous angular

velocity of the crank (rad $s^{-1}$) was measured every 2° using three independent sensors sampling in series (measurement uncertainty of <3%). Effective force (*i.e.* the propulsive force applied perpendicularly to the crank arm) was determined by the ratio between torque and the constant length of the crank arm (170 mm). Before the experiments began, a classical calibration procedure (with known mass) was performed and a zero adjustment was done before each session.

1RM test: after familiarization (see below), 1RM was determined directly for two lower-body exercises (Squat and Deadlift) as the maximum resistance that could be lifted once throughout the full range of motion maintaining a correct execution form. Before attempting a 1RM, participants performed a standard warm up as per ACSM guidelines (29). Then, a series of 3-5 single repetitions with increasing loads was performed until failure to complete one movement with correct form over the full range of motion (29).

Isometric strength test: All isometric contractions were performed on a custom-built isometric rack that allowed the bar to be fixed at any height above the floor. The isometric rack was placed over a force plate (Advanced Mechanical Technologies, Newton, MA), which sampled at 600 hz. All participants performed a minimum of two familiarization-testing sessions one week before the initiation of the actual study to ensure that maximal isometric attempts were completed. A standardized warm-up based upon previous literature was utilized (12). The position for each isometric pull was established before each trial with the use of a goniometry to ensure a knee angle of 140±5° with the barbell placed at the mid-thigh position (i.e. the position that allows the highest force generation during a whole-body exercise (12)). Once the position was established, participants were strapped to the bar in order to avoid any movement. With each trial, participants were instructed to pull as hard and as fast as

possible. Each participant performed four isometric mid-thigh pulls separate by a 3-minutes recovery between trials. The best attempt was used for further analysis.

*Data analysis*

Ramp incremental test: Gas exchange threshold (GET), respiratory compensation point (RCP), peak $VO_2$ ($VO_{2peak}$) and peak PO ($PO_{peak}$) were determined as previously described (22). Briefly, $VO_{2peak}$ was determined as the highest $VO_2$ obtained over a 30s interval and $PO_{peak}$ was defined as the highest mechanical power output achieved at termination of the RI exercise. GET and RCP were estimated by visual inspection from gas exchange variables by three blinded expert reviewers (40).

Constant work rate exercise: $VO_2$ kinetics was modeled using non-linear least-squares regression (OriginPro 7.5, OriginLab Corp., Northampton, MA, USA). Breath-by-breath $VO_2$ was filtered for errant breaths (*i.e.* values resulting after sighs, swallows, coughs etc., defined as residing outside of 99% prediction limits) and interpolated to 1 s intervals (36). Responses from the three exercise transitions were ensemble averaged to improve the signal-to-noise and averaged into 5 s bins for non-linear regression fitting. Previous studies have concluded that this "extra" oxygen component is of independent and delayed onset with respect of the phase II of the $VO_2$ kinetics response (7,30) and, additionally, using a fixed fitted interval (e.g., 3-6 min difference) did not accurately estimate the slow component amplitude (7). Therefore, oxygen uptake kinetics parameters were determined using a two-phase response with independent time delays as follow:

$$VO_2(t) = VO_{2Bsl} + A_p\left(1 - e^{-\frac{(t-TD_p)}{\tau_p}}\right) + A_s\left(1 - e^{-\frac{t-TD_s}{\tau_s}}\right)$$

where $VO_2$ (t) is whole body oxygen consumption at time t, $VO_{2Bsl}$ is pre- transition $VO_2$ , $A_p$ and $A_s$ are the primary and slow component amplitudes respectively, $TD_p$, $TD_s$, $\tau_p$ and $\tau_s$ are their respective time delays and time constants. The first 20 s of

data were removed before modeling to reduce the influence of the venous-return component (37).

The NIRS-derived [HHb] data during the Δ50 exercise ([HHb]) were time aligned and averaged to 5-s bins. This [HHb] response has been described to consist of a time delay at the onset of exercise, followed by an exponential-like increase in the signal (28). The time delay (TD) for the [HHb] data was determined as previously described (27). The [HHb] data were modeled from the end of the TD-[HHb] with a single exponential model as described as follow:

$$\Delta[HHb](t) = [HHb]_{Bsl} + A_p\left(1 - e^{-\frac{t - TD_p}{\tau_p}}\right)$$

where [HHb] (t) is the [HHb] response at time t, $[HHb]_{Bsl}$ is pre- transition [HHb] value, $A_p$ is the primary amplitude of the response, $TD_p$, and $\tau_p$ are time delays and time constant of the response, respectively.

The second-by-second [HHb] and $VO_2$ data were normalized for each subject (0–100% of the response). The normalized $VO_2$ was left shifted by 20 s to account for the phase I-phase II transition as previously described (36). Data were further averaged into 5-s bins for statistical comparison of the rate of adjustment for [HHb] and $VO_2$. Additionally, an overall [HHb]-to-$VO_2$ ratio for the adjustment during the exercise on-transient was derived for each individual as the average value from 20s until the appearance of the slow component (determined by the individual $TD_s$ resulted from the $VO_2$ kinetics fitting procedure). The start point was selected to be 20 s to begin beyond the physiological TD-[HHb] derived from NIRS. An end point equal to $TD_s$ was selected to ensure that both the [HHb] and $VO_2$ signals (for the primary component of the response) had already reached 100% of their amplitudes.

The raw EMG signals were rectified and smoothed using a fourth-order band-pass Butterworth digital filter with a frequency range set between 20 and 500 Hz. The onset and offset of EMG activity were obtained by a mathematical method where the onset of muscle activation was determined as the signal with an amplitude of at least 2 standard deviations beyond the mean EMG value at baseline; this was adopted as threshold criteria for determination of the muscle activation – deactivation dynamics. The root mean square (RMS) was calculated as the 30 s average of the muscle activation phase (excluding the offset dynamic) and was used as an index of the total muscle activation (35). The mean power frequency (MDF) was used as an indicator of the distribution of the frequency content within the EMG signal (8) and was calculated from the raw EMG signal. All EMG parameters were all expressed as a percentage of the 3rd minute of exercise (comparatively to the onset of the $VO_{2SC}$). The EMG signal was analyzed using a custom-made program written in MATLAB software (MathWorks Inc., Natick, MA).

Effective force expressed to the pedals (*i.e.* the propulsive force applied perpendicularly to the crank arm) was determined by the ratio between torque and the constant length of the crank arm (170 mm) using a custom-made program written in MATLAB software (MathWorks Inc., Natick, MA). Data was time-aligned to the onset of the constant work rate exercise and expressed as 30 s average from the onset to the end of exercise. The force applied to the pedal during the constant work rate exercise was expressed in percent relative to the peak force recorder during the ramp incremental exercise ($F_{peak}$). The average force expressed over the whole the step transition ($F_{avg}$) was calculated as representative of the force required to sustain the applied mechanical load.

Isometric strength test: Based on the vertical force component recorder by the force platform, two isometric variables were calculated. The Isometric Peak Force (IPF) was determined as the highest force value (N) recorder during the isometric strength maximal attempts. Contractile rate of force development (RFD) was defined as the slope of the force–time curve ($\Delta_{force}/\Delta_{time}$) from the onset of contraction up to the IPF. Isometric Peak RFD (IPRFD) was determined as the highest RFD value ($N \cdot s^{-1}$) recorded from the onset of contraction up to the IPF in incrementing time periods of 10 ms. The onset of contraction was defined as the instant when the force signal exceeded the baseline by 7 N (4).

*Training program*

Before the beginning of the study, all the participants took part of a 2-week familiarization period, which consisted of 6 strength-training sessions on non-consecutive days and performed with no overload to avoid any possible adaptations that could interfere with the main sub-sequent intervention (29). During this period, each participant received close supervision and instruction on proper exercise technique and training principles.

Subjects in the intervention group trained in a weight room 3 times per week (90 min each session) on non-consecutive days, for 5 weeks and performed a total of 15 training sessions. All training session were supervised and instructed by a qualified strength coach with an instructor/participants ratio of 1/4. The training exercises (three fundamentals whole-body exercises and two complementary exercises) were performed with Olympic barbell and plates (Eleiko, Sweden) in a power rack. As *per* ACSM guideline for novice lifters, the load modulation over time was conducted using a linear model that implies a decreasing of training volume while increasing intensity (1). Training characteristics are detailed in table 1.

*Statistics*

After assumptions verification (*i.e.*, outliers, normality, homogeneity of variance and covariance and sphericity, tested respectively using studentized residuals analysis, Q-Q plot, Levene's test, Box's test and Mauchly's test), a two-way mixed ANOVA (2x2; BW) was performed to assess whether differences existed between independent groups (between-subjects factor: intervention *vs.* control) over time (within-subjects factor: pre *vs.* post) in the measured statistics. F-statistics for both higher and lower order effects, were interpreted using the Greenhouse-Geisser correction (39) and, when significant, pairwise comparisons were performed to detect any intra and inter-factor differences. The adjusted α level for every pairwise comparison was calculated using Student-Newman-Keuls's method (13). The required sample size was calculated based on an expected effect size estimation (medium effect size) on the primary dependent variable of interest (the absolute change in the $VO_{2SC}$ amplitude), using G-power package (http://gpower.hhu.de) and ensuring 1-β >80%.

Data are presented as means ± SD. 95% Confidence intervals around mean differences (95% ΔCIs [lower limit, upper limit]) and effect sizes of those differences (Cohen's *d*, ranked as trivial (0-0.19), small (0.20-0.49), medium (0.50-0.79) and large (0.80 and grater) (12)) are also reported as objective and standardized measures of magnitude of effects and as alternative metrics of meaningfulness (41). For the effect size calculation, the SD in the control group at baseline, was used to standardize the mean difference for each contrast (13).

Regarding regression analyses (*i.e.*, double exponential model) the goodness of fit was assessed using the residual sum of squares (representing the degree of inaccuracy in the fitting), the model sum of squares (representing the improvement in prediction resulting from using a double-exponential model rather than a single exponential

model) and the $R^2$ (interpreted as the proportion of improvement using a double-exponential model).

To assess any statistical differences over time in the force applied on the pedal and in the EMG signals (*i.e.* iEMG, RMS and MPF) during the Δ50% exercises, a two-tailed z-distance was calculated between the 3rd and the 6th minute of exercises within each signal. A z-distance between two points higher than 1.96 was considered statistically significant at the 0.05 level.

All statistical analyses were performed using STATA (Version 14, Texas, USA) and α was set in advance at the 0.05 level; statistical significance was accepted when $p <$ α.

**Results**

The same sample of participants has been previously used for a different study purpose. For this reason, data from the ramp incremental exercises have been already presented elsewhere. However, here are displayed for descriptive purposes. Participants' characteristics and pre training exercises statistics are listed in table 2. Changes in the morphological (body mass and BMI) and functional ($VO_{2peak,}$ $PO_{peak}$, GET, RCP and $F_{peak}$) statistics measured during the ramp incremental exercise as a function of group and time are presented in table 2.

*Training program and adherence:* Participants successfully completed the strength program with a compliance of 94±1% (14/15 training sessions) and no injuries were reported. Volume (number of repetitions) and average intensity (% 1RM) performed as a function of weeks are displayed in Table 1.

*Force:* As shown in table 2, the ability to produce force increased significantly in the intervention group for 1RM tests (squat: 14±10% and deadlift: 11±5%), isometric peak force (IPF: 19±7%) and the rate of force development measured

during the mid-thigh pull test (IPRFD: 42±26%), whereas no significant changes were observed in the control group (average differences: 1±4%). No differences were detected at baseline between groups in any of the above measured statistics (see table 2 for statistical comparisons).

*Force applied to the pedal:* Whereas a significant increase in the maximal force applied to the pedal over the ramp incremental exercise was observed in the intervention group (12±4%, table 2), no changes were detected in the control group (0.5±4%). These changes were accompanied by a significant post-training reduction in the relative force applied to the pedal to sustain the mechanical load (table 3) during the constant work rate exercise (in percent relative to the $F_{peak}$) in the intervention group (figure 1). No changes were detected in the control group (figure 1).

*$VO_2$ kinetics:* Group average responses during the Δ50 exercise for the $VO_2$ kinetics, the force applied on the pedal, the [HHb] and the EMG signals are displayed in figure 1. The parameters estimates describing the above responses for all participants are presented in table 3. $VO_2$ kinetics parameters at baseline were not different between groups (all $d < 0.55$ and all $p > 0.05$; table 3). Before the strength training intervention, all subjects exhibited a significant slow component ($d = 3.05$, p = 0.001, 95% CI [0.37, 0.88]) (table 3 and figure 1). After training a significant reduction of the $VO_{2SC}$ was observed in the intervention group (ΔCI [-606, -351], $d$ = -4.01, p = 0.001) with no difference detected for the control group (ΔCI [-220, 145], $d$ = -0.21, p = 0.68). The $VO_{2SC}$ reduction in the training group was associated with a 74±56% (8±5 min) increase in the time to reach exhaustion (table 3) while no comparable gain was detected in the control group (3±17% or 0.5±2 min) (table 3).

No significant differences between groups were observed for other $VO_2$ kinetics parameters post strength training (table 3).

*[HHb] kinetics*: The overall time course of the [HHb] (as reflected by τ[HHb]) and the calculated TD[HHb] at baseline were similar between groups (all $d < 0.45$ and all $p > 0.35$; table 3). No changes in response to training were observed for both statistics in both the intervention and the control group (table 3). Before training, τ[HHb] adjustment was ≈50% (≈ 13 s) faster than $\tau VO_2$ in both groups (all $d > 1.47$ and all $p < 0.02$; table 3) which resulted in the calculated $[HHb]/VO_2$ ratio displaying a transient "overshoot" during the exercise on-transient ($d = 5.66$, p = 0.001, 95% CI [1.44, 1.96]; table 3 and figure 1). After 5 weeks of either strength training or control period, τ[HHb], $\tau VO_2$ and the $[HHb]/VO_2$ peak did not change significantly (table 3).

*EMG:* No between-group differences were detected between the 3rd and the 6th minute of exercise in the MPF, iEMG and RMS signals neither before, nor after the 5-week intervention/control period (figure 1). The calculated two-tailed z-distances in the EMG signals did not reach the required threshold (*i.e.* >1.96) to declare statistical differences (all *z*s < 1.96 and all *p*s > 0.05) as a function of time in any conditions.

**Discussion**

The present study tested the hypothesis that strength training, by increasing maximal force and potentially reducing the recruitment of high-threshold motor units to sustain a given absolute exercise intensity, would reduce the $VO_2$ slow component observed during a constant work rate exercise in young males. The primary finding of this investigation was that a 5-week strength-training program that significantly improved the ability to produce force (*i.e.* 1RM, IPF and IPRFD) in young healthy individuals, resulted in: *i)* a significant reduction in the magnitude of the $VO_2$ slow component (92±8% or 481±170 $ml \cdot min^{-1}$) that was accompanied by an increase in the

time to exhaustion of 74±56% during the constant work rate exercise and *ii)* a reduction in the average force (relative to $F_{peak}$; 12±7%) needed to sustain the same absolute exercise intensity after training.

This is the first study indicating a role of strength training in the reduction of the $VO_2$ slow component observed during a constant work rate cycling exercise. The pre-training results indicated a significant development of a $VO_2$ slow component as a function of time. The overall amplitude of the slow component in the present investigation is in line to those previously reported (range between 500 and 950 ml $min^{-1}$) (8,11,21). Although various mechanisms such as increased ventilatory and cardiac work, lactate clearance, stimulation from circulating hormones, and increased temperature have been proposed to contribute to the $VO_2$ slow component (10), the total contribution from these processes appears to be relatively small (30) and $O_2$ consumption from the locomotors muscles has been indicated to account for more than 85% of the $VO_{2SC}$ (31). During constant cycling exercise, within the working muscles, the genesis of the $VO_{2SC}$ might be attributed to fatigue. Fatigue might develop on the initially recruited oxidative muscle fibers (i.e. type I) causing a progressive recruitment of different, preferentially type II, fibers (6). As an alternative explanation fatigue can occur simultaneously on the recruited type I and type II muscle fibers (11). The most prevalent hypothesis is that the $VO_{2SC}$ might be the result of an increased recruitment of motor units innervating less oxidatively efficient type II fibers, which has been shown to occur at exercise intensities at or above Δ50% (21)). For example, whereas at intensities of about 40% $VO_{2peak}$ type I fibers are almost exclusively recruited, and at intensities of about 60% $VO_{2peak}$ both type I and type II fibers are activated, higher intensities of exercise above 75% $VO_{2peak}$ (which closely resembles the intensity of exercise evaluated in this study) a predominance of

type II fibers recruitment is observed (24). Further support to the idea that increased recruitment of motor units innervating type II fibers is connected to the slow component of $VO_2$ is provided the strong correlation between the percent of type II fibers and the magnitude of $VO_{2SC}$ (16)). Additionally, a positive correlation between the mechanical muscle inefficiency during cycling and the percentage distribution of fibers types has been reported (6), so that participants with higher proportion of type II fibers had lower efficiency for muscle contraction compared to those with a lower proportion of type II fibers. This factor might also contribute to the development of the $VO_2$ slow component at higher intensities of exercise.

The observed increase in strength after 5-weeks of training in the present study is similar to that reported in previous studies (5,29) and resulted in attenuation the $VO_{2SC}$ during constant work rate cycling exercise. These results suggest that the mechanism(s) responsible for this training-induced enhancement in the mechanical efficiency of muscle contraction might also be those involved in strength gain (14). There are several adaptations that likely occur after strength training, and that might have induced an improvement in the work efficiency of exercising skeletal muscle, and thus a reduction in the $VO_{2SC}$ in the present study. First, following strength training there are fiber-specific changes that might be involved in the observed attenuation of the $VO_{2SC}$. A reduction in myosin heavy chain IIb and an increase in myosin heavy chain IIa (mirroring a change in fiber type composition, with a reduction in the percentage of type IIb fibers and an increase in the percentage of type IIa fibers (2)) has been observed during the early phase of strength training (23,38). These adaptations, along with a shift in metabolism to more aerobic fiber type recruitment during exercise, would reduce the metabolic cost related to muscle tension and enhance contractile efficiency (5). Finally, the kinetics of force

development after strength training can also play a role in altering metabolic demand of the exercising muscle during cycling (4). Specifically, metabolic cost is greater at the beginning of a muscle contraction compared with the maintenance phase of the contraction (32). The strength training gain observed in this study induced a change in the rate of force development, which in turn improves the ability to generate force more rapidly, and lengthens the portion of the contraction phase devoted to the maintenance of developed force. This might potentially reduce the metabolic demand of the muscle, and enhance intramuscular work efficiency during exercise (4).

Reductions in the magnitude of the $VO_{2SC}$ in response to endurance training have been previously reported (21,42). In fact, the slow component of $VO_2$ has been shown to be attenuated very rapidly in response to endurance training, with a reduction of about 50% evident just after 2 weeks of training (19), and several adaptive changes within the muscles have been proposed to the observed reduction in the $VO_2$ slow component (25,43,44). Although this information is important, this study is the first to investigate the effect of strength training in the reduction of the $VO_{2SC}$. Unlike what is typically observed after endurance training, the present strength training intervention resulted in a reduction of the $VO_2$ slow component without concomitant changes in other cardiovascular indexes of fitness (*i.e.*, $VO_{2peak}$) or exercise intensities boundaries (*i.e.*, GET and RCP) (table 2). Similarly, the [HHb] kinetics response and the [HHb]/$VO_2$ ratio during exercise did not change after training, suggesting that the balance between microvascular blood flow and muscle $O_2$ utilization was not affected by this strength training intervention. Overall, these results might indicate that changes in the patter of muscle fiber recruitment, rather than “metabolic” adaptations following strength training, were mainly responsible for

the reduction in the slow component of $VO_2$ observed at higher intensities of cycling exercise.

An additional finding of the present study was an absence of electromyography evidence for a progressive recruitment of type II fibers during a heavy exercise bout exhibiting a $VO_{2SC}$ (Figure 1). Additionally, 5-weeks of strength training did not result in a significant change in any of the measured EMG variables. Despite our initial hypotheses, the results of the present investigation suggested that an alteration in fiber recruitment was not present in concomitance with the development of the $VO_{2SC}$. The high variability in the EMG signal, as displayed in figure 1, may partly explain the lack of any statistical significant results before and after training. In addition, the lack of temporal change in the EMG signals cannot rule out a fiber recruitment hypothesis as a mechanistic basis for the slow component development. As discussed by Cannon and colleagues (11), the relatively small shift in recruitment that might occur during the development of the $VO_{2SC}$ and the possible recruitment exchange between fatiguing type I and type II fibers may result in a unchanged EMG data as observed in the present study. Therefore, muscle fiber recruitment patterns may have changed throughout the exercise bout and following strength training in the present study, but these changes may not be identified by the measure of EMG.

Over the past decades various attempts have been made to relate the $VO_{2SC}$ with a progressive recruitment of glycolytic type II muscle fibers during constant work rate exercise, by interpreting the development of the temporal-associated surface electromyography signal. Some authors found and increase in both the integrated electromyogram (iEMG) and in the root mean square (RMS) to be associated to the $VO_2$ slow component, suggesting a likely recruitment of additional type II fibers

(8,9). However, other authors indicated no relationship between EMG signals and the slow component of oxygen consumption during cycling (8,11). Therefore, due to these controversial findings, it should be highlighted that the intrinsic limitation in the EMG analysis may have hindered the interpretation of some of the results and conclusions presented in the current study. The technical difficulties of EMG measurement are well recognized, and the reproducibility of EMG remains a topic of discussion (18). Furthermore, the interpretation of EMG during cycling exercise poses some challenges as the dynamic nature of this movement could have induced some alterations in electrodes positioning and so affected the records of surface EMG (3).

From a health benefits perspective, the reduction in the oxygen excess associated with higher intensities of exercise observed in the present study after strength training is an important training adaptation that significantly improved exercise tolerance. Such extended ability to sustain exercise might impacts compliance to aerobic activities thus improving the total amount of volume that can be performed at a given intensity of exercise and the total energy expenditure. From this perspective, strength training could significantly impacts the ability to protract aerobic exercise for an adequate amount of time and/or to extend the volume of exercise at a given exercise intensity, being a primary training component in health promotion and athletes development.

**Conclusions:**

In conclusion, data from this study suggest that the training-induced decrease in the magnitude of the $VO_2$ slow component and the increase in exercise tolerance observed during cycling above the lactate threshold can be at least partly explained by the reported increase in the ability to produce force after strength training. However, the data presented have indicated no association between the development of the slow

component and a shift in EMG signals during heavy cycling exercise. While the complexities of the electromyography measurement may have influenced the ability to elucidate the involvement of a progressive recruitment of type II fibers in the development of the $VO_{2SC}$ further study are warranted in order to better elucidated the possible influence of strength training in affecting muscle fibers recruitment during exercise in the heavy intensity domain.

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**Table 1**: Daily training regimen characteristics and average daily volume (kg) and relative intensity (% 1RM) are displayed as a function of the training weeks.

| | **Week 1-2** rep x set@RI | | | **Week 3** rep x set@RI | | | **Week 4** rep x set@RI | | | **Week 5** rep x set@RI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | A | B | C | A | B | C | A | B | C |
| 1.**Squat** | 8x4@65% | 8x4@65% | 4x6@75% | 4x6@75% | 2x2@80%<br>2x2@85%<br>1x2@90%<br>1x3@95% | 4x4@80% | 3x4@90% | 7x3@65%<br>5x3@70% | 5x5@75% | 4x1@80%<br>3x2@83%<br>2x3@87%<br>3x2@83% | 3x2@80%<br>3x3@87%<br>3x2@83% | 2x2@85%<br>2x2@87%<br>1x4@95%<br>3x1@85% |
| **2.Bench** | 8x4@65% | 8x4@65% | 4x6@75% | 4x6@75% | 2x2@80%<br>2x2@85%<br>1x2@90%<br>1x3@95% | 4x4@80% | 3x4@90% | 7x3@65%<br>5x3@70% | 5x5@75% | 4x1@80%<br>3x2@83%<br>2x3@87%<br>3x2@83% | 3x2@80%<br>3x3@87%<br>3x2@83% | 2x2@85%<br>2x2@87%<br>1x4@95%<br>3x1@85% |
| **3.Deadlift** | 8x4@65% | 8x4@65% | 4x6@75% | 4x6@75% | 2x2@80%<br>2x2@85%<br>1x2@90%<br>1x3@95% | 4x4@80% | 3x4@90% | 7x3@65%<br>5x3@70% | 5x5@75% | 4x1@80%<br>3x2@83%<br>2x3@87%<br>3x2@83% | 3x2@80%<br>3x3@87%<br>3x2@83% | 2x2@85%<br>2x2@87%<br>1x4@95%<br>3x1@85% |
| **4.Pull ups** | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM | 5x4@6RM |
| **5.Push Press** | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM | 4x8@10RM |
| Total Volume | 148 | 148 | 124 | 124 | 91 | 100 | 88 | 160 | 127 | 118 | 115 | 97 |
| Average Intensity | 65% | 65% | 75% | 75% | 87.5% | 80% | 90% | 67.5% | 75% | 83% | 83% | 89% |

**Table 2.** Participants' morphological and physiological characteristics as a function of group and time.

Data are presented as mean ± SD.

| | Intervention group | | | | Control group | | | |
|---|---|---|---|---|---|---|---|---|
| | Pre | Post | $\Delta_{CIs}$ [LL, UL] | *p* | Pre | Post | $\Delta_{CIs}$ [LL, UL] | *p* |
| Age (yrs) | 24±2 | 24±2 | [-2, 2] | 1.00 | 28±2 | 28±2 | [-2, 2] | 1.00 |
| Height (cm) | 177±8 | 177±8 | [-9, 9] | 1.00 | 176±6 | 176±6 | [-6, 6] | 1.00 |
| Body mass (kg) | 72.7±15.2 | 73.6±13.9 | [-1.1, 2.8] | 0.35 | 76.0±5.6 | 75.9±5.7 | [-0.7, 0.8] | 0.87 |
| $VO_{2peak}$ ($L \cdot min^{-1}$) | 3.31±0.40 | 3.29±0.44 | [-0.43, 0.47] | 0.92 | 3.86±0.56$^{\#}$ | 3.71±0.68 | [-0.82, 0.52] | 0.63 |
| $PO_{peak}$ (watt) | 309±30 | 325±42 | [-5, 36] | 0.11 | 346±37 | 352±52 | [-13, 28] | 0.38 |
| $F_{peak}$ (N) | 327±12 | 373±29 | [22, 70] | **0.01** | 361±16 | 356±24 | [-27, 16] | 0.63 |
| GET (W) | 132±19 | 135±31.3 | [-17, 11] | 0.63 | 164±27$^{\#}$ | 167±30 | [-16, 43] | 0.32 |
| GET ($L \cdot min^{-1}$) | 1.90±0.27 | 1.84±0.28 | [-0.35, 0.23] | 0.66 | 2.31±0.48 | 2.27±0.49 | [-0.55, 0.47] | 0.87 |
| RCP (W) | 201±27 | 210±31 | [-7, 25] | 0.21 | 243±29$^{\#}$ | 251±54 | [-26, 39] | 0.63 |
| RCP ($L \cdot min^{-1}$) | 2.51±0.36 | 2.42±0.34 | [-0.46, 0.28] | 0.61 | 2.90±0.51 | 2.84±0.51 | [-0.61, 0.49] | 0.82 |
| 1RM Squat (kg) | 99±19 | 112±19$^{*}$ | [6, 20] | **0.01** | 104±17 | 103±16 | [-4, 3] | 0.82 |
| 1RM Deadlift (kg) | 100±22 | 111±22$^{*}$ | [6, 15] | **0.01** | 110±9 | 110±12 | [-4, 4] | 0.86 |
| IPF (N) | 1498±283 | 1749±272$^{*}$ | [7, 496] | **0.04** | 1627±239 | 1603±230 | [-116, 68] | 0.55 |
| IPRFD ($N \cdot s^{-1}$) | 10165±3022 | 13564±2596$^{*}$ | [542, 6257] | **0.03** | 11196±2863 | 12350±2393 | [-1393, 1701] | 0.82 |

Note: $VO_{2peak}$: peak oxygen consumption; $PO_{peak}$: peak power output; GET: gas exchange threshold; RCP: respiratory compensation point; $t_e$: time to exhaustion; 1RM: one repetition maximum; IPF: isometric peak force; IPRFD: isometric peak rate of force development; $\Delta_{CIs}$: confidence interval around the mean difference. $^{*}$ indicate a significant difference with respect to the pre training condition ($p < 0.05$). $^{\#}$ underlies any difference between groups measured at baseline ($p < 0.05$).

15 **Table 3.** Groups parameters estimate as a function of time during the Δ50 exercise for the VO2 kinetics, the force applied to the pedal, [HHb]

16 and the EMG responses. Data are presented as mean ± SD.

| | | Intervention group | | | | Control group | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pre | Post | $\Delta_{CIs}$ [LL, UL] | *p* | Pre | Post | $\Delta_{CIs}$ [LL, UL] | *p* |
| $VO_2$ | Bsl (ml $min^{-1}$) | 0.96±0.11 | 1.08±0.14 | [-0.02, 0.25] | 0.08 | 1.15±0.19 | 1.19±0.20 | [-0.17, 0.25] | 0.69 |
| | $TD_p$ (s) | 12±3 | 14±6 | [-3.08, 7.09] | 0.41 | 11±6 | 10±5 | [-6.92, 4.92] | 0.73 |
| | $\tau_p$ (s) | 26±6 | 26±3 | [-5.08, 5.08] | 0.99 | 20±7 | 21±6 | [-5.99, 7.99] | 0.76 |
| | $A_p$ (ml $min^{-1}$) | 1.76±0.22 | 1.55±0.31 | [-0.49, 0.08] | 0.14 | 1.88±0.32 | 1.92±0.36 | [-0.32, 0.41] | 0.82 |
| | $TD_s$ (s) | 183±41 | 186±54 | [-48, 54] | 0.90 | 160±54 | 165±30 | [-41, 52] | 0.82 |
| | $\tau_s$ (s) | 223±35 | 188±57 | [-85, 15] | 0.16 | 186±43 | 183±32 | [-43, 38] | 0.87 |
| | $A_s$ (ml $min^{-1}$) | 0.52±0.17 | 0.04±0.01 | [-0.61, -0.35] | **0.01** | 0.71±0.20 | 0.68±0.13 | [-0.21, 0.16] | 0.73 |
| | $t_e$ (min) | 11±2 | 19±5 | [4, 12] | **0.01** | 10±2 | 10±2 | [-3, 2] | 0.63 |
| [HHb] | τ (s) | 10±3 | 13±4 | [-1, 7] | 0.12 | 10±4 | 13±6 | [-2, 8] | 0.26 |
| | TD (s) | 12±3 | 10±4 | [-6, 2] | 0.28 | 13±2 | 13±2 | [-2, 2] | 0.85 |
| | Δ[HHb]/Δ$VO_2$peak | 1.7±0.3 | 1.6±0.3 | [-0.4, 0.2] | 0.51 | 1.8±0.7 | 1.6±0.3 | [-0.8, 0.4] | 0.47 |
| | Δ[HHb]/Δ$VO_2$auc | 17±7 | 13±8 | [-12, 4] | 0.31 | 15±7 | 10±8 | [-13, 3] | 0.20 |
| PFM | $\%F_{peak}$ | 79±11 | 67±9 | [-22, -2] | **0.02** | 84±16 | 86±13 | [-13, 18] | 0.78 |

17 Note: For $VO_2$: Bsl: baseline; A, TD and τ: amplitude, time delay and time constant of the primary (p) and secondary (s) component respectively. For [HHb]: TD: time delay; τ: time
18 constant; peak: peak value; auc: area under the curve. $\Delta_{CIs}$: confidence interval around the mean difference. * indicate a significant difference with respect to the pre training condition
19 ($p < 0.05$). # underlies any difference between groups measured at baseline ($p < 0.05$)

Figure 1

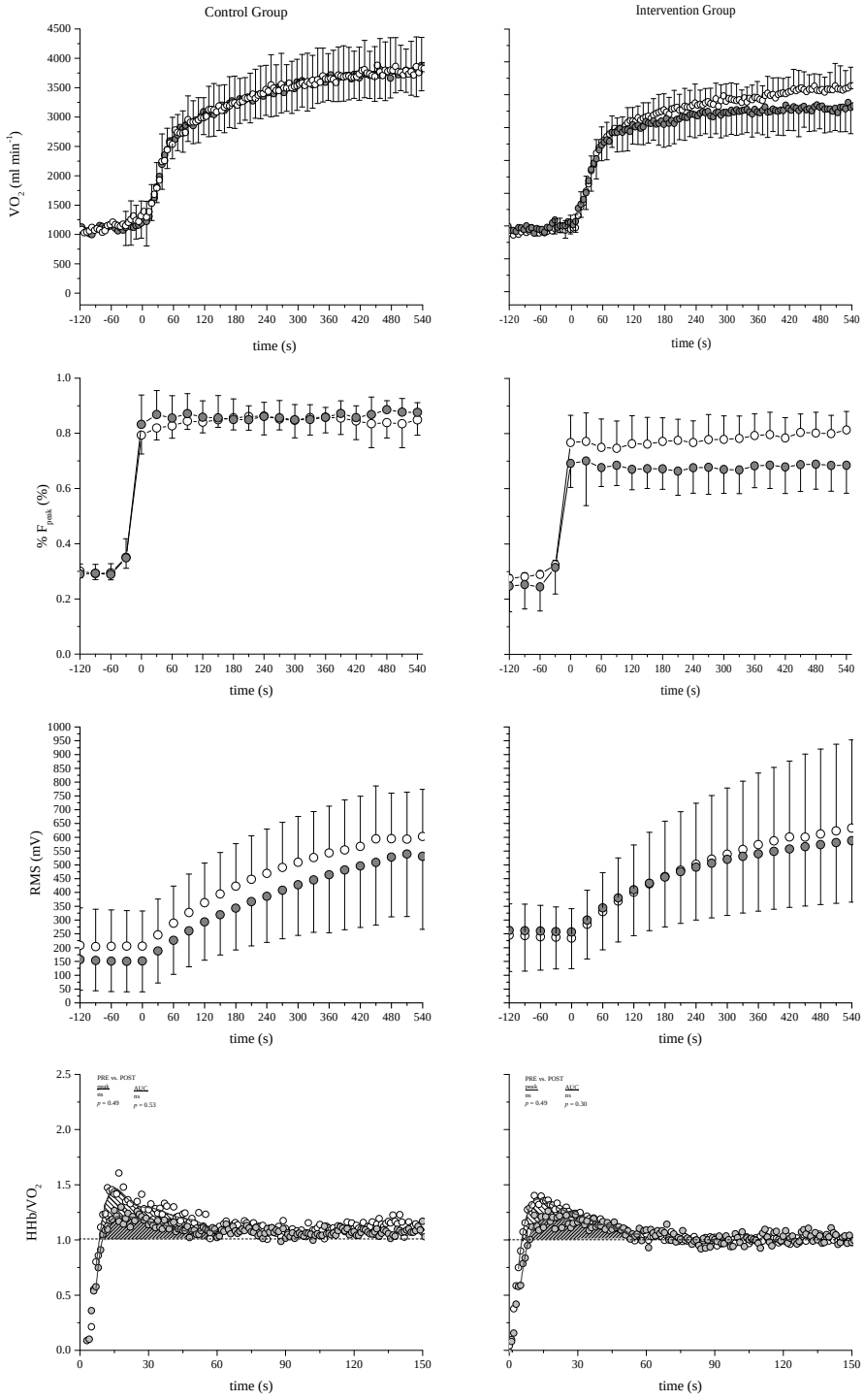


Figure 1: Groups' average data have been calculated based on the results of the fitting procedures and displayed respectively (top to bottom), $VO_2$ ($ml \cdot min^{-1}$),

relative force applied on the pedal with respect to the peak force (%$F_{peak}$ (%)) Root mean square (mV) and for the HHb/$VO_2$ ratio as a function of time (seconds). Data are presented for the pre (white circles) and the post (grey circles) condition and for the control (left column) and the intervention group (right column). Statistical comparisons are detailed in the result section.

# Chapter 7

# General Discussion

**Study one:**

For years, the concept of threshold-based exercise intensity has been used to assess and stratify cardiorespiratory fitness and health, for exercise prescription, and to quantify the outcomes of specific interventions (14). Yet, numerous physiological 'thresholds' exist in the literature (each with unique nomenclature and methodology for determination) creating ambiguity regarding their physiological-bases and relevance (6, 15). Each of these "paradigms" uses different methods of measurement and may be preferred based on consistencies with previous work, available instruments, and the test population, lending to confusion regarding which one represents (or should represent) the 'ceiling' of tolerable endurance exercise and whether they are physiologically equivalent.

It is possible that each of the above mentioned "thresholds" might be the result of similar underlying physiological and metabolic processes? Does these different "thresholds" reflecting a common level of aerobic metabolism beyond which there is a progressive loss of homeostasis? Before the mechanisms underlying these indices of exercise intensity can be examined, it first must be determined whether the intensity at which each occurs is similar (in terms of $VO_2$) within a single group of subjects.

This first study tested the hypothesis that Critical Power (CP), Maximal Lactate Steady State (MLSS), Respiratory Compensation Point (RCP) and the breaking point observed in the deoxyhemoglobin (*deoxy*Hb) derived from NIRS signal ($[HHb]_{BP}$) occur at the same metabolic rate (i.e., $VO_2$).

The main finding was that the $VO_2$ values associated with CP, MLSS, RCP, and $[HHb]_{BP}$ were not different suggesting that each functional index of exercise intensity could provide a method by which to identify the boundary between *heavy* and *very heavy* exercise domains. This is the first study to directly demonstrate a commonality between these 'thresholds' in a single group of

subjects substantiating the notion of a 'critical metabolic rate' as the highest metabolic rate at which exercise is well tolerated for long durations.

Results demonstrate that there is a relationship between the metabolic/physiological responses and the $VO_2$ values associated to each of these thresholds suggesting that they may share a common underlying mechanistic link.

Finally, the current study has demonstrated that the concepts of CP, MLSS, RCP, and $[HHb]_{BP}$ may be unified by determining the $VO_2$ (rather than the Power Otput (PO)) associated with each functional index of exercise intensity substantiating the existence of a "metabolic boundary" partitioning *heavy* from *very heavy* exercise domains. These data suggest that the above mentioned constructs may be physiologically equivalent and, providing optimal design and appropriate determination, each could theoretically represent the highest $VO_2$ at which the metabolic response to exercise (Lactate concentration $[La]_b$ and $VO_2$) can be stabilized, and thus the "boundary" of sustainable versus unsustainable constant-power exercise.

This is of valuable practical importance as the interchange-ability of each index may provide exercise physiologists, sport scientists and clinicians with a number of options for determining the limits of tolerable endurance exercise depending upon the test population, the available resources and the desired intensity target for exercise prescription.

**Study two**

As underlined in the previous study, the concept of exercise intensity provides a framework for research and training design in the field of exercise physiology. Traditionally, exercise intensity is expressed as a percentage of maximal oxygen uptake ($VO_{2max}$) and it is known that as exercise intensity increases in any individual, unique thresholds exist that demarcate boundaries associated with specific physiological and metabolic profiles (16, 19). The rationale for delimiting these threshold intensities is controversial as disagreements exist in the scientific community in terms of both a theoretical basis for their existence, as well as an appropriate methodology for their determination (11). Despite these

disagreements, the concept of intensity-dependent thresholds has been used for years in athletes, healthy sedentary population, and patients to assess cardiovascular or pulmonary health, to stratify individuals based on fitness status, to determine and monitor exercise intensities, and to quantify the outcomes of specific interventions(14).

In the previous study and in recent investigations the utilization of near infrared spectroscopy (NIRS) technology has been proposed for the determination of threshold intensities during exercise. This technology provides the means to non-invasively collect high-resolution data and can be used in both laboratory and field settings (1). Specifically, it has been shown in a relatively small group of healthy male adults (study #1) that a deflection point (DP) in the NIRS-derived *deoxy*Hb signal during incremental cycle exercise occurred at the same $VO_2$ as that associated with CP, MLSS and RCP, indicating that the boundary between sustainable and unsustainable exercise intensity could be accurately determined using quantitative measures of *deoxy*Hb.

The present study tested in a larger and more heterogeneous group of men whether the occurrence of the *deoxy*$Hb_{DP}$ coincided with the RCP during incremental cycle exercise to exhaustion.

The main finding was that $VO_2$ values observed at the *deoxy*$Hb_{DP}$ were highly correlated with and not significantly different from those observed at the RCP, suggesting that NIRS-derived measurements can be used to accurately estimate the exercise intensity associated with the RCP as an alternative to ventilatory and gas exchange-based measurements. Compared to ventilatory-based techniques, *deoxy*$Hb_{DP}$ offers the advantage of being objective and independent from irregularities of breathing pattern that can heavily affect the former. Furthermore, its non-invasive nature, the ability to evaluate even small muscle masses and the high sampling frequency allow the characterization of the response to exercise even in subjects with a limited exercise capacity and/or motivation (17).

The relevance of the present study resides in providing an objective, reliable, and easily detectable demarcation of an exercise intensity associated to the RCP through the use of NIRS and the *deoxy*Hb signal.

Considering the recent development of low cost portable, and easy to wear NIRS devices, and taking into account that none of the more invasive or gas exchange derived measures completely satisfies the requirements of accuracy, precision and overall economy of testing for determination of exercise threshold intensities, the use of the NIRS-derived methodology becomes appealing.

In conclusion, our data from the study two, suggest that the RCP, a variable commonly used to prescribe exercise training intensities and to determine exercise performance, can be easily, objectively and accurately detected using non-invasive NIRS-derived measures.

**Study three**

Among indices of exercise capability, Critical Power (CP) provides a simple, sensitive and clear marker for functional evaluation of exercise capability/tolerance (10). It can be used to prescribe and evaluate the effectiveness of training programs, predict endurance performance in high-standard and amateur athletes, and in disease populations (8).

The appropriateness of the term "critical power" for the demarcation of the edge between heavy and severe exercise intensity domains has been recently questioned, because of emphasis on the word "power" that assesses external output (20). In cycling, that output is adversely affected if pedalling rate is not controlled. Furthermore, as demonstrated in the study 1, the internal load, as described by the absolute value of $VO_2$ might be the common denominator of several indexes of the heavy to severe intensity boundary. Hence use of the term "critical intensity" has been proposed as an elegant and appropriate way to describe the intensity of exercise at which lactate accumulation steadily exceeds lactate removal, independently of the exercise form, and to reconcile the apparent discrepancy between different indexes of the heavy-to-severe-intensity boundary (21) (*see* study 1).

As a demarcation index between heavy and severe exercise intensity domains, critical intensity represents a key determinant of endurance performance (10). The reference method adopted for its determination however, requires repeated

laboratory visits, trials to exhaustion and high motivation, which limit its applicability for both diagnostic and research purposes. Several alternative approaches have allowed fewer trials required for critical intensity estimation (2, 4, 18). Yet the requirement for an exhaustive effort has not been eliminated. To overcome this limitation, in this study we developed a sub-maximal 3-min test as a possible alternative for critical intensity estimation.

The present study developed and tested the suitability of a single, 3 min non-exhaustive cycle-ergometer test as an alternative to traditional critical intensity determination.

In agreement with our hypothesis: *i)* there was a linear relationship between delta lactate and % of validated critical intensity and *ii)* critical intensity during cycling was accurately and precisely estimated from the delta lactate measured at the 3$^{rd}$ minute of a single, sub-maximal and constant-intensity bout of cycling exercise in healthy adults; *iii)* the results are generalizable outside our sample (*i.e.* indicating a good cross validity) as indicated by the high adjusted $R^2$ from the regression model and by the narrow confidence intervals around the mean estimated critical intensity value; *iv)* the predictive power of our model (explained as the mean difference between the true (validated critical intensity) and the estimated critical intensity values), remains valid across a wide range of relative exercise intensities (*i.e.* from 55 to 95% peak power output) and *v)* the within-subject variability in the estimated critical intensity values, when testing the participant at different power outputs, showed a mean value of 7W (corresponding to roughly 3% in the present sample).

Finally, the data from this study, conducted on a homogeneous group of young to middle age men, indicates that critical intensity can be accurately and precisely predicted based on delta blood lactate measured at the 3$^{rd}$ minute of a single sub-maximal, non-exhaustive exercise trial performed at a constant power output over a wide range of relative exercise intensities (55-95% peak power output). Based on these observations, this new 3-min sub-maximal test offers an economical, practical and valid alternative to traditional determination of critical intensity.

**Study four**

The ability to reconcile different "thresholds" of exercise intensity to a similar underlying physiological and metabolic processes, reflecting a common level of aerobic metabolism (as demonstrated in study 1), allows us to investigate the body response to exercise above this "ceiling", beyond which there is a progressive loss of homeostasis (11). Additionally, the ability to identify this threshold with new technology and/or different and time effective approaches (as explored in study 2 and 3) in now more than ever can be conducted with ease.

During ramp incremental cycle exercise to exhaustion, there is a linear increase in $VO_2$ relative to the mechanical power output (PO), with a functional gain ($\Delta_{VO2}/\Delta_{PO}$) that varies between 8 and 12 $ml \cdot min^{-1} \cdot W^{-1}$ (3). A homogeneous linear relationship is often assumed across the whole exercise intensity spectrum (13). However, when the exercise exceeds a critical intensity (*i.e.* "threshold" phenomena), the rise in $VO_2$ as a function of work rate displays an increased slope that justifies the description of $VO_2$/PO relationship as a double-linear as opposed to a single-linear function (3). The development of this so-called "excess $VO_2$" in the heavy-intensity domain of incremental exercise entails a progressive loss of efficiency (3). The more pronounced the excess $VO_2$ is, the earlier $VO_{2peak}$ will be reached and task failure will ensue, in turn causing a reduction/impairment of exercise tolerance.

Based on our baseline (i.e., pre-training) results of a steeper and progressive increase in the electromyography ($\Delta_{RMS}/\Delta_{PO}$) relationship after the appearance of a threshold response (a similar response of that of the $VO_2$), might underlies a significant increase in the recruitment of motor units containing presumably less efficient type II fibers occurs at exercise intensities above approximately 75% of $VO_{2peak}$ that accompanies the rise in $VO_2$. This rapid and steeper increase in EMG observed as a function of power output during a ramp incremental exercise above the critical intensity would represent the point at which an increased contribution from fast twitch motor units occurs to maintain the required energy supply for muscle contraction. This phenomenon may occur as a result of a change in the

pattern of motor unit recruitment from predominantly slow twitch motor units to fast twitch motor units.

The present study tested the hypothesis that strength training, by increasing maximal force and affecting the recruitment of high-threshold motor units to sustain a given exercise intensity, would reduce the excess $VO_2$ observed during a ramp incremental exercise in young males.

The primary finding of this investigation was that a 5-week strength training program significantly improved the ability to produce force in young healthy individuals. In addition, in agreement with our hypothesis, these changes were associated with: *i)* a significant and large reduction in the magnitude of the excess $VO_2$ accompanied by a change in the $PO_{peak}$ by an average of 16±10 watt (5±3%); and *ii)* a temporal-associated large reduction in the intensity of muscle activation during a ramp incremental exercise. This is the first study indicating a role of strength training in the reduction of the excess $VO_2$ observed during a ramp incremental cycling exercise.

Five weeks of strength training resulted in an attenuation of both the excess $VO_2$ and the increase in the EMG signal as a function of the mechanical power output, observed during a ramp incremental exercise, suggesting that the mechanism(s) responsible for this training-induced enhancement in the mechanical efficiency of muscle contraction might also be those involved in strength gain.

From a health benefits perspective, the reduction in the oxygen excess associated with higher intensities of exercise is an important training adaptation that improves exercise tolerance and/or metabolic efficiency. A reduction in the magnitude of the excess $VO_2$ in response to endurance training has been previously reported. Several adaptive changes within the muscles after endurance training might contribute to the observed reduction in the excess $VO_2$.

Although this information is important, no previous study has investigated the effect of strength training in the reduction of the excess $VO_2$ during ramp incremental exercise in young healthy subjects

Data from this study suggest that the training-induced decrease in the magnitude of the non-linearity in the $\Delta_{VO2}/\Delta_{PO}$ relationship above the critical intensity can be

at least partly explained by the observed increase in the ability to produce force after strength training, which results in an improvement of muscle work efficiency and leads to an enhancement in metabolic stability during cycling incremental exercise.

**Study five**

The body response to exercise in the heavy intensity domain (*i.e.* above the threshold that divide a sustainable vs. an unsustainable intensity) that has been studied in study 4 during a ramp incremental exercise, can be investigated even during a constant work rate exercise.

The $VO_2$ slow component ($VO_{2SC}$) represents an increase in oxygen consumption ($VO_2$) during constant work rate exercise that projects above the expected $VO_2$ steady-state for a given workload (9). This $VO_{2SC}$ occurs when exercise is performed in the heavy (above the critical intensity). The magnitude of the slow component can account for ~25% of the total increase in $VO_2$ above the pre-exercise baseline, or even rise until $VO_{2peak}$ is reached and exercise tolerance is impaired.

Regardless of the mechanisms that are responsible for the development of the $VO_2$ slow component (apparently similar to those involved in the development of the "excess" $VO_2$ during a ramp incremental exercise (7)), several interventions have been shown that it can be attenuated or eliminated, and most of these interventions involved endurance exercise training (13).

Interestingly, despite the interest for performing exercise interventions that would help reduce the $VO_{2SC}$ and elucidate its mechanisms, the role of strength training interventions to the $VO_{2SC}$ has never been evaluated. This is surprising as strength training, with the aim to increase maximal force, should reduce the recruitment of high-threshold motor units to sustain mechanical power output during cycling exercise at a given exercise intensity (5, 12) (as demonstrated in study 4 during a ramp incremental exercise).

Therefore, the aim of this study was to evaluate the possible effect of strength training on the $VO_{2SC}$ in healthy young individuals. We tested the hypothesis that

a 5-week strength training intervention would attenuate the development of the $VO_{2SC}$ and increase exercise tolerance, likely due to a reduction in the recruitment of high-threshold, type II motor units during constant work rate cycling exercise in the heavy intensity domain.

The primary finding of this investigation was that a 5-week strength-training program that significantly improved the ability to produce in young healthy individuals, resulted in: *i)* a significant reduction in the magnitude of the $VO_2$ slow component (92±8% or 481±170 $ml \cdot min^{-1}$) that was accompanied by an increase in the time to exhaustion of 74±56% during the constant work rate exercise and *ii)* a reduction in the average force needed to sustain the same absolute exercise intensity after training.

This is the first study indicating a role of strength training in the reduction of the $VO_2$ slow component observed during a constant work rate cycling exercise. The pre-training results indicated a significant development of a $VO_2$ slow component as a function of time.

From a health benefits perspective, the reduction in the oxygen excess associated with higher intensities of exercise observed in the present study after strength training is an important training adaptation that significantly improved exercise tolerance. Such extended ability to sustain exercise might impacts compliance to aerobic activities thus improving the total amount of volume that can be performed at a given intensity of exercise and the total energy expenditure. From this perspective, strength training could significantly impacts the ability to protract aerobic exercise for an adequate amount of time and/or to extend the volume of exercise at a given exercise intensity, being a primary training component in health promotion and athletes development.

In conclusion, data from this study suggest that the training-induced decrease in the magnitude of the $VO_2$ slow component and the increase in exercise tolerance observed during cycling above the lactate threshold can be at least partly explained by the reported increase in the ability to produce force after strength training. However, the data presented have indicated no association between the development of the slow component and a shift in EMG signals during heavy cycling exercise. While the complexities of the electromyography measurement

may have influenced the ability to elucidate the involvement of a progressive recruitment of type II fibers in the development of the $VO_{2SC}$ further study are warranted in order to better elucidated the possible influence of strength training in affecting muscle fibers recruitment during exercise in the heavy intensity domain.

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# Appendix

## Pedal Force Measurement (PFM) analysis

The Matlab® script can be found following the sequent link:

*https://www.dropbox.com/sh/z9139p8cyp08iom/AAAm9yFhnGfCOR6n_5t-owSsa?dl=0*

## Electromyography (EMG) analysis

The Matlab® script can be found following the sequent link:

*https://www.dropbox.com/sh/z9139p8cyp08iom/AAAm9yFhnGfCOR6n_5t-owSsa?dl=0*