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The Influence of Baseline Metabolic Rate and Exercise Training on Cardio-Respiratory Dynamics

Coordinator

Chiar.mo Prof. Salvatore Squatrito

Supervisor

Chiar.mo Prof. Salvatore Squatrito
Dr. Enrico Tam

Candidate

Mariasole Da Boit

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"the night is always darkest just before the dawn"

-Anonymous-

"Discovery consists in seeing what everyone else has seen

and thinking what no-one else has thought"

- Albert Szent-Györgyi-

'I cannot teach anybody anything, I can only make them think.'

- Socrates-

Symbols and abbreviations

Δ	difference
Δ[HHb]/Δ$\dot{V}_{O_2}$	index of O_2 extraction per $\dot{V}_{O_2}$ increment
%Δ	% difference between GET and $\dot{V}_{O_{2max}}$
A	exponential response amplitude
ADP	adenosine diphosphate
ATP	adenosine triphosphate
CaO_2	arterial O_2 contents
CvO_2	venous O_2 contents
CK	creatine kinase
CO_2	carbon dioxide
CP	critical power (i.e., asymptote of the power/time hyperbola)
eNOS	endothelial nitric oxide synthase
ET	endurance training
GET	gas exchange threshold
H^+	hydrogen ion/proton
Hb	haemoglobin
HbO_2	oxygenated haemoglobin

Hb_{tot}	total haemoglobin
HHb	deoxygenated haemoglobin
HI	high intensity interval training
HR	heart rate
K^+	potassium ion
LBF	leg blood flow
L-NAME	N^G-nitro-L-arginine methyl ester
Mb	myoglobin
MRT	mean response time (approximated by τ + TD of an exponential)
N_2	nitrogen
NIRS	near-infrared spectroscopy
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NOS	nitric oxide synthase
O_2	oxygen
PCr	phosphocreatine (or creatine phsophate)
P_i	inorganic phosphate
PO_{2m}	muscle oxygen pressure
$\dot{Q}$	cardiac output

$\dot{Q}_{cap}$	capillary blood flow
$\dot{Q}_{m}$	muscle blood flow
RST	repeated sprint training
SV	stroke volume
τ	time constant (time to reach 63% of an exponential response)
TD	exponential response time delay
$\dot{V}_{CO_2}$	carbon dioxide output
$\dot{V}_{E}$	pulmonary ventilation (expired)
VeO_2	volumes of expired O_2
ViO_2	volumes of inspired O_2
$\dot{V}_{O_2}$ or $\dot{V}_{O_{2p}}$	pulmonary oxygen uptake
$\dot{V}_{O_{2m}}$	muscular oxygen uptake
$\dot{V}_{O_{2max}}$	maximum oxygen uptake
$\dot{V}_{O_{2peak}}$	peak oxygen consumption
W	watt
WR	work rate

ABSTARCT

Speeding the $\dot{V}_{O_2}$ kinetics results in a reduction of the O_2 deficit and thus a diminution in metabolic perturbation, improving the performance in athletes (Burnley and Jones 2007), and the functional capacity in the elderly, diseased and sedentary individuals (Schalcher et al. 2003, Grassi et al. 2006), by enhancing exercise tolerance.

Two main factors have been discussed as possible determinants of $\dot{V}_{O_2}$ kinetics: the oxygen delivery to exercising muscle (Hugson et al. 1982**,** Tschakovsky and Hughson 1999) and a 'metabolic inertia' at the muscle level (Grassi et al. 1996, Rossiter et al. 1999). However the underlying mechanism is still matter of debate.

Therefore, the main focus of these experiments was to investigate what influences $\dot{V}_{O_2}$ kinetics during moderate-intensity exercise which has the grater application for the population.

Purpose: The aim of these studies was to assess the variations in cardio-pulmonary parameters (study 1 and 2) and muscle oxygenation (study 2) during rest to work and/or work to work exercise transitions within the moderate domain.

In the study 1 we investigated $\dot{V}_{O_2}$ kinetics and cardiovascular system adaptations during step exercise transitions in different regions of the moderate domain.

In the study 2 we investigated $\dot{V}_{O_2}$ kinetics, cardiovascular system and muscle oxygenation adaptations during step exercise tests before, after and over a 4 week period of aerobic endurance training.

Study 1 methods: Seven active subjects (4 male; 26 ± 8 yr; 176 ± 5 cm; 69 ± 6 kg; $\dot{V}_{O2max}$ 44 ± 6 ml·kg^{-1}·min^{-1}) performed 4 types of cycling step transition in the moderate domain from rest (0-50W; 0-100W) or elevate baseline (25-75W; 25-125W) in a randomised order. GET and $\dot{V}_{O2max}$ were assessed before the step exercise tests. Breath-by-breath $\dot{V}_{O2}$ uptake, beat-by-beat $\dot{Q}$ were measured during the testing and the responses were fitted by using mono or bi-exponential models after an appropriate treatment.

Study 2 methods: 10 moderately active subjects (6 male; 25 ± 4 yr; 175 ± 9 cm; 71 ± 12 kg; $\dot{V}_{O2max}$ 41 ± 7 ml·kg^{-1}·min^{-1}) performed 3 cycling step transition tests (0 to 100 W), separated by 5 minutes of recovery, before, after and during 4 weeks of endurance training (ET). $\dot{V}_{O2max}$ and gas exchange threshold (GET) were assessed at the beginning and at the end of the ET which consisted in 40 minutes performed 3 times a week at an intensity of about 60% of $\dot{V}_{O2max}$. Breath-by-breath $\dot{V}_{O2}$ uptake, beat-by-beat $\dot{Q}$ and deoxyheamoglobin were measured during the testing and the values of each single experiment were normalised, aligned and ensemble-averaged. Responses were fitted by using mono or bi-exponential models.

Study 1 results: $\dot{V}_{O2}$ kinetics time constant (τ) and the functional gain ($\Delta\dot{V}_{O2}/\Delta$WR) were grater in transitions reaching the upper compared to the lower regions of the moderate domain. $\dot{Q}$ increased more abruptly when the exercise required started from rest however the Phase II $\dot{Q}$ time constant was faster (P <0.05) than $\dot{V}_{O2}$ τ for each exercise step.

Study 2 results: The time constant (τ) of the fundamental Phase of $\dot{V}_{O_2}$ kinetics became faster during ET (25%; before 28 ± 5 s, after 21 ± 4 s; *P* <0.05) and particularly after 1 training session (4%; τ = 26 ± 4; *P* <0.05). The weekly rate of decrease of τ was related to GET absolute variation (R = 0.84). $\dot{Q}$ kinetics changed after 4 training sessions nevertheless it was always faster than $\dot{V}_{O_2}$ τ. A gradually attenuation in Δ[HHb] /Δ$\dot{V}O_2$ was detectible over the period of training.

Conclusion: The results of these investigations suggest that muscle fibres recruitment exerts a strong influence on the pulmonary O_2 response within the moderate-intensity domain either during different forms of step transition exercise or in modulating the adaptations following a period of endurance training.

Chapter I : INTRODUCTION

I. $\dot{V}o_2$ KINETICS AND BIOENERGETIC

Skeletal muscle may be considered a biological engine able to carry out almost instantaneous changes of mechanical performance. To do this it needs to utilize energy sources which are essential to activate an energetic process in order to produce adenosine triphosphate (ATP) which is the only substrate that can supply the chemical energy necessary to drive contraction.

There are three energetic systems which meet this request immediately or at some later time to prevent a dramatic reduction in muscle ATP concentration, since ATP in human muscle is limited and is exhausted in few seconds after onset of exercise.

The energy supplied to sustain exercise of short duration and high intensity derives from the immediate energy system which includes intramuscular high energy phosphate: ATP stored and ATP rebuild by phosphocreatine (PCr) breakdown along with by-products of ATP (adenosine diphosphate and inorganic phosphate; ADP and P_i, respectively) in what is termed Lohmann reaction. The energy to supply ATP during intense exercise derives mainly from store muscle glycogen through anaerobic glycolysis which metabolises glucose with resulting lactate formation, from the reduction of pyruvic acid, and hydrogen ion (H+) release. Accumulation of these deleterious metabolic products and the limited substrates allow sustaining a work only for a short period of time because of the muscle fatigue process.

For every molecule of glucose there is a production of 2 ATP. That conveys the low gain in terms of energy production and shows clearly why this energetic system cannot sustain long duration exercise.

When an exercise lasts beyond several minutes it is necessary a more efficient mechanism which is able to use all the available substrate such as carbohydrate and fat. The aerobic metabolism provides most of the energy transfer for this condition producing 38 ATP molecules from a glucose molecule without accumulation of metabolic by-products. That makes the oxidative metabolism the principal means by which human organism generates energy.

As result either aerobic or anaerobic (even if in a really little amount) show the importance of O_2 delivery from the lungs to mitochondria across the blood stream (oxygen cascade).

II. O_2 UPTAKE

Several investigations have been conducted in humans to understand the adjustment of oxidative reaction. In fact the human body has an extraordinary capacity to increase its metabolic rate in response to energetic challenges, in order to sustain high request of ATP.

At onset of exercise the energetic requirements of the contracting muscles increase immediately in what has been termed a “square-wave” fashion but Krogh and Lindhard (1913) and Hill and Lupton (1923) had noticed that the onset of constant-load exercise the O_2 uptake lags behind the mechanical work and the release of

aerobic energy. Pulmonary and muscle $\dot{V}_{O_2}$ responses with an increase after a period of inertia. After this lag O_2 rises in a mono-exponential fashion profile until it meets the energy requirements and so attains a steady state from 2 to 12 minutes (depend on work intensity). Ideally, oxygen consumption should be measured at mitochondrial level in order to evaluate the real response but, unfortunately, this is not possible because of technical limitations.

Thus $\dot{V}_{O_2}$ uptake is commonly measure at mouth level and can be described using the Fick equation:

$$\dot{V}_{O_2} = \dot{Q} \times (CaO_2 - CvO_2)$$

Where $\dot{Q}$ represents tissue blood flow and CaO_2 and CvO_2 represent arterial and venous O_2 contents respectively. $\dot{Q}$ will depend upon cardiac output and the degree of vasodilatation in the tissue vascular beds as well as the pressure differential acting across the tissue. CaO_2 is related to haemoglobin concentration and the degree of saturation of haemoglobin with O_2. The difference between CaO_2 and CvO_2 represents the amount of oxygen extracted from the arterial blood by the tissue in order to support energetic processes in mitochondria. The $\dot{V}_{O_2}$ of every tissue therefore depends both upon “central” factors (i.e. delivery) and “peripheral” factors (i.e. extraction and utilization of O_2 in tissue).

III. O_2 DEFICIT:

As explained above at the onset of exercise an abrupt increase in ATP is required to perform the movement. This process it is provided principally by energy released through PCr and anaerobic glycolysis and a small contribution from $\dot{V}_{O_2}$ stores (myoglobin, venous blood), since the aerobic system responds slowly. This can be observed by the gradual increase in $\dot{V}_{O_2}$ uptake with a time constant (τ: time to reach the 63% of its final amplitude) of 20-45 s (healthy individuals) with a subsequent attainment of a steady state value in 3-4 minutes (for exercise below ventilatory threshold). This utilization of energetic sources different from aerobic system is called "O_2 deficit" and it is calculated as follow:

Deficit $O_2 = \Delta\dot{V}_{O_2} \times \tau$

Where $\Delta\dot{V}_{O_2}$ is in $L \cdot min^{-1}$ and τ in the fraction of a minute.

Consequences of a deficit is an intracellular perturbation due to a greater production of lactic acid as well as a depletion of high-energy phosphate and a reduction in muscle PH, which have been implicated in the muscle fatigue process. Thus clearly appears as the rate at which $\dot{V}_{O_2}$ uptake rises following the onset of exercise influences the magnitude of O_2 deficit and so the perturbation of homeostasis and the exercise tolerance. Consequently for healthy and/or fit individuals the faster is the $\dot{V}_{O_2}$ response (τ smaller) the smaller is the O_2 deficit, instead for unhealthy or unfit people the larger is τ and the higher is the O_2 deficit.

Chapter II: REVIEW OF THE LITTERATURE

I. OXYGEN UPTAKE COMPONENTS

Phase I or cardio-dynamic Phase

Whipp and Wasserman in 1972 were the first to identify this initial response characterized by an abrupt rise in $\dot{V}_{O_2}$ as a result of increased venous return via muscle pump and an increased right ventricular output elevating pulmonary blood flow without any appreciable changes in venous partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2) and respiratory exchange ratio (RER). First remarkable changes appear after a delay of 10-20s when the deoxygenated blood from active muscles arrives at the pulmonary sites for gas exchange. In the meanwhile the use of body gas stores allows a cellular O_2 up take to be satisfied without alteration in vinous O_2 concentration.

Phase II primary component (also called fast or fundamental)

It is characterized by a sharply increase in O_2 uptake as result of further rise in O_2 extraction from the blood perfusing the contracting muscles. The time for this deoxygenated blood to reach the pulmonary capillary bed determines a delay (TD) which is signalled at the lung by the modifications in the RER, an augmentation in end-tidal PCO_2 and a decrease in end-tidal PO_2.

Phase III steady state (SS)

This Phase is present only during moderate exercise when cardiac output ($\dot{Q}$) plateaus and venous O_2 content reaches a stable value. For exercise at higher intensities the attainment of a steady state might be delayed or absent.

Slow component

During exercise above gas exchange threshold (GET) an additional cost of oxygen is required and therefore there is a further increase in $\dot{V}_{O_2}$, following the fundamental component, with an exponential response profile to reach the required intensity.

This phase has been recognized 40 years ago (Åstrand and Saltin 1961) but a lot of experts still ignore it thinking that also above GET $\dot{V}_{O_2}$ rises reaching a SS at every power outputs. The slow component usually appears after 90-180s after the onset of exercise and its typical amplitude represents the 10-20% of the total $\dot{V}_{O_2}$ response even if its magnitude has been noticed to be less during treadmill running than other modes of exercise. In order to study this phenomenon it is common to set the intensity at 50% of the difference between GET and $\dot{V}_{O_{2max}}$ since it is at this intensity that the slow component appears. Hence the presence of a slow component may be at the centre of the fatigue process in both heavy and severe exercise (Burnley and Jones 2007): so the larger is the slow component the shorter is the exercise tolerance.

II. EXERCISE INTENSITY DOMAIN

It is important to standardize the intensity of exercise in order to be aware of which kind of physiological changes are involved.

Thus three important parameters have been used to identify four intensity domains:

- Gas exchange threshold: it is the point at which the blood lactate concentration starts to accumulate (generally at 45-60% of $\dot{V}_{O_{2max}}$) and it is also the highest level at which a $\dot{V}_{O_2}$ slow-component is not still present;

- Critical power (CP): is the maximal sustainable power output at the so called "maximal lactate steady state" (MLSS);

- Maximal oxygen consumption ($\dot{V}_{O_{2max}}$): maximal amount of oxygen which the organism can utilize to synthesize ATP aerobically.

Domains:

Moderate: all work rates under GET in which blood lactate is not elevated and the $\dot{V}_{O_2}$ levels off (Phase III). Within this domain the speed at which $\dot{V}_{O_2}$ uptake increase has been noticed to be relatively constant at every work rate but accelerated or slowed by some specific conditions such as training, ageing and diseases.

Heavy: all work rates between GET and CP. In this domain it is evident the presence of a slow component of the $\dot{V}_{O_2}$ kinetics which begins after about 2 minutes of exercise and $\dot{V}_{O_2}$ stabilizes after 10-20 minutes.

Severe: all work rates between CP and $\dot{V}_{O_{2max}}$. At this intensity blood lactate continues to increase and the $\dot{V}_{O_2}$ slow component doesn't level off and if the exercise is sustained for enough time $\dot{V}_{O_{2max}}$ can be reached. The rapidity with which $\dot{V}_{O_2}$ slow component rises is dependent on the proximity of the power output to CP. Therefore the duration of exercise in this domain depends on three parameters: $\dot{V}_{O_{2max}}$, slow component and anaerobic capacity.

Extreme: all work rates closed to $\dot{V}_{O_{2max}}$. A slow component is not evident and $\dot{V}_{O_2}$ may rise with an exponential profile that is truncated at $\dot{V}_{O_{2max}}$. Normally the time of duration is less than 120 seconds.

III. $\dot{V}_{O_2}$ KINETICS AND PERFORMANCE

The traditional parameters of endurance performance such as $\dot{V}_{O_{2max}}$, exercise economy, lactate threshold and critical power are strictly linked with $\dot{V}_{O_2}$ kinetics influencing its behaviour through the different intensity domains of exercise and comprehensively modifying the exercise tolerance and thus limit the endurance performance. This is evident especially for the slow component as it represents an additional energy cost: the lactate threshold determines the existence of a slow component and the rate of increasing is dependent on the proximity of the power output to the critical power. Moreover in the severe domain, above critical power, $\dot{V}_{O_2}$ uptake is connected to the $\dot{V}_{O_{2max}}$ which determine its rate of raising and in this way the time to exhaustion (Burnley et al. 2007, Jones et al. 2009).

IV. $\dot{V}_{O_2}$ KINETICS AND PATHOLOGICAL CONDITIONS

It is well known that pulmonary $\dot{V}_{O_2}$ kinetics are slower in patients affected by pathologies such as heart failure, peripheral vascular disease, chronic obstructive pulmonary disease, type II diabetes suggesting an impaired capacity by lungs, heart and blood vessels to delivery O_2 to muscle. On the other and it is well known that also skeletal muscles are metabolically compromised in these patients imply a further factor of slowdown.

In heart transplant recipient an acceleration of cardiac output kinetics, employing prior exercise, does not facilitate O_2 uptake (Grassi et al. 1997) leading to a consideration for peripheral limitation. To discern from between two types of limitations would be an important acknowledge for the clinicians to better address therapeutic interventions. This topic needs clarifications giving that pulmonary $\dot{V}_{O_2}$ kinetics is considered a stronger prognostic tool compared to $\dot{V}_{O_2\ max}$ (Grassi et al. 2006).

V. MODELLING

F.M. Henry (1951) was the first to introduce the concept that $\dot{V}_{O_2}$ uptake would increase in an exponential manner during a transition to constant-load work. Thus the O_2 consumption could be calculated with the follow first-order equation:

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2}(b) + A(1 - e^{-(t-TD)/\tau}) \qquad \text{[equ 1]}$$

where $\dot{V}_{O_2}$ (t) is O_2 uptake at any point in time, $\dot{V}_{O_2}$ (b) is the baseline, A is the steady state (SS) amplitude of the $\dot{V}_{O_2}$ response and $(1 - e^{-(t-TD/\tau)})$ is the exponential function in which t is time, TD is the time delay before the start and τ (time taken to achieve 63% of $\Delta\dot{V}_{O_2}$) is the time constant and it describes the rate at which $\dot{V}_{O_2}$ is rising towards the SS. Thus when 2 time constants have elapsed $\dot{V}_{O_2}$ reaches the 86%, after 3 approximately 95% and after 4 more than 98%.

This system would be characterised by predictable oxidative response behaviour: an unchanged velocity constant throughout the whole transition. But subsequently the same researcher tested O_2 consumption at different level of intensity and published findings that challenged the previous notion, thus suggesting that an equation with two exponential components was necessary to describe the rise of O_2 uptake during exercise (Henry and DeMoor 1956). Nevertheless this two exponential components process was applied excluding the lag which lies between muscle and lung (Krogh and Lindhard 1913).

Whipp et al. (1982) carried out a remarkable study revolutionising the research about O_2 uptake. They undertaken a rigorous analysis of pulmonary gas exchange kinetics in the moderate domain during work to work cycle exercise and introduced 2 important concepts:

- repeated-trial protocol which allow to average the transitions in order to assess the physiological response;
- the identification and elimination of the Phase I since it does not reflect the muscle O_2 uptake.

However the utilisation of a monexponential function through the collected data has been reintroduced by Linnarsson (1974) to characterize a combination of cardiodynamic

Phase and the fundamental Phase in order to assess the comprehensive increasing in cellular respiration. It is called the mean response time (MRT) and it is calculated summing time constant and time delay (τ+ TD).

PHASE I

Fitting $\dot{V}_{O_2}$ pulmonary data starting from the beginning of exercise will result in a distorted fit (Whipp et al. 1982) which doesn't represent the real O_2 uptake.

In order to avoid this problem the first method utilized was to detect the start of the inflection point which denotes the end of the Phase I. This point is not easy to trace, due to "noise" in the pulmonary signal, so other methods have been applied by researchers. Some of them have chosen to model also the cardio-dynamic Phase using a bi-exponential model others attempt to not consider it eliminating the first 20 s after exercise onset: the mean transit time from muscle capillary to lung is ~17 s before exercise and ~10-12 s after 10 s of exercise (Krustrup et al. 2009).

PHASE II

An accurate description of this phase is necessary as it reflects the muscle O_2 consumption (Grassi et al. 1996, Bangsbo et al. 2000).

The changes in oxygen consumption involved in this Phase can be well fitted by the exponential function (equ. 1). Values of Phase II $\dot{V}_{O_2}$ τ around 20 s in young healthy

subjects represent a “fast” $\dot{V}_{O_2}$ response kinetic while values of τ around 50 s mean a “slow” response.

SLOW COMPONENT

When a slow component is present it is necessary to add another exponential term to have a good fitting as follow:

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2}(b) + Ap\,(1 - e^{-(tp-TDp)/\tau p}) + As(1 - e^{-(ts-TDs/\tau s)}) \qquad \text{[Equ. 2]}$$

where $\dot{V}_{O_2}$ (t) is O_2 uptake at any point in time, $\dot{V}_{O_2}$ (b) is the baseline and A, t, TD and τ are respectively the amplitude, the time, the time delay and the time constant of the $\dot{V}_{O_2}$ (“p” stands for primary component and “s” slow component).

VI. MEASUREMENT OF BREATH BY BREATH $\dot{V}_{O_2}$

During the past half century the invention and production of new gas analysers and economical flow meters have changed the data collection technique from periodic collection of Douglas bags to breath-by-breath gas exchange. Moreover with the development of gas exchange algorithms changes in lung gas stores between two breathes can be taken into account, even if it generates some problems relates to the high variability between breaths and to a low signal-noise ratio.

First algorithms:

- expiratory flow method as used for calculation of $\dot{V}_{O2E}$ requires measurements of expiratory flow and expired gas fractions and assumes nitrogen (N_2) balance at the mouth but this is invalid for single respiratory cycles;

- Calculation of the difference between actually measured volumes of inspired and expired O_2 (ViO_2-VeO_2) but since Vi-Ve is a function of N_2 exchange therefore also this algorithm is affected by breath by breath changes.

The problem is still the alveolar O_2 stores.

Therefore Auchincloss at al. (1966) proposed an algorithm for O_2 consumption at alveolar level correcting O_2 uptake at mouth level for breath by breath changes in alveolar O_2 stores. Moreover Di Prampero and Lafortuna (1989) showed that the alveolar $\dot{V}_{O2}$ kinetic was influenced by the end respiratory gas volume before the beginning of the successive breath (Vai-1).

In order to resolve this problem Grønlund (1984) propose an algorithm in which the traditional concept of respiratory cycle is changed: it is defined as the interval elapsing between two equal expiratory gas fractions in two successive breaths without any calculations about Vai-1. Recently, Cautero et al. (2002) found that the Grønlund algorithm could assess $\dot{V}_{O2}$ a kinetics with a better precision than Auchincloss's one.

VII. OXYDATIVE CONTROL SYSTEM ANLAYSIS

oxidative control by convective/diffusive O_2 conductance at limb level

It is well know that, after an augmentation in work load, variables related to O_2 delivery (e.g. heart rate, cardiac output, muscle blood flow) adjust to a new requirements in a slower manner compared to metabolic demand. This could represent a partial determinant of the slow rate of increase in oxidative phosphorylation at exercise onset. Consequently for ages the main question has been whether the pulmonary O_2 consumption might have reflected the oxygen muscle uptake, as was proposed by Whipp and colleagues (1982), and to understand whether the adjustment of O_2 delivery might have limited O_2 utilization in working muscle.

Thus adequacy of O_2 delivery has been assessed by a comparison of the temporal profiles of blood flow muscle ($\dot{Q}_m$) and $\dot{V}_{O_2}$ muscle ($\dot{V}_{O_{2m}}$).

Two methods are usually employed to measure the muscle blood flow: thermodilution method and Doppler ultrasound. The former is based on the premise that when an indicator substance is added to circulating blood, the rate of blood flow is inversely proportional to the change in concentration of the indicator over time. The indicator is a fluid with a different temperature than blood (thermodilution method).

The latter is a non-invasive instrument able to assess, employing the Doppler Effect, whether structures are moving towards or away from the probe, and its relative velocity. By calculating the frequency shift of a particular sample volume, for example flow in an artery or a jet of blood flow over a heart valve, its speed and direction can

be determined. Thus it is possible to measure femoral artery blood flow (LBF) kinetics and in particular mean blood velocity and femoral artery diameter.

The first study, in which muscle blood flow and $\dot{V}_{O_{2m}}$ kinetics could be closely inferred in exercising humans using the thermodilution technique, was conducted by Grassi in 1996 in which he concluded that during the on-transition the kinetics of $\dot{Q}_m$ and $\dot{V}_{O_{2m}}$ were similar, indicating that bulk delivery of O_2 to the working muscles is not limiting leg kinetics. Moreover he demonstrated that the $\dot{V}_{O_{2m}}$ and pulmonary $\dot{V}_{O_2}$ ($\dot{V}_{O_{2p}}$) kinetics have the same response: a delay Phase (I) followed by a mono-exponential increase to steady state, during moderate domain. For this Phase II $\dot{V}_{O_{2m}}$ reflects $\dot{V}_{O_{2p}}$ within ± 10% at the onset of exercise since the absolute $\dot{V}_{O_2}$ measured at the lung will always be higher than that measured at the working muscle level due to an additional cost of cardiac and ventilatory work, the posture, etc. (Barstow et al. 1990).

Two years later Grassi (1998) carried out another similar study utilizing a dog gastrocnemius preparation to enhance O_2 delivery to the muscle to see whether this produces faster muscle $\dot{V}_{O_2}$ kinetics during rest to submaximal contraction (60-70% $\dot{V}_{O_{2peak}}$). He applied two conditions: spontaneous adjustment of blood flow and a pump-perfused muscle to eliminate every adjustment of O_2 delivery. Also in this case not significant differences were found provided further evidences that O_2 delivery to muscle is not a limiting factor on $\dot{V}_{O_{2m}}$ kinetics.

These findings were confirmed in subsequent studies when it was shown that Phase II pulmonary O_2 measurement also closely reflects the kinetics of muscle O_2 uptake also during high-intensity exercise (Bangsbo et al. 2000)

Taking together these results imply that pulmonary O_2 uptake reflects muscle O_2 uptake, confirming the hypothesis of Whipp et al. (1982) and that $\dot{V}_{O_2}$ delivery not influence O_2 muscle uptake.

The latter assumption might not be true at intensity above GET and for this reason Grassi et al. (2000) performed another similar research during transitions from rest to 100% of $\dot{V}_{O_{2peak}}$ and found that a slightly faster $\dot{V}_{O_{2m}}$ kinetics; perfectly in agreement with other evidences obtained in humans (Gerbino et al. 1996).

Thus GET might represent the point above which O_2 delivery could contribute to limit $\dot{V}_{O_{2m}}$.

oxidative control by convective/diffusive O_2 conductance at muscle level

However it is presently unknown whether the limb (conduit artery) blood flow following exercise onset are representative of blood flow microcirculation where gas exchange occurs.

Recently, an important technique has been invented which allows to measure oxidative muscle metabolism in humans: Near-infrared spetroscopy (NIRS).

This non-invasive optical method allows to monitor the oxygenation of tissue by means of the infra-red light which is absorbed differently based on the amount of haemoglobin saturated with O_2: oxyhaemoglobin ($\Delta[O_2Hb]$) and deoxyhaemoglobin concentration ($\Delta[HHb]$). The latter has been used to estimate fractional O_2 extraction within the microcirculation, thereby assessing the balance between O_2 delivery and O_2 utilization. The signal is derived mainly from the small blood vessels, especially from

capillaries (Poole et al. 2005), since the bigger vessels contain a large molar of blood which absorbs all the light (Pereira et al. 2007). This innovative device permitted a new series of interesting researches focused into the kinetics of oxidative metabolism during exercise transitions.

Harper et al. (2006) estimated capillary blood flow ($\dot{Q}_{cap}$) by rearranging the Fick equation, using the primary component of $\dot{V}_{O2p}$ to present $\dot{V}_{O2m}$ and deoxyhaemoglobin measured by NIRS as surrogate for (a-v) O_2. They found that MRT of $\dot{Q}_m$ were faster than those of $\dot{V}_{O2}$ for moderate exercise but in contrast the kinetics of $\dot{Q}_{cap}$ were significantly slower than those of $\dot{V}_{O2m}$ and $\dot{Q}_m$. This suggests that it may be inappropriate to use $\dot{Q}_{cap}$ to represent the kinetics of oxygen delivery to the microcirculation in assessing the dynamic adequacy of $\dot{Q}_m$ to $\dot{V}_{O2m}$ matching.

However Grassi et al. (2003), using NIRS, reported that deoxyhaemoglobin is relatively constant at the onset of exercise (6-10s of no change), followed by a rapid increase to a constant plateau, as already showed by De Lorey et al. (2003). Moreover a significant correlation was described between the mean response time of the primary component of Δ[HHb] on-kinetics and the time constant of the pulmonary Phase II.

The constant muscle oxygenation during the initial phase of the on-transition indicates a tight coupling between increases in O_2 delivery and O_2 utilization.

To work out what really happens inside cells, the site of gas exchanging, a relatively recent technology, Phosphorescence quenching technique, has been used. It allows to measure muscle oxygen pressure (PO_{2m}) determined by the relationship between $\dot{Q}_m$ and $\dot{V}_{O2m}$.

Basing on this technique Walsh et al. (2005) measured the intracellular oxygen pressure (PO_2) in isolated myocytes at onset of constant work-rate. They found that despite an adequate concentration of O_2 a time delay of 6-12 sec exist s before PO_2 falls in a linear manner with increasing stimulation frequency. Also modifying the extracellular PO_2 from 20 to 60 torr does not affect the time delay but, at the same work-rate, the steady state gradient was smaller and τ was faster at 20 torr. A reason for this may be that some "critical PO_2" is being reached as PO_2 approaches 0 torr inducing a fall in PO_2.

This delay argues against the hypothesis of the oxygen plays a deterministic role in setting the initial metabolic response and it is in agreement with the results previously reported by Kinding et al. (2003)

Even analyzing type I and II muscle fibers, in order to determine whether fiber type affect the O_2 exchange at the microcirculatory level after contraction, both fibers exhibited a delay followed by a mono-exponential decrease in PO_{2m} to steady state but oxidative fibers demonstrated a blunted PO_{2m} response. This behaviour might be due to an advantageous transfer from blood to tissue in order to preserve a higher capillary O_2 pressure: a more precise matching of $\dot{Q}_m$ to $\dot{V}_{O2m}$ dictates the microvascular O_2 pressure and so intracellular PO_2 attenuating intracellular perturbation (Behnke et al. 2003).

Considering these findings together they suggest the following mechanism: at the beginning of muscle contractions Δ[HHb] τ is stable because at the level of capillaries blood flow increases enough to match $\dot{V}_{O2m}$ uptake, which instead increases without time delay, and this maintains PO_{2m} constant up to 20 sec.. But after few seconds blood flow plateaus, consequently O_2 extraction augments to sustain $\dot{V}_{O2m}$. Then the

blood flow response matches again $\dot{V}_{O_{2m}}$ and Δ[HHb] becomes constant (Jones and Poole 2005).

metabolic inertia

There are several possible factors that might contribute to 'metabolic inertia' hypothesis in modulating $\dot{V}_{O_2}$ kinetics.

Phosphocreatine (PCr)

Bessman in 1960 proposed the “creatine phosphate shuttle” theory in which creatine kinase (CK) catalyzes the reversible Lohmann reaction rephosphoryilate ADP, using PCr, and it provides a temporary buffer for ATP. Therefore it would “buffering” also the increment in ADP concentration, which is well known to be a powerful stimulator of mitochondrial respiration. Thus it emerges that higher level of PCr would slow the activation of oxidative phosphorylation by delaying key energetic controlling signal(s) between sites of ATP hydrolysis and mitochondria (Meyer et al. 1984).

Piiper, Di Prampero and Margaria were the very first to describe a linear relationship between PCr and $\dot{V}_{O_{2m}}$ in steady state condition, suggesting some link between these two variables. In particular Margaria hypothesized a regulatory role of PCr hydrolysis on $\dot{V}_{O_2}$ kinetics (Margaria et al. 1965).

Since studies by Mahler (1985) have shown a direct proportionality between the kinetics of PCr fall and $\dot{V}_{O_{2m}}$ suggesting that use of PCr as a proxy variable for the kinetics of $\dot{V}_{O_{2m}}$ is justified, at least at moderate work rate.

Recently Rossiter et al. (1999) were the first to compare the kinetic of phosphocreatine with the rise in $\dot{V}_{O_{2p}}$, using a whole body ^{31}P MRS technique, simultaneously during moderate exercise. The result was an indistinguishable temporal profile of the fall in PCr and rise in $\dot{V}_{O_{2p}}$ (i.e. to within 10%) as previously proposed by Barstow et al. (1990), based on their computer modelling. Same results have been elucidated during heavy-intensity exercise (Poole et al. 2007).

Considering these findings and since $\dot{V}_{O_{2m}}$ τ is not different from phase II $\dot{V}_{O_{2p}}$ τ, it seems reasonable to assume that $\dot{V}_{O_{2p}}$ response provide a good estimate for intramuscular PCr fall during exercise.

Further evidence in favour of this concept has been reported by Kinding et al. (2005). After an inhibition of creatine kinase (CK) the time course of the change in ATP/ADP+P_i was more rapid: lets to a faster PO_2 kinetics. Interestingly the abolition of a time delay demonstrates that, in the absence of a PCr buffering, O_2 consumption is stimulated immediately at the onset of contraction suggesting changes in phosphorylation.

Dietary supplementation with Cr has been shown to modify intramuscular levels of PCr+Cr and enhance performance during high-intensity exercise. Based on this knowledge a study was carried out to investigate the physiological role of PCr and Cr in oxidative metabolism after Cr supplementation. They found that the influence of PCr is more potent than that of Cr, acting not only as a buffer but also as modulator of ATP production: PCr decreases the sensitivity of mitochondrial respiration to ADP, the opposite effect of Cr; a finding fully compatible with the creatine shuttle theory.

Thus an increase in Cr concentration after supplementation would lead to a reduced PCr/ Cr ratio, enhancing the sensitivity of mitochondrial respiration to ADP (Walsh et al. 2001).

Summarizing these data suggest that the CK reaction influences changes in ADP, resulting in one of the possible principal signals which accelerate the oxidative phosphorylation.

Pyruvate dehydrogenase (PDH)

It is the first component enzyme of pyruvate dehydrogenase complex (PDC) which contributes to catalyze the decarboxylation of pyruvate into acetyl-CoA by a process called pyruvate decarboxylation, linking glycolitic and oxidative metabolism. Pharmacological activation of pyruvate dehydrogenase with dichloroacetate (DCA) reduces substrate-level phosphorylation suggesting and enhancement of the contribution of oxidative phosphorylation to energy turnover.

Grassi et al. (2002) tested the hypothesis in isolated dog gastrocnemius during transition from rest to 60-70% of $\dot{V}_{O2peak}$. DCA infusion resulted in a significant activation of PDH but it did not significantly effect "anaerobic" energy provision and $\dot{V}_{O2p}$ kinetics. Similar conclusions were drawn by another study in which it is confirmed that either $\dot{V}_{O2p}$ or PCr kinetics were not faster after DCA administration (Rossiter et al. 2003). These authors observed only an amelioration of O_2 deficit for the same work load due to a lower $\dot{V}_{O2}$ and PCr amplitude and less blood lactate accumulation.

Thus the low energy of contractions and the lower amplitudes could explain in part a spare of PCr without influences on $\dot{V}_{O_2}$ kinetics (Grassi et al. 2006).

Nitric oxide (NO)

Nitric oxide, known as the "endothelium-derived relaxing factor", is biosynthesized endogenously from L-arginine, oxygen and NADPH by various nitric oxide synthase (NOS) enzymes. There are three isoforms of the NOS enzyme: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS) (Stamler and Meissner 2001).

Nitric Oxide is of critical importance as a mediator of vasodilation and therefore a controller of blood flow. It is induced by several factors, and once synthesized by eNOS, it results in phosphorylation of several proteins that cause smooth muscle relaxation. These vasodilatory actions play a key role also in renal control of extracellular fluid homeostasis, essential for the regulation of blood pressure.

Furthermore NO has the potential to inhibit several mitochondrial enzymes and to compete with O_2 the binding site at cytochrome c oxidase. With these two effects of vasodilatation and $\dot{V}_{O_2}$ inhibition NO may serve as part of a mechanism necessary to reduce O_2 extraction and increase O_2 delivery to meet the increase in $\dot{V}_{O_{2m}}$, maintaining higher intramyocyte PO_2 levels during exercise.

Pharmacological inhibition of the NOS enzymes via L-NAME administration, which would alleviate the inhibitory effect of NO on mitochondrial respiration (Brown and Cooper 1994) has been used to assess $\dot{V}_{O_2}$ uptake kinetics. Faster Phase II O_2 kinetics

after L-NAME has been reported during moderate intensity exercise (Jones *et al*., 2003) and supra-maximal (Wilkerson *et al*. 2004) exercise in humans.

Controversially Grassi et al. (2005b) was not found in dog gastrocnemius preparation. A possible explanation is that in this study they kept $\dot{Q}_m$ constantly elevated during exercise avoiding any possibility of a vasoconstriction, and thus a restriction in O_2 delivery, dues to a NO inhibition.

As for the L-NAME administration, the influence of increasing the NOS substrate, by the means of L-arginine, on O_2 kinetics is unclear. Koppo *et al*. (2009) reported a small (< 2 s) but significant, speeding in Phase II O_2 kinetics during moderate exercise with L-arginine, a finding that clashes with the speeding in Phase II O_2 kinetics observed following L-NAME administration aforementioned.

Summary

There are some conditions, such as hypoxia or B-blockade (O_2 supply restricted), supine exercise, exercise above GET and arm exercise above heart level (reduced muscle perfusion pressure), older age or some diseases, in which $\dot{V}_{O_2}$ availability might be considered to be among the factors responsible for a slower $\dot{V}_{O_2}$ kinetic.

On the other hand, as it has been previously described, in healthy people performing normal forms of exercise there are evidences against the concept that bulk O_2 delivery limits $\dot{V}_{O_2}$ kinetics. Thus modifications at muscle level seem to be more likely in influencing the oxygen uptake responses.

VIII. WORK TO WORK STEP TRANISTIONS

It has always been matter of discussion whether the time constant of Phase II of O_2 kinetics evidences a linear-order behaviour throughout the continuum of exercise.

The notion of dynamic linearity, which obeys at the low of superposition (Fujihara et al. 1973), that is the output can be predicted from the input. In terms of $\dot{V}_{O_2}$ kinetics the time constant and gain of the primary component should be the same at every work rate (moderate, high or severe).

work-work below GET

Firstly Di Prampero et al. (1970) found that work -work transitions were determined by a faster response kinetics (t ½ 17s) compared to full transitions (unloaded-work), suggesting that the influence of O_2 stores, which may play a role in the latter condition, were absent during transitions from an elevate baseline. Other researchers have conducted studies comparing full transitions versus work to work transitions in the moderate domain but they have reported contrasting results. A faster kinetic was also pointed out by Davies et al. (1972) comparing full transitions and work to work transition from moderate to heavy domain during exercise on treadmill. Conversely no differences were found using a similar protocol on a cycloergometer by Diamand et al. (1997) and in another study (Linnarson 1974).

In 1982 Whipp et al. carried out a study in order to assess the precise non steady-state parameters of ventilatory gas exchange, in which 6 subjects undertook 8 repetitions of 100w constant load cycling in the moderate domain either starting from rest or from

unloaded cycling. They found that for the Phase I the response was much more abrupt during rest to work compare to unloaded condition, but it does not change in its duration or in the constant of the subsequent Phase II.

Hugson at al. (1982) attempted, with a remarkable study, to clarify the previous findings analyzing a wild range of full and work to work transitions in the moderate domain. They found slower kinetics of $\dot{V}_{O_2}$ during work-to-work transitions than during rest-work, especially in the upper compared to lower region of moderate domain, along with slower HR kinetics, thus suggesting a lack in O_2 delivery.

Considering all these studies together it is evident that there is not a unified concept whether O_2 uptake kinetics are delayed, advanced or remain unchanged during transitions below GET, therefore this situation needed some clarifications.

In order to do that R. Hugh Morton (1987) carried out a critical examination of the previous researches and suggested a series of methodological considerations which can be summarized as follow:

- Wrong chosen experimental design in terms of limited work range and/or not clear level of intensity (above or below GET) or different type of exercise (Di Prampero et al. 1970, Davies et al. 1972);

- Incorrect statistical inferences and/or mathematically incomplete modelling procedures (imposition of a TD: Diamand et al. 1997, Hugson at al. 1982; a single-exponential model constrained to start at the onset of exercise: Di Prampero et al. 1970).

The invention of new and more reliable technology, such as breath-by-breath method, along with Morton's insightful review permitted a new approach on work-to-work investigations to the purpose of clear up the confusion.

The first remarkable study was conducted by Brittain et al. (2001) with the intent to clarify the issue with respect to moderate domain dynamic linearity. They created a protocol composed of either full or two half steps to and from 90% GET, with multiple like-transitions, to improve the confidence in parameter estimation. Using a breath-by-breath analysis and non-linear least squares method utilised by Hugson and Morrisey (1982) they found a slower τ on $\dot{V}_{O_2}$ kinetics in the upper compared to the lower region of moderate domain. Moreover the G response was different for all the three transitions. This study confirm that oxygen uptake kinetics do not follow a linear behaviour even below GET and Brittain and colleagues suggested that the reason might be attributed to a progressively involvement of muscle fibres, following the hierarchical manner recruitment (Henneman et al. 1965), as there are more than the three well known type (type I, type IIX and type IIA) which have gradual differences in bioenergetic proprieties (Schiaffino and Reggiani 2011).

In the light of two main conflicting theories, slower O_2 supply vs fibres recruitment, MacPhee et al. (2005) addressed the issue utilizing NIRS, to monitor muscle oxygenation, pulmonary $\dot{V}_{O_2}$, leg blood flow (LBF) and heart rate (HR) to understand whether O_2 availability was responsible for slowing $\dot{V}_{O_2}$ kinetics. They observed that during two-legged knee-extension transition from 3W work to 90% GET a slower $\dot{V}_{O_2}$ kinetics in the upper compared to lower regions (as it shown by Brittain et al. 2001) along with slower HR, LBF and Δ[HHb] supporting the theory of Hughson and Morrissey (1982): a slower adaptation of O_2 delivery. However it has to be noticed that a sluggish in HR or LBF responses does not necessarily means a lack of O_2 availability at muscle fibres level since it has been demonstrated that enhancing O_2 delivery to the muscle during rest to submaximal contraction (60-70% vo2 peak) does

not produce faster muscle $\dot{V}_{O_2}$ kinetics. Moreover the kinetics of capillary blood flow is significantly slower than the femoral artery blood flow during knee-extension exercise (Harper et al. 2006) and a lack of a fall in PO_2 at onset of exercise suggests that muscle $\dot{Q}$ O_2 is not limiting $\dot{V}_{O_{2m}}$ kinetics (Welsh et al. 2005). The different MacPhee's findings might be related to the modality of exercise involved even if in another research (Koga et al. 2005) showed a LBF severalfold faster than pulmonary $\dot{V}_{O_2}$ kinetics during moderate knee-extension exercise.

Recently Spencer et al. (2011) assessed work to work exercise transition utilizing the same protocol of MacPhee but utilizing a cycle ergometer. He had the same results but the HR was unchanged for all conditions accompanied by a constant O_2 deficit suggesting, also in this case, a difference in muscle fibres recruitment.

Contrasting results have been reported by Bowen et al. (2011). To assess oxygen uptake after a prior exercise he included either full or half step transitions but two types of full transitions (from 20W to 90% GET) one with two like-step separated by 12 minutes of rest and another one separated by only 30 seconds to maintain an elevated metabolic rate. Surprising $\dot{V}_{O_2}$ kinetics was slower not only after an half step, where either work rate or metabolic rate were raised, but also when initiated from an increase metabolic rate alone. This result along with a constant presence of a high muscle oxygenation rules out either the hypothesis of a limitation in O_2 delivery or a work rate effect, and thus an involvement of different muscle fibres, instead lets to consider an influence of muscle energetic state.

In conclusion the majority of the studies report that the time constant for the Phase II of $\dot{V}_{O_2}$ kinetics does not follow a system linear behaviour showing a trend which is slower at higher work rate but the reason is still unrevealed.

work- work above GET

An equal panorama is present for work transition above GET: some studies show no variations between the time constant of $\dot{V}_{O_2}$ kinetics for heavy/severe compared to moderate exercise (Barstow and Mole 1991, Ozeyner et al. 2001) but the majority show a slower kinetics as it has been pointed out by Jones and Poole (2005) who analysed a pool of data from 25 investigations. It is clear that a linear first order system is lacking in the primary component $\dot{V}_{O_2}$ kinetics when exercise is performed above the gas exchange threshold. A slower Phase II at higher work rates has always been considered as a limitation in O_2 availability, since at 70% of $\dot{V}_{O_{2max}}$ cardiac output reaches its maximal value. But recently it has been proposed that the reason of a slower Phase II and a fall in the primary component gain may be related to an increase in fast muscle fibres recruitment (hierarchy theory) (Poole et al. 2008).

It has been demonstrated that muscle fibres react differently when recruited: the slow fiber has a blunted PO_{2m} dynamics that probably reflect an augmented ability to match O_2 delivery to the metabolic demand ($\dot{V}_{O_2}$) of the muscle compared to fast fibers. Moreover they demonstrate a different reaction moving from a lower to a higher intensity: fiber I increases $\dot{V}_{O_2}$ enhancing $\dot{Q}_m$ whereas fiber II increases O_2 extraction. Thus when an exercise involves more fast than slow fibers, the latter may be O_2 limited slowing the $\dot{V}_{O_2}$ kinetics. (Behnke et al. 2003).

Millet et al.(2009) reported that $\dot{V}_{O_{2p}}$ responses in different way during running and cycling in the same subjects reflecting fundamental differences in muscle contraction and so different use of fibres. Alteration in motor unit recruitment may sustain the reduced $\dot{V}_{O_2}$ slow component observed for the same work rate following intervention

such as endurance training, priming exercise and glycogen depletion of the type I fibre population (Poole et al. 2008). This is in agreement with the metabolic response that would be expected when a population of high-order fibres is recruited to reach the augmented muscle force-production.

However it should be taken into consideration that these two effects, oxygen delivery limitation and muscle fibres recruitment, might both occur and are not reciprocally exclusive (Behnke et al. 2003).

IX. ENDURANCE TRAINING

It is well recognized that endurance training (ET) elicits both central and peripheral adaptations. It alters cardiovascular parameters, neuronal recruitment patterns, and causes profound changes in muscle bioenergetics such as an increasing in muscle glycogen stores, and glycogen sparing at sub maximal workloads via increased fat oxidation, enhanced lactate kinetics and provides morphological alterations such as mitochondrial biogenesis, fast-to-slow fibre-type transformation and capillarization (Coffey and Hawley 2007).

The aforementioned modifications enhance the classical parameters of aerobic performance such as $\dot{V}_{O_2\,max}$, lactate threshold, critical power and exercise economy.

An increase in lactate threshold leads to an extended range of power outputs therefore higher workloads can be sustain without developing a slow component.

Additionally ET shifts the critical power allowing higher power output to be sustained without further accumulation of lactate, as well as an increase in $\dot{V}_{O_{2max}}$ gives more

"space" for the slow component and exercise economy is improved permitting less O_2 consumption. All these traditional parameters are strictly correlated with O_2 uptake influencing its behaviour through the different intensity domains of exercise, leading to a comprehensively amelioration of exercise tolerance and endurance performance (Burnley and Jones 2007).

ET has important effects in a reduction of the fundamental component of oxygen consumption but effects on the time constant are even more significant. Studies have shown that Phase II $\dot{V}_{O_2}$ τ can be reduced up to 50% after only 4-6 weeks of endurance training (Fukuoka et al. 2002, Berger et al. 2006) with profound enhancement of exercise tolerance.

A study conducted by Phillips et al. (1995) tested the progressive effect of endurance training on $\dot{V}_{O_2}$ consumption. They were the very first to assess the kinetics of O_2 uptake at various times (pre-training, after 4, 9, 30 days and post-training) during step test from 25W to 60% of $\dot{V}_{O_{2peak}}$ for a period of an endurance training program of 2 h/day at 60% of $\dot{V}_{O_{2peak}}$ for 30 days. The novel finding of this study was that a modification in $\dot{V}_{O_2}$ kinetics, increased as early as 4 days after a training program was commenced but a further acceleration of the $\dot{V}_{O_2}$ response was seen only at the end of 30 days of training. Conversely muscle citrate synthase activity was measured after 5 days showing no significant increase in its activity; however, a further 26 days of training resulted in a 50% increase.

A protocol comparing two types of endurance training, moderate intensity continuous training (LO) with high intensity interval training (HI), were carried out to determine the influences on $\dot{V}_{O_2}$ uptake during moderate and heavy step tests. Both kind of training were 6 weeks long and matched for total work done. The LO group exercised

for 30 min per session at a work rate requiring 60% $\dot{V}_{O_{2peak}}$, whereas the HI group were prescribed 20 x 1-min exercise bouts at 90% $\dot{V}_{O_{2peak}}$ separated by 1-min rest periods. The result was that either LO or HI would enhance $\dot{V}_{O_2}$ on-kinetics at both intensities suggesting that the nature and intensity of training are less important that the total volume performed (Berger et al. 2006). Another research was conducted to test the influences of 8 sessions of high-intensity interval training (8–12 x 1-min intervals at 120% $\dot{V}_{O_{2max}}$ separated by 1 min of rest) and low-intensity continuous endurance training (90–120 min at 65% $\dot{V}_{O_{2max}}$) on $\dot{V}_{O_2}$ uptake. Subjects completed step transitions to a moderate-intensity work rate (90% GET) before training and after every 2 training days. This study demonstrated that $\dot{V}_{O_2}$ kinetics was faster during moderate-intensity exercise after only 2 days of training, regardless of the type of exercise training program and the total exercise training volume (high intensity = 1,800 kJ; low intensity = 8,500 kJ). Interestingly the time course of [HHb] was not changed with training, despite a speeding of $\dot{V}_{O_2}$ kinetics, suggesting that microvascular O_2 delivery was faster and remained "matched" to muscle O_2 utilization also after training (McKay et al. 2009).

On the other hand Bailey et al. (2009) reported no changes neither in pulmonary $\dot{V}_{O_2}$ kinetics nor in Δ[HHb] kinetics after 2 weeks of continuous training while a significant improvement in both parameters after 2 weeks of repeated sprint training, even if the total work done was matched.

Murias et al. (2010) investigated the mechanism of adaptation for fundamental phase of $\dot{V}_{O_2}$ kinetics in older and younger men after 12 weeks of endurance training. All the subjects started with a continuous training (45min 70% $\dot{V}_{O_{2peak}}$) and after 10 weeks they were split randomly in two groups: one followed the same continuous training

and the other one undertook a high intensity interval training (10-12 bouts, 1min long at 90-100% $\dot{V}_{O2peak}$ with min rest between 2 bouts). $\dot{V}_{O_2}$ uptake and muscle deoxygenation were assessed during step transition from 20w to 90% GET before and after 3, 6, 9 and 12 weeks of training. They reported a decrease in the Phase II $\dot{V}_{O_2}$ time constant within the first 3 weeks of training in both older and younger, without further modifications throughout the training. In addition after 3 weeks of training attenuation in the initial Δ[HHb]/Δ$\dot{V}_{O_2}$ overshoot during step test was present, suggesting an amelioration of microvascular blood flow, O_2 distribution and muscle O_2 utilization. Previous findings by the same group showed a progressively attenuated overshoot as time constant of pulmonary $\dot{V}_{O_2}$ became smaller (Murias et al. 2011).

While the improvement in $\dot{V}_{O_2}$ uptake after endurance training is well identified, it is presently not clear which type of adaptation might be involved and at what extent the stimulus upon $\dot{V}_{O_2}$ kinetics might be dependent on the specific nature of the training program performed (intensity volume and frequency).

CHAPTER III: GENERAL INTRODUCTION

The ATP turnover increases immediately at exercise onset while O_2 uptake lags behind the mechanical work and the release of aerobic energy. This difference between ATP requirements and the ATP production by oxidative metabolism is known as O_2 deficit (Krogh and Lindhard 1913) and it is compensated by the utilisation of other energetic sources: PCr degradation, anaerobic glycolisis and a small contribution of O_2 stores.

Speeding the $\dot{V}_{O_2}$ kinetics results in a reduction of the O_2 deficit and thus a diminution in metabolic perturbation improving the performance by enhancing exercise tolerance.

Interventions which enhance $\dot{V}_{O_2}$ kinetics, such as training, allow to improve not only exercise performance in athletes but also functional capacity in the elderly, diseased and sedentary individuals in order to improve their quality of life. Therefore, $\dot{V}_{O_2}$ kinetics is identified, along with the four traditional aerobic function ($\dot{V}_{O_{2max}}$, exercise economy, lactate threshold and critical power), as a measure to predict endurance performance in athletes (Burnley and Jones 2007) and it can be also used as a tool to evaluate exercise capacity during a metabolic state that mimics habitual physical activities, providing important information in assessing the disease severity and predicting the prognosis (Cross et al. 1995; Schalcher et al. 2003, Grassi et al. 2006).

For these reasons to understand the mechanism/s which influence/s $\dot{V}_{O_2}$ kinetics would be an important acknowledge to better address exercise training as well as therapeutic interventions.

For a long time 2 main factors have been discussed as possible determinants of $\dot{V}_{O_2}$ kinetics: the oxygen delivery to exercising muscle (Hugson et al. 1982**,** Tschakovsky

and Hughson 1999) and a 'metabolic inertia' at the muscle level (Grassi et al. 1996, Rossiter et al. 1999).

Since it has still matter of debate the main focus of these experiments was to investigate what influences $\dot{V}_{O_2}$ kinetics during moderate-intensity exercise which has the grater application for the population.

To address this issue we measured the $\dot{V}_{O_2}$ kinetics in different regions of the moderate domain in order to assess whether $\dot{V}_{O_2}$ kinetics responds with the same fashion (study 1) and to assess whether $\dot{V}_{O_2}$ kinetics are affected by an endurance training intervention (study 2).

INTRODUCTION STUDY 1

An abrupt increase in work rate is accompanied by instantaneous adaptations of the cardiovascular and respiratory system in order to arise the amount of O_2 delivered into the muscles and thus permits to the oxidative phosphorylation to meet the required energy turnover.

In the moderate domain the oxygen uptake response to dynamic exercise rising in an exponential manner to reach a steady state within 2-3 minutes. Two components are identified: a Phase I, characterised by a rapid cardiodynamic response, and a slower Phase (fundamental Phase or primary component) which reflects the influence of muscles metabolic rate.

It has always been matter of discussion whether the time constant of the fundamental Phase of $\dot{V}_{O_2}$ ($\dot{V}_{O_2}$ τ) evidences a linear-order behaviour throughout the continuum of

exercise (Di Prampero et al. 1970, Linnarsson et al. 1974). This concept, which obeys at the low of superposition (Fujihara et al. 1973), is that the output can be predicted from the input. Applied to $\dot{V}_{O_2}$ kinetics the τ and gain (G = $\Delta\dot{V}_{O_2}$ /ΔWR, where $\Delta\dot{V}_{O_2}$ is the adjustment in oxygen uptake and ΔWR the adjustment in work rate) of the primary component should be the same at every work rate (moderate, high or severe).

While for the intensity above the ventilatory threshold (GET) it has been demonstrated that a linear first order system is lacking in the primary component due to a development of additional constrains which determine the slow component (Wilkerson et al. 2004, Jones and Poole 2005), below GET is still not clear and contrasting results have been reported. Some studies show a similar τ for pulmonary $\dot{V}_{O_2}$ kinetics (Linnarsson et al. 1974, Casaburi et al 1977, Diamond et al. 1977, Whipp et al. 1982); in contrast other researchers found a slower response in the upper compared to the lower region of moderate domain (Di Prampero et al. 1970, Davies et al. 1972, Hughson et al. 1983).

R. Hugh Morton (1987) carried out a critical examination of the previous researches, suggesting a series of methodological considerations such as not clear level of intensity at which exercise was performed (Di Prampero et al. 1970, Davies et al. 1972) or incorrect statistical inferences and/or mathematically incomplete modelling procedures (Di Prampero et al. 1970, Diamand et al. 1997, Hugson at al. 1982), which bring their results into question.

Based on these considerations recently studies have been designed to resolve this issue and the same finding was reported: the $\dot{V}_{O_2}$ uptake response is slower at higher intensity (Brittain et al. 2001, McPhee et al. 2005, Spencer et al. 2011, Bowen et al.

2011) compare to lower intensity or even full transitions within the moderate domain, demonstrating that oxygen uptake does not follow a liner behaviour neither under GET. In order to explain this mechanism Hugson and Morrisey (1982) proposed that an insufficient oxygen delivery at higher work rate intensity occurs slowing the O_2 consumption, since they noticed a more sluggish heart rate response as recently confirmed by another study (MacPhee et al. 2005).

On the other hand a slow $\dot{V}_{O_2}$ kinetics time constant might be related to a progressively involvement of muscle fibres, following a hierarchical manner recruitment (Henneman et al. 1965), thus only less efficient fibres are available to a higher work rate (Brittain et al. 2001, Spencer et al. 2011), or an increase in ATP production along with a reduction in intracellular energy state (Bowen et al. 2011).

Therefore, according to the majority of studies the time constant for the Phase II of $\dot{V}_{O_2}$ kinetics seems to not follow a system linear behaviour neither below GET, showing a trend which is slower at higher work rate but the reason is still unrevealed.

A clarification on this topic has important implications in understanding the underlying mechanisms which might speed or slow O_2 uptake and, therefore, influence the oxygen deficit and thus the performance.

The aim of this study was to assess the adaptations of pulmonary oxygen consumption and cardiovascular parameters during rest to work and work to work exercise transitions in different region of the moderate domain. We hypothesised that the time constant of Phase II $\dot{V}_{O_2}$ uptake would be slower in the upper region either from a rest or a prior work state. Moreover we postulate that the adaptation of the cardiovascular system would response in a more consistent manner following rest to work than work to work transition without influencing O_2 uptake in any condition.

INTRODUCTION STUDY 2

At the onset of exercise a rapid increase in the work rate and ATP turnover is followed by a slower mono-exponential rise in $\dot{V}_{O_2}$ uptake to the required energy demand.

This response consists in a Phase I (15-20s), also called cardiodynamic Phase, a Phase II characterized by a time constant (τ) which arises exponentially to meet the amplitude and a Phase III which is the plateau of the new steady state reached within 3-4 minutes of the onset of moderate exercise (Whipp et al. 1982).

It has been demonstrated that time constant, describing the rate of adaptation of $\dot{V}_{O_2}$ uptake, influences the O_2 deficit and consequently has an impact upon the performance (Burnley et al. 2007, Jones et al. 2009). A lower τ means a faster adjustment and, on the contrary, a higher τ means a slower O_2 consumption; a shift from a condition to another is influenced by different situations such as training status (Phillips et al. 1995, Koppo et al. 2004,) and diseases (Sietsema et al. 1994, Cross et al. 1995). However which type of adaptations might modulates this response are still not clear.

The rise of the Phase II closely reflects the kinetics of adjustment of oxidative metabolism within the skeletal muscle (Whipp et al. 1982, Grassi et al. 1996, Grassi et al. 1998) suggesting that O_2 delivery does not influence muscle O_2 uptake as it has been hypothesized (Hughson et al. 1982). Although the bulk O_2 delivery does not appear to be a boundary in muscle O_2 consumption ($\dot{V}_{O_{2m}}$) the microcirculation at capillary level ($\dot{Q}_{O_2}$) seems to be slower than muscular blood flow and muscular O_2 uptake (Harper et al. 2006), thus adequacy of $\dot{Q}_{O_2}$ to $\dot{V}_{O_{2m}}$ might be critical (Murias et al. 2010). Conversely other investigators support the “metabolic inertia” theory by

which a slower activation on the intramuscular oxidative machinery is fundamental in setting the limit for the $\dot{V}_{O_2}$ kinetics (Grassi et al. 2001, Walsh et al. 2005).

Endurance training, involving either continuous exercise (CO) at submaximal work rates and/or high-intensity interval exercise (HI), is a potent stimulus in modifying performance parameters (e.g. $\dot{V}_{O_{2max}}$, GET) related to O_2 uptake. For this reason a variety of studies have been carried out to assess whether this type of training might influence also the time constant of $\dot{V}_{O_2}$ kinetics (Hickson et al. 1978, Phillips et al. 1995, Fukuoka et al. 2001, Berger et al. 2006, Bailey et al. 2009).

It has been reported that after only few weeks of endurance training modifications in $\dot{V}_{O_2}$ kinetics are present during transition to moderate intensity exercise (Phillips et al. 1995, Berger et al. 2006, Murias et al. 2010) but it is still unclear whether the type of exercise and/or the training volume might influence in different manner (Berger et al. 2006, Bailey et al. 2009, McKey et al. 2009). Moreover only few studies have evaluated the early time course of the training-induced adaptations. Phillips et al. (1995) demonstrated that after only 4 days of continuous endurance training Phase II $\dot{V}_{O_2}$ τ became progressively faster and same results have been found after 2 days either following a CO or HI (McKey et al. 2009), although nobody has never tested possible changes as early as after one session of endurance training.

Thereby, while the improvement in $\dot{V}_{O_2}$ uptake after endurance training is well recognised, the underlying mechanism is still matter of debate (Zoladz et al. 2006, McKay et al. 2009, Murias et al. 2010).

Given that a faster $\dot{V}_{O_2}$ kinetics improves exercise performance and enhances physical function and that endurance exercise is able to speed the O_2 uptake, the determination of the impact of a moderate intensity continuous training could be very

important for people who are not able to sustain higher power output such as elderly and subjects with pathologies, in whom high-intensity exercise might be contraindicate.

The purpose of this study was, therefore, to assess the influences of 4 weeks of moderate intensity continuous endurance training on $\dot{V}_{O_2}$ kinetics, cardiac output and muscle deoxygenation during moderate exercise step transition.

We hypothesised that following this program of training influences on pulmonary $\dot{V}_{O_2}$ consumption will be detected. Moreover, since it has been demonstrated that $\dot{V}_{O_2}$ τ increases after only 2 days of training but it is present unknown whether earlier adaptations might be occur, we evaluated oxygen consumption kinetics as early as after only one session of training. Additionally we hypothesised that early modifications might be already discernable after one session.

Chapter IV: GENERAL METHODS

I. GENERAL EXPERIMENTAL PROCEDURES

The investigations that comprise the experimental chapters of this thesis required the administration of exercise tests that were conducted at the "Record" exercise physiology laboratory, Sport Science department - Alma Mater Studiorum - University of Bologna. All of the exercise tests and training sessions were conducted in an air conditioned laboratory with an ambient temperature of 18-22°C. All the procedures were approved by the University Ethics Committee prior to the commencement of testing.

Subjects

All subjects taking part in the investigations in this thesis volunteered to participate. Subjects were non-smokers that were free from diseases and recreationally active at various levels, including regular structured exercise and/or competitive sport, although none were elite-level athletes. Subjects were instructed to report to the laboratory in a rested state, having completed no strenuous exercise within the previous 24 hours and having abstained from food, alcohol and caffeine for the preceding three hours. Testing was conducted at the same time of day (±2 hours) for each subject and subjects were familiarised with the mode(s) of exercise and experimental procedures prior to the initiation of testing.

Informed consent

Prior to agreeing to participate in a study, subjects were provided with a verbal explanation f the study and the experimental procedures . Also an information sheet, which outlined the requirements associated with their participation and the potential risks and benefits, was given to every subject. Subjects were assured that even though group results would be available for public inspection and individual response profiles might be presented to depict a representative response, their anonymity would be strictly preserved and all data safety stored. Subjects also understood that they were free to withdraw from the investigation at any time without disadvantage.

Any additional questions or concerns that subjects had were answered and they subsequently gave their written informed consent to participate.

Health and safety

Great care was taken to ensure that the laboratory provided a clean and safe environment that was appropriate for exercise testing of human subjects. All respiratory apparatus was disinfected according to manufacturers' recommendations.

II. MEASUREMENT PROCEDURES

Descriptive data

For all investigations, each subject's stature and mass were measured and these parameters along with age were recorded prior to the initiation of testing. In all experiments the $\dot{V}_{O2max}$ and GET as well as the power output and $\dot{V}_{O2}$ at the GET were also determined during the preliminary exercise testing session.

Cycle Ergometer

Cycle ergometer was the exercise modality employed to investigate the physiological and performance parameters of interest. All these cycle tests were performed on an electronically-braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands) which can administer work rate in various functions. The ergometer functions that were used in the series of experiments that comprise this thesis include the step and proportional. The ergometer was calibrated regularly by a laboratory technician in accordance with the manufacturer's guidelines.

Pulmonary gas exchange

During all exercise tests pulmonary gas exchange and ventilation were measured. the analyses were performed using a metabolic cart (Quark B2 Cosmed, Italy). Gases were

sampled continuously through a capillary line inserted in the outer frame of the flowmeter and analysed by fast-response O_2 (chemical) and CO_2 (infrared) sensors. The software operating the metabolic cart allowed to record gas and flow signals (sampling frequency: 25 Hz) and save them as text files.

The analyzers and the propeller were calibrated before each experimental test using a gas mixture of known composition (FO_2= 0.16; FCO_2=0.04; N_2 as balance) and ambient air; the volume sensor was calibrated using a 3-liter syringe (Hans Rudolph, Kansas City, MO). Following the procedures indicated by the manufacturer. Subjects breathed through a low-dead-space, low-resistance mask.

After each test, raw breath-by-breath gas exchange data was exported and analysed at a later date.

Cardiovascular parameters

During all exercise tests, except for the last study, continuous recordings of beat-by-beat mean arterial pulse pressure were obtained at a fingertip of the right arm by means of a non-invasive cuff pressure recorder (Portapres; FMS, Amsterdam, The Netherlands). This instrument is also a beat to beat haemodynamic monitoring system with the capability of automatically retrieving other important haemodynamic parameters, such as heart rate, stroke volume, cardiac output, periferical resistences and mean arterial pressure by means of the Modelflow method (Wesseling et al. 1993) applied off-line to the pulse pressure profiles using the BeatScope® software package (FMS). With the Modelflow method, aortic blood flow from arterial blood

pressure pulsation can be instantaneously reconstructed by simulating a three-element (resistive, elastic and inductive) non-linear and time varying model of the vascular tree. Numerical integration of flow during systole yields the stroke volume and as a consequence all the other parameters.

Near-infrared Spectroscopy

During the exercise tests a near-infrared spectroscopy (NIRS) system was used to assess the oxygenation status of the m. vastus lateralis of the right leg (Nimo (R) Nirox, Nirox optoelectronics, Brescia, Italy). NIRS provides a non-invasive method by which the concentration changes in oxygenated and deoxygenated haemoglobin can be assessed based upon changes in near-infrared light absorption by the tissue under interrogation. This technique is based on the transparency of tissues to light in the NIRS region where the typical red spectrum of a white light travels through a biological tissue. Four different wavelength laser probes (775, 810, 850 and 910 nm) provided the light source. An emission probe irradiates laser beams to the tissue and the light returning from the tissue is captured by a detection probe positioned 33 millimetres from the emission probe. Changes in light intensities were recorded continuously at 40 Hz and online estimation of the concentration changes in oxyhaemoglobin (HbO_2), deoxyhaemoglobin (HHb), and total haemoglobin (Hbtot) from the resting baseline were displayed and recorded. The physical principles of tissue spectroscopy are described in detail by Takafumi et al. (2007) and the manner in which these are applied have been explained by DeLorey et al. (2003).

For these measurements, the leg was shaved and cleaned with alcohol around the belly of the muscle (midway between the lateral epicondyle and greater trochanter of the femur) and the probes, housed in an optically dense plastic holder, were secured on the skin surface with adhesive tape then covered with an optically dense black vinyl sheet, thus minimizing the intrusion of extraneous light. The thigh was wrapped with an elastic bandage to minimize movement of the probes. Pen marks were made around the probes to enable precise replacement in subsequent tests. Before starting each test NIRS-derived signal was zero set and baseline values were recorded with the subject at rest with leg extended at downstroke in a seated position for upright cycling.

Following each test, raw NIRS data were exported for later analysis. Given the uncertainty of the optical path length in the m. vastus lateralis at rest and during exercise, NIRS data are presented as absolute and normalized (0-100% full response).

III. TESTING PROCEDURES

Preliminary exercise testing

Prior the commencement of every study a preliminary testing was performed.

It comprise a 'ramp' incremental cycling test to exhaustion while maintaining the cadence of 80 rev·min^{-1}. These incremental tests consisted of three minutes of pedalling at 0 W, followed by a continuous ramped increase in work rate of 25 W·min-1 (30 W·min-1 in the third study) until exhaustion. Saddle and handlebar heights were

recorded and the same settings were reproduced on all subsequent tests. Pulmonary gas exchange and heart rate were measured throughout these incremental tests.

Determination of O_{2peak} and GET

To calculate GET the breath-by-breath values of O_2 uptake were exported from the software, smoothed and averaged into 5-s bins and O_{2peak} was defined as the highest 30-s rolling average value. The GET was assessed by applying the V-slope method (a steeper increase of $\dot{V}_{CO_2}$ as compared to $\dot{V}_{O_2}$), along with an increase in $\dot{V}_E/\dot{V}_{O_2}$ increase with no increase in $\dot{V}_E$ / $\dot{V}_{CO_2}$ and an increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension, as indicated by Wasserman et al. (1999)

Experimental testing

For all investigations, constant-load tests were used to assess O_2 kinetics. These tests involved an abrupt transition from a lower (or rest) to higher work rate on the ergometer used for the study. In the first study the constant-load tests were performed at four different combinations of a pre-determined work rates based on watts. The workloads were the same for every subject and assigned randomly. In the second study the transitions were from rest to a pre-fixed work rate of 100 watts.

IV. DATA ANALYSIS PROCEDURES

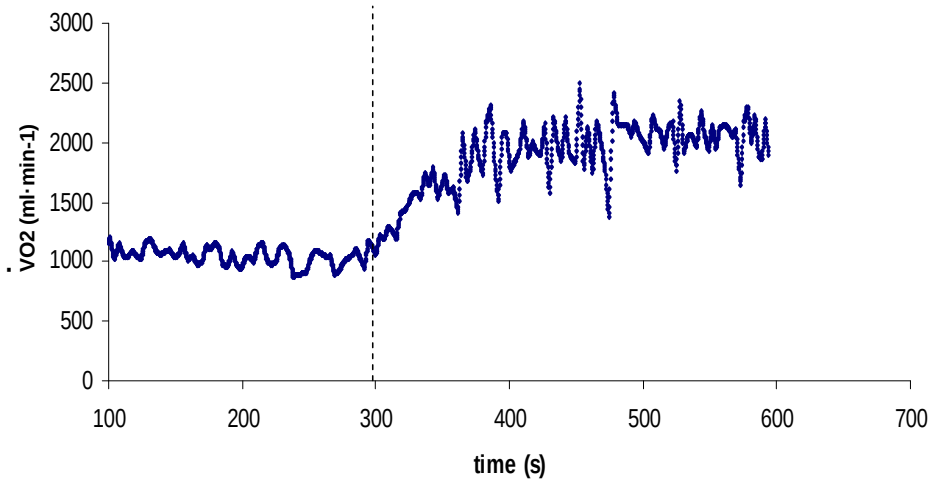
Enhancing signal-to-noise ratio

The raw breath-by-breath O_2 data displays inherent irregularities that produce fluctuations (i.e. 'noise') and, furthermore, outliers (errant breaths caused by coughing, swallowing, premature ending of a breath, etc.) are often present. These impair the precision of characterizing non steady-state gas exchange kinetics during exercise.

The kinetics can be viewed as the sum of two components: an underlying physiology response, and the noise, whose magnitude proves to be much greater in some subjects than in others. The noise produces statistical uncertainty in the estimation of its parameters and the noisier the data is, the lower will be the confidence in the values (Lamarra et al. 1987). Based on these considerations raw data from each test were examined and aberrant data were excluded. Data points lying more than four standard deviations from the five-breath local rolling average were removed from the raw pulmonary O_2 response for each individual transition prior to analysis (Lamarra *et al*. 1987). During this process, great care was taken to only remove definitive outliers.

Single breath alveolar O_2 uptake was calculated from the original gas and flow traces, by means of the algorithm of Grønlund, which takes into account variation of the gas lung's stores the occur from one breath to another. In particular, the N_2 + Ar concentration was estimated as 100% minus the sum of measured O_2 and CO_2 concentrations as also utilized by Clemensen at al. (1994). The algorithm was

implemented in a computerised procedure written in the object-oriented G language, implemented in the developing environment Labview 6.0 (National Instruments, USA). Once this editing was complete, data were linearly interpolated using a dedicated algorithm to provide a value every 0.25 seconds, thus permitting to average the series of the identical transition undertaken, necessary to increase the signal- to-noise ratio which increases the confidence in the results obtained from the exponential analysis (Lamarra et al. 1987).



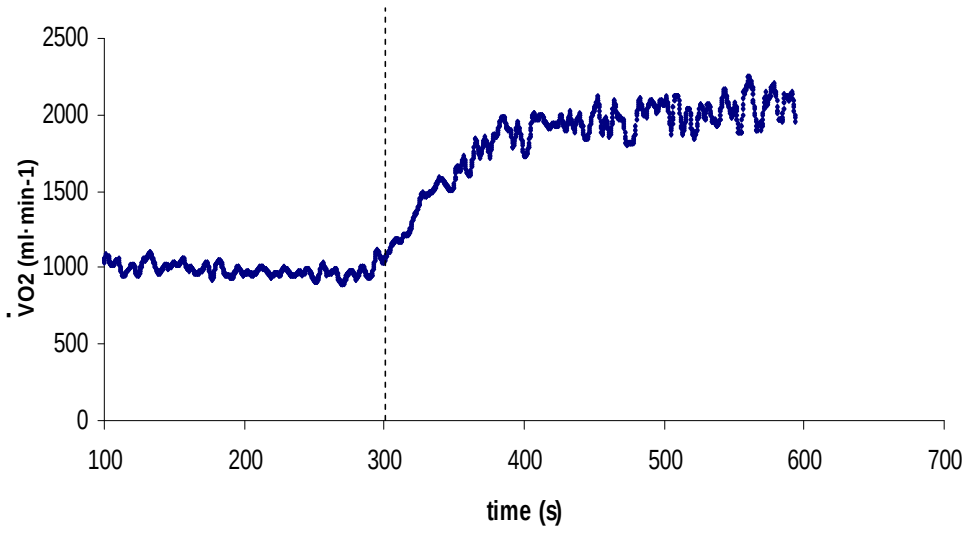


Figure 1: Pulmonary $\dot{V}_{O_2}$ response during moderate exercise prior to and following preliminary data analysis procedures. An untreated pulmonary $\dot{V}_{O_2}$ response during one bout of moderate-intensity cycle exercise is shown in the upper panel. The amalgamated response of four individual moderate $\dot{V}_{O_2}$ responses that have been filtered and ensemble-averaged is shown in the lower panel. The dashed vertical lines indicate the point of work rate imposition. Note that in the lower panel, where the data have been subjected to preliminary data analysis procedures, the fitting is clearer due to an enhanced signal-to-noise ratio.

For the same reason Lamarra pointed out that breath-to-breath variability in the O_2 signal diminishes when a response amplitude is increased; therefore the number of like transitions required to obtain the requisite confidence in the parameter estimates will vary depending on the magnitude of the work rate imposed (Figure 1).

According to these observations for the work-to-work transitions in the second study three repetitions of an identical trial were performed while four like-repetitions per condition in the first one, since the work rate required was higher.

The final averaged kinetics of pulmonary oxygen consumption was time-aligned with the onset of the work rate transitions and treated by subtracting the $\dot{V}_{O_2}$ values that were calculated by averaging the data obtained the three last minutes of the basal condition.

Mathematical Modelling

Once the filtering, the linear interpolation and the averaging of the breath-by-breath data were completed the files obtained were imported into a modelling program that described the O_2 response using a nonlinear least-square regression algorithm. This

program employs an iterative process that minimises the sum of the squared error between the fitted function and the observed data.

The $\dot{V}_{O_2}$ data were modelled as a bi-exponential function as follow:

$$Y(t) = H(t-td_1) \times A_1 \times (1 - e^{-(t-td1/\tau1)}) + H(t-td_2) \times A_2 \times (1 - e^{-(t-td2/\tau2)}) \quad \text{(Equ. 1)}$$

Where $H(t-t_d)$ is the Heaviside function defined as

$$H(t-t_d) = \begin{cases} 0 & \text{if } t < t_d \\ 1 & \text{if } t \geq t_d \end{cases}$$

Y(t) represents the increase of $\dot{V}_{O_{2p}}$ at the onset of the exercise, A_1 and A_2 indicate the amplitude terms, td_1 and td_2 are the time delays elapsed from the onset of the exercise and τ_1 and τ_2 represent the time constants of the two exponentials.

The Heaviside function inserted in the equation 1 accounts for the fact that each component does not come into play before an interval of time equal to the corresponding time delay.

Moreover the MRT has been calculated as follow:

$$MRT = [A_1 / (A_1+A_2) \cdot (\tau_1+TD_1)] + [A_2 / (A_1+A_2) \cdot (\tau_2+TD_2)] \quad \text{(Equ. 2)}$$

Where A is the amplitude, τ is time constant and TD is the time delay; 1 and 2 indicate respectively the Phase I and II.

Mathematical Modelling of cardiovascular Data

The cardiovascular data were linearly interpolated to provide a value every 0.25 seconds such that it could be time-aligned to the start of exercise and ensemble-averaged. A single- (equ. 3) or bi- exponential (equ. 1) model similar to the one used to fit the O_2 data was also employed to characterise the physiological response/s.

$$Y(t) = H(t\text{-}td_1) \times A_1 \times (1 - e^{-(t\text{-}td1/\tau 1)}) \qquad \text{(Equ. 3)}$$

Mathematical Modelling of Δ[HHb] data

Same procedure of time-aligned and averaging has been taken for the 0.25 s Δ[HHb] data, which were normalized for each subject (0-100% of the response) before continuing with an interpolation. Δ[HHb] kinetics parameters of the exponential models were estimated using a weighted non-linear least squares fitting procedure according to Carson et al. (1983) and implemented with Labview 6.0 software (National Instruments, Austin, TX, USA). Δ[HHb] profile has been described to consist of a time delay at the onset of exercise, followed by an increase in the signal with an "exponential-like" time course (DeLorey et al. 2003). The time delay for the Δ[HHb] response (Δ[HHb] TD) was determined with second-by-second data, and corresponded to the time between the onset of exercise and the first point at which the Δ[HHb] signal started to systematically increase. The Δ[HHb] data were modelled from the end of the Δ[HHb] TD to 120 s of the transition with an exponential model as described in

equ. 3. The Δ[HHb] τ describes the time course for the increase in Δ[HHb]. We calculate and overall time course of Δ[HHb] MRT from the onset of exercise as (Δ[HHb] MRT = Δ[HHb] TD + Δ[HHb] τ). The second-by-second ΔHHb and $\dot{V}_{O_2}$ kinetics data were normalized for each subject (0–100% of the response). The normalized $\dot{V}_{O_2}$ was left shifted by a time corresponding to $\dot{V}_{O_2}$ Phase II TD to account for the Phase I-Phase II transition so that the onset of exercise coincided with the beginning of Phase II $\dot{V}_{O_2}$, which has been previously described to coincide with muscle $\dot{V}_{O_2}$ within 10% (Rossiter at al. 1999).

The ratio of Δ[HHb] amplitude to $\dot{V}O_2$ amplitude (Δ[HHb]/Δ$\dot{V}O_2$) was used as an index of O_2 extraction during various phases of the response. An end point of 240 s was selected to ensure that both the Δ[HHb] and Δ$\dot{V}O_2$ signals had already reached 100% of their amplitudes.

Since prior research indicates that [HbO_2] responses do not approximate an exponential (DeLorey et al. 2007), NIRS-derived [HbO_2] data collected were not modelled. However the sum of [HbO_2] and Δ[HHb] was used to provide an estimate of changes in total haemoglobin ([HHbtot]).

Statistical methods

All statistical analyses within the experimental chapters of this thesis were conducted with either Microsoft Excel (paired t-tests and correlations) or the Statistical Package for Social Sciences (one-way repeated measures analysis of variance). Further

information relating to the particular statistical tests, that were employed for the different investigations, is provided within each of the experimental chapters.

Before any statistical tests were carried out, the data were screened for normal distribution using standard procedures. Statistical significance was accepted at $P < 0.05$. All data are presented as means ± SD.

METHODS STUDY 1

Subjects

Seven healthy, non smoking, subjects (4 male, mean ± SD: age = 25.9 ± 7.5 yr, height = 175.6 ± 5.1cm, body mass = 69.1 ± 5.9 kg) volunteered to participate in this investigation. The subjects participated in exercise at recreational level, but were not highly trained. They were instructed to avoid strenuous exercise in the 24 h preceding each test, to arrive after a light meal and in a hydrated condition. Subjects were also instructed to refrain from caffeine and alcohol in the 24 h preceding each testing session. Subjects provided their written informed consent to participate after all procedures and risks associated with the experimental testing were explained. All procedures were approved by the University of Bologna Research Ethics Committee.

Experimental overview

The subjects reported to the laboratory on three occasions over a 2 week period to complete exercise testing on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). On the first visit to the laboratory, subjects completed a ramp incremental test for the determination of the peak O_2 uptake ($\dot{V}_{O_{2peak}}$) and the gas exchange threshold (GET). On the two remaining laboratory visits pulmonary O_2 uptake ($\dot{V}_{O_2}$) kinetics and cardiovascular dynamics were determined during a series of step-exercise tests where the baseline metabolic rate (rest or cycling at 25 W) and the work rate increment (50 or 100 W) were manipulated.

Incremental test

The ramp incremental cycle test was preceded by a two minute period of 'unloaded' cycling at 0 W. Thereafter, the work rate was linearly increased at a rate of 25 $W \cdot min^{-1}$ until the limit of tolerance. Subjects were instructed to cycle at 80 rpm during the test and the test was terminated when the cadence dropped > 10 rpm below the required cadence. The seat height and pedal configuration were noted and reproduced in subsequent tests. During this incremental exercise test, pulmonary $\dot{V}_{O_2}$ was collected on a breath-by-breath basis and averaged over consecutive 10-s periods. The $\dot{V}_{O_{2peak}}$ was taken as the highest 30-s average value attained prior to the subject's volitional exhaustion in the test. The GET was determined from a cluster of measurements including 1) the first disproportionate increase in CO_2 production ($\dot{V}_{CO_2}$) from visual

inspection of individual plots of $\dot{V}co_2$ vs. $\dot{V}O_2$, 2) an increase in expired ventilation ($\dot{V}_E$) /$\dot{V}O_2$ with no increase in $\dot{V}_E$ /$\dot{V}co_2$, and 3) an increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension.

Step transition tests

After arriving at the laboratory on the remaining two testing sessions subjects rested for 10 min whilst seated on the cycle ergometer. Pulmonary $\dot{V}o_2$ and cardiovascular parameters were assessed over a 4 min period to establish basal values for these parameters. Thereafter, subjects completed two blocks of four step cycle tests interspersed by a passive rest period (30-40 min) to ensure the cardio-respiratory parameters had returned to basal values. The step tests under investigation in this study, which were administered in a randomised order, included: rest to 50 W (0-50), rest to 100 W (0-100), 25 W to 75 W (25-75) and 25 W to 125 W (25-125) (Figure 1). Each step test was preceded by 5 min of passive rest (0-50 and 0-100 conditions) or baseline cycling at 25 W (25-75 and 25-125 conditions) before an immediate transition to the required work rate. The step increment in work rate was maintained for 5 min before the work rate was restored to 25 W or the subject ceased cycling. This sequence was repeated until the subject had completed four repetitions of the step exercise test. The pulmonary $\dot{V}o_2$ and cardiovascular responses during the four repetitions for each step exercise test were averaged prior to analysis to increase the signal- to-noise ratio which, in turn, enhances the confidence in the results obtained from the exponential analysis (Lamarra et al. 1987).

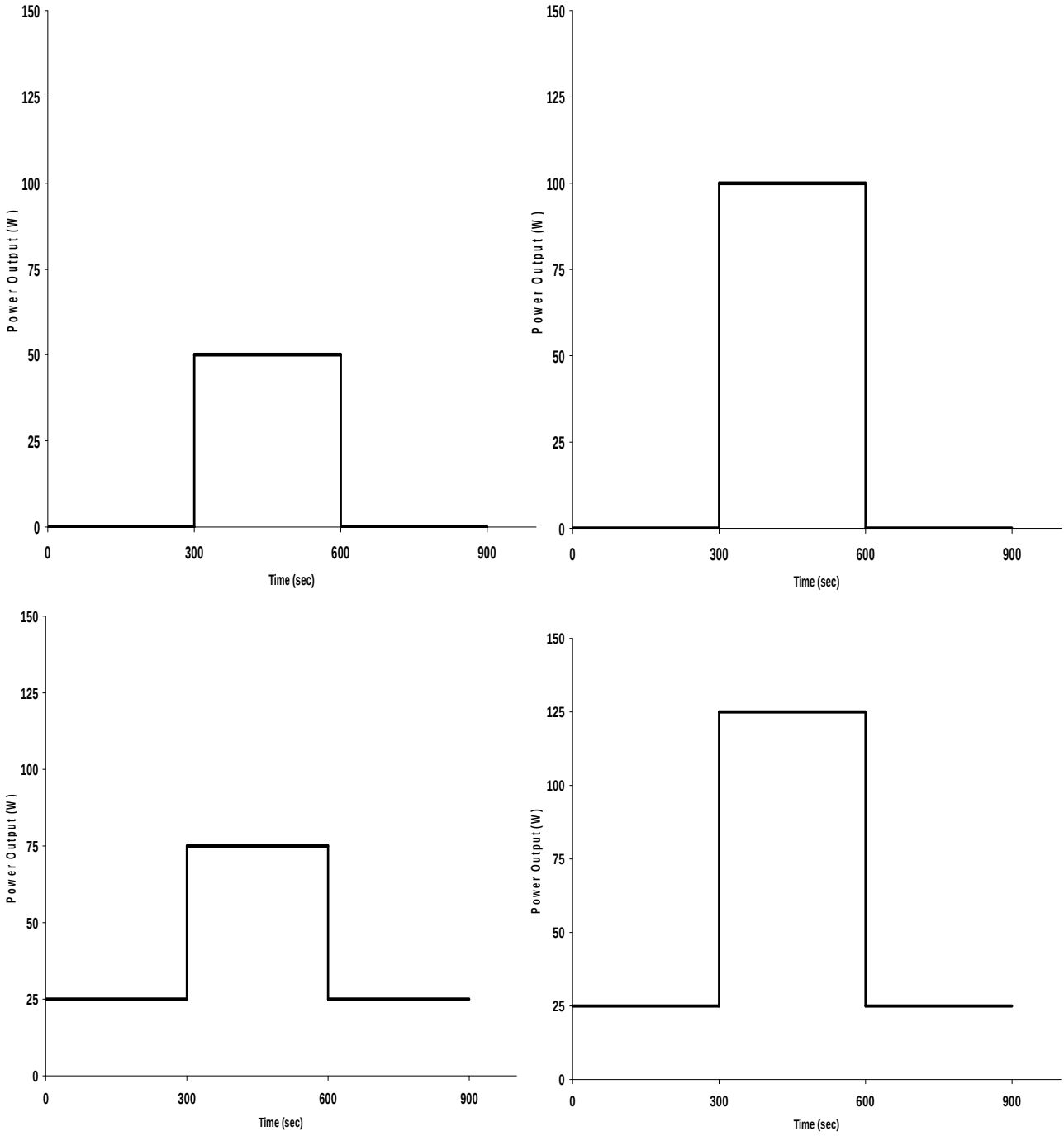


Figure 1. step transition tests; upper panel: 0-50W (left), 0-100W (right); lower panel: 25-75 (left), 25-125 (right).

Measurements

Pulmonary gas exchange and ventilation were determined on breath-by-breath basis during all tests, using a metabolic cart (Quark B2 Cosmed, Italy). Ventilation was assessed by a bi-directional digital turbine that was housed in the face mask worn by the subject and expired gases were sampled continuously through a capillary line inserted in the outer frame of the flowmeter and analysed by fast-response O_2 (chemical) and CO_2 (infrared) sensors. The gas analyzer was calibrated before each test with gases of a known concentration and the turbine transducer with a 3 L syringe (Hans Rudolph, Kansas City, MO), in line with the manufacturer's guideline.

Mean arterial pressure was recorded at a fingertip on the right arm by means of a noninvasive infrared cuff pressure recorder and a sensor which measures the hydrostatic height difference between the measured finger and heart level to compensate slow changes in blood pressure due to hydrostatic effects (Portapres; FMS, Amsterdam, The Netherlands). These values were collected online in raw form and stored on a personal computer using the BeatScope® software package (FMS). Stroke volume, heart rate, cardiac output and peripheral resistances were determined using Modelflow method (Wesseling et al. 1993). The data were exported in a spreadsheet file for subsequent analysis.

Data treatment

Individual raw pulmonary $\dot{V}_{O_2}$ files were examined and outliers, which were not reflective of the physiological responses, were removed by visual inspection. Single breath alveolar O_2 uptake was calculated from the original gas and flow traces, by means of the algorithm of Grønlund (1994), which takes into account variation of the gas lung's stores the occur from one breath to another. The algorithm was implemented in a computerised procedure implemented in the developing environment Labview 6.0 (National Instruments, USA).

These data were subsequently linearly interpolated to provide a value every 0.25 seconds and time-aligned to the onset of exercise. The $\dot{V}_{O_2}$ responses from the same experimental conditions were averaged and imported into a modelling program that described the $\dot{V}_{O_2}$ response using a nonlinear least-square regression algorithm. This program employs an iterative process that minimises the sum of the squared error between the fitted function and the observed data.

The $\dot{V}_{O_2}$ data were modelled as a bi-exponential function as follow:

$$Y(t) = H(t-td_1) \times A_1 \times (1 - e^{-(t-td1/\tau1)}) + H(t-td_2) \times A_2 \times (1 - e^{-(t-td2/\tau2)}) \qquad \text{(Equ. 1)}$$

Where H(t-td) is the Heaviside function defined as

$$H(t-td) = \begin{cases} 0 & \text{if } t < td \\ 1 & \text{if } t \geq td \end{cases}$$

Y(t) represents the increase of $\dot{V}_{O_2}$ at the onset of the exercise, A_1 and A_2 indicate the amplitude terms, td_1 and td_2 are the time delays elapsed from the onset of the exercise and τ_1 and τ_2 represent the time constants of the two exponentials.

The Heaviside function (inserted in the equation 1) accounts for the fact that each component is not manifest before an interval of time equal to the corresponding time delay.

The cardiovascular data were also linearly interpolated to provide a value every 0.25 seconds such that it could be time-aligned to the start of exercise and ensemble-averaged. A single exponential (equation 2), or bi- exponential model (equation 1), identical to the one used to fit the $\dot{V}_{O_2}$ data, was also employed to characterise the physiological responses.

$$Y(t) = H(t-td_1) \times A_1 \times (1 - e^{-(t-td1/\tau1)}) \qquad \text{(Equ. 2)}$$

Statistics

Cardio-respiratory data were analysed using a one-way repeated-measures ANOVA. Where analyses revealed a significant difference the origin of these effects was explored using follow-up t-tests using Fishers's LSD. Relationships between the $\dot{V}_{O_2}$ and cardiovascular parameters was explored using Pearson's correlation coefficient. All data are presented as mean ± SD unless otherwise stated and statistical significance was accepted as $P<0.05$.

METHODS STUDY 2

Subjects

Eleven healthy, non smoking, subjects (6 male, mean ± SD: age = 24.8 ± 4.2 yr, height = 174.9± 9 cm, body mass = 70.5 ± 11.7 kg) volunteered to participate in this investigation. The subjects participated in exercise at recreational level, but were not highly trained. They were instructed to avoid strenuous exercise in the 24h preceding each test, to arrive after a light meal and in a hydrated condition. Subjects were also instructed to refrain from caffeine and alcohol in the 24 hours preceding each testing session. Subjects provided their written informed consent to participate after all procedures and risks associated with the experimental testing were explained. All procedures were approved by the University of Bologna Research Ethics Committee.

Experimental overview

The subjects were required to report to the laboratory on two occasions before and after four weeks of endurance exercise training. On the first laboratory visit subjects completed a ramp incremental test for determination of the peak O_2 uptake ($\dot{V}_{O2peak}$) and the gas exchange threshold (GET), while pulmonary O_2 uptake ($\dot{V}_{O2}$) kinetics, muscle deoxygenation kinetics and cardiovascular dynamics were determined during step-exercise tests on the second laboratory visit (T0, T5). To track the changes in cardio-respiratory dynamics with exercise training, subject also completed step

exercise tests at weeks 1 (T1: after 1 training session), 2 (T2: after 4 training sessions), 3 (T3 after 7 training sessions) and 4 (T4 after 10 training sessions) of the training intervention. All step exercise tests were completed on an electronically-braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands) at the same absolute work rate. Subjects were instructed to maintain their habitual levels of physical activity, throughout the study.

Incremental test

The ramp incremental cycle test was preceded by a two minute period of 'unloaded' cycling at 0 W. Thereafter, the work rate was linearly increased at a rate of 25 $W \cdot min^{-1}$ until the limit of tolerance. Subjects were instructed to cycle at 80 rpm during the test and the test was terminated when the cadence dropped > 10 rpm below the required cadence. The seat height and pedal configuration were noted and reproduced in subsequent tests. During this incremental exercise test, pulmonary $\dot{V}_{O_2}$ was collected on a breath-by-breath basis and averaged over consecutive 10-s periods. The $\dot{V}_{O_{2peak}}$ was taken as the highest 30-s average value attained prior to the subject's volitional exhaustion in the test. The GET was determined from a cluster of measurements including 1) the first disproportionate increase in CO_2 production ($\dot{V}_{CO_2}$) from visual inspection of individual plots of $\dot{V}_{CO_2}$ vs. $\dot{V}_{O_2}$, 2) an increase in expired ventilation ($\dot{V}_E$) /$\dot{V}_{O_2}$ with no increase in $\dot{V}_E$ /$\dot{V}_{CO_2}$, and 3) an increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension.

Step transition tests

The kinetics of pulmonary $\dot{V}_{O_2}$ and muscle deoxygenation, and cardiovascular dynamics were determined during four step exercise tests. Each step test was preceded by 5 min of passive rest before an immediate transition to the required work rate of 100 W. The step increment in work rate was maintained for 5 min before the subject ceased cycling. This sequence was repeated until the subject had completed three repetitions of the step exercise test. The pulmonary $\dot{V}_{O_2}$, muscle deoxygenation and cardiovascular responses during the three repetitions for each step exercise test were averaged prior to analysis to increase the signal- to-noise ratio which, in turn, enhances the confidence in the results obtained from the exponential analysis (Lamarra et al. 1987).

Training Intervention

The endurance training program consisted of three session per week for a total duration of four weeks. Every session was 40 minutes long (plus 10 minutes of a light warm up and 10 minutes of a warm down). Each exercise training session was performed in the laboratory using commercially available cardio fitness machines (Technogym, Cesena, Italy), under the supervision of an expert. Subjects completed 10 min of exercise on a cycle ergometer, motorised treadmill, cross trainer and step machine. To set the intensity of the training subjects were provided with a heart rate monitor (Polar S610, Polar Electro Oy, Kempele, Finland) and instructed to exercise at an intensity that would elicit a heart rate corresponding to 60% of $\dot{V}o_{2peak}$ attained in

the incremental test. This method of exercise intensity assignment is in accordance with the guidelines of American College of Sports medicine (ACSM guidelines 2008).

Measurements

Pulmonary gas exchange and ventilation were determined on breath-by-breath basis during all tests, using a metabolic cart (Quark B2 Cosmed, Italy). Ventilation was assessed by a bi-directional digital turbine that was housed in the face mask worn by the subject and expired gases were sampled continuously through a capillary line inserted in the outer frame of the flowmeter and analysed by fast-response O_2 (chemical) and CO_2 (infrared) sensors. The gas analyzer was calibrated before each test with gases of a known concentration and the turbine transducer with a 3 L syringe (Hans Rudolph, Kansas City, MO), in line with the manufacturer's guideline.

During the exercise tests a near-infrared spectroscopy (NIRS) system was used to assess the oxygenation status of the m. vastus lateralis of the right leg (Nimo (R) Nirox, Nirox optoelectronics, Brescia, Italy). NIRS provides a non-invasive method by which the concentration changes in oxygenated and deoxygenated haemoglobin can be assessed based upon changes in near-infrared light absorption by the tissue under interrogation (Takafumi et al. 2007). An emission probe irradiates laser beams to the tissue and the light returning from the tissue is captured by a detection probe positioned 33 millimetres from the emission probe. Changes in light intensities were recorded continuously at 40 Hz and online estimation of the concentration changes in

oxyhaemoglobin (HbO_2), deoxyhaemoglobin (HHb), and total haemoglobin (Hbtot) from the resting baseline were displayed and recorded.

For these measurements, the leg was shaved and cleaned with alcohol around the belly of the muscle (midway between the lateral epicondyle and greater trochanter of the femur) and the probes, housed in an optically dense plastic holder, were secured on the skin surface with adhesive tape then covered with an optically dense black vinyl sheet, thus minimizing the intrusion of extraneous light. The thigh was wrapped with an elastic bandage to minimize movement of the probes. Pen marks were made around the probes to enable precise replacement in subsequent tests. Before starting each test NIRS-derived signal was zero set and baseline values were recorded with the subject at rest with leg extended at downstroke in a seated position for upright cycling.

Following each test, raw NIRS data were exported for later analysis. Given the uncertainty of the optical path length in the m. vastus lateralis at rest and during exercise, NIRS data are presented as absolute and normalized (0-100% full response).

Mean arterial pressure was recorded at a fingertip on the right arm by means of a noninvasive cuff pressure recorder and a sensor which measures the hydrostatic height difference between the measured finger and heart level to compensate slow changes in blood pressure due to hydrostatic effects (Portapres; FMS, Amsterdam, The Netherlands). These values were collected online in raw form and stored on a personal computer using the BeatScope® software package (FMS). Stroke volume, cardiac output, heart rate and peripheral resistances were determined using Modelflow method (Wesseling et al. 1993). The data were exported in a spreadsheet file for subsequent analysis.

Data treatment

Individual raw pulmonary $\dot{V}_{O_2}$ files were examined and outliers, which were not reflective of the physiological responses, were removed by visual inspection. Single breath alveolar O_2 uptake was calculated from the original gas and flow traces, by means of the algorithm of Grønlund (1984), which takes into account variation of the gas lung's stores the occur from one breath to another. The algorithm was implemented in a computerised procedure implemented in the developing environment Labview 6.0 (National Instruments, USA).

The gas exchange, cardiovascular and muscle oxygenation responses were linearly interpolated to provide a value every 0.25 seconds such that they could be time-aligned to the start of exercise, ensemble-averaged and imported into a modelling program that described the response using a nonlinear least-square regression algorithm. This program employs an iterative process that minimises the sum of the squared error between the fitted function and the observed data.

The $\dot{V}_{O_2}$ data were modelled as a bi-exponential function as follow:

$$Y(t) = H(t-td_1) \times A_1 \times (1 - e^{-(t-td1/\tau1)}) + H(t-td_2) \times A_2 \times (1 - e^{-(t-td2/\tau2)}) \quad \text{(Equ. 1)}$$

Where $H(t-t_d)$ is the Heaviside function defined as

$$H(t-td) = \begin{cases} 0 & \text{if } t < td \\ 1 & \text{if } t \geq td \end{cases}$$

Y(t) represents the increase of $\dot{V}_{O_2}$ at the onset of the exercise, A_1 and A_2 indicate the amplitude terms, td_1 and td_2 are the time delays elapsed from the onset of the exercise and τ_1 and τ_2 represent the time constants of the two exponentials.

The Heaviside function (inserted in the equation 1) accounts for the fact that each component is not manifest before an interval of time equal to the corresponding time delay.

Cardiovascular data were modelled as a single exponential (Equ. 2), or bi- exponential model (Equ. 1), identical to the one used to fit the $\dot{V}_{O_2}$ data, was also employed to characterise the physiological responses.

$$Y(t) = H(t\text{-}td_1) \times A_1 \times (1 - e^{-(t\text{-}td1/\tau 1)}) \qquad \text{(Equ. 2)}$$

Δ[HHb] data were modelled as a single exponential (Equ. 2) and the profile has been described to consist of a time delay at the onset of exercise, followed by an increase in the signal with an "exponential-like" time course (DeLorey et al. 2003). The time delay for the Δ[HHb] response (Δ[HHb] TD) corresponded to the time between the onset of exercise and the first point at which the Δ[HHb] signal started to systematically increase. The Δ[HHb] τ describs the time course for the increase in Δ[HHb]. We calculate and overall time course of Δ[HHb] MRT from the onset of exercise as (Δ[HHb] MRT = Δ[HHb] TD + Δ[HHb] τ). The Δ[HHb] and $\dot{V}_{O_2}$ kinetics data were normalized for each subject (0–100% of the response). The normalized $\dot{V}_{O_2}$ was left shifted by a time corresponding to Phase II $\dot{V}_{O_2}$ TD to account for the Phase I-Phase II transition so that the onset of exercise coincided with the beginning of Phase II $\dot{V}_{O_2}$,

which has been previously described to coincide with muscle $\dot{V}_{O_2}$ within 10% (Rossiter at al. 1999).

The ratio of Δ[HHb] amplitude to $\dot{V}O_2$ amplitude (Δ[HHb]/Δ $\dot{V}O_2$) was used as an index of O_2 extraction during various phases of the response. An end point of 240 s was selected to ensure that both the Δ[HHb] and Δ$\dot{V}O_2$ signals had already reached 100% of their amplitudes.

Statistics

Cardio-respiratory and muscle oxygenation data were analysed using a one-way repeated-measures ANOVA. Where analyses revealed a significant difference the origin of these effects was explored using follow-up t-tests using Fishers's LSD. Relationships between the $\dot{V}_{O_2}$ and cardiovascular and muscle oxygenation parameters was explored using Pearson's correlation coefficient. All data are presented as mean ± SD unless otherwise stated and statistical significance was accepted as $P<0.05$.

Chapter V: RESULTS AND DISCUSSIONS

RESULTS STUDY 1

Subjects characteristics are shown in table 1.

Table 1 . Subject characteristics and peak exercise responses

Subject	Age (yr)	Height (cm)	Body Mass (kg)	GET W	VO2max ml/min	VO2max ml/kg/min	MPA W
1	25	175	70	145	3083	44	269
2	22	178	71	150	2508	37	273
3	21	181	75	150	3430	51	265
4	19	176	67	150	4038	54	405
5	21	168	62	150	2507	40	215
6	35	170	62	150	2793	44	256
7	38	181	77	145	3110	39	290
Mean	26	176	69	149	3067	44	282
SD	8	5	6	2	545	6	59

During the ramp incremental test, subjects attained a peak work rate of 282 ± 59 W and a $\dot{V}_{O_{2peak}}$ of 44 ml·kg^{-1}·min^{-1}. The $\dot{V}_{O_2}$ and work rate values at the GET were 1762 ± 207 ml·min^{-1} and 149 ± 2 W. The work rate at the GET was higher that the work rates administered in this study for all seven subjects (20 W, 50 W, 75 W, 100 W and 125 W

was respectively at 17%, 34% (± 1), 50% (± 1), 67% (± 1) and 84% (± 1.4) of GET) and can therefore be categorized as moderate-intensity exercise.

Pulmonary oxygen uptake

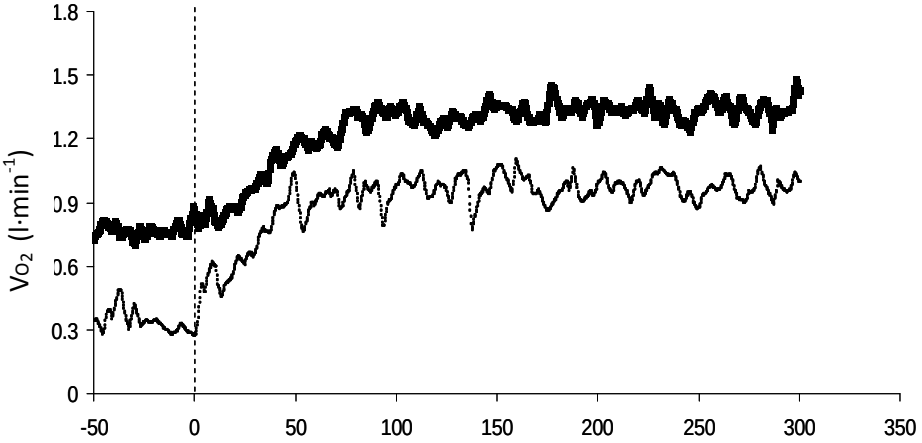
The parameters derived from the exponential model fitting of pulmonary $\dot{V}_{O_2}$ are presented in table 2, the $\dot{V}_{O_2}$ response for a representative individual is shown in figure 2.

Table 2. the kinetics parameters of $\dot{V}O_2$ for every step exercise condition

		Step 0-50	Step 0-100	Step 25-75	Step 25-125
A 1	l/min	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1*	0.2 ± 0.1*§
τ 1	s	1.6 ± 1.6	1.2 ± 0.8	1.8 ± 2.1	1.2 ± 1.1
TD 1	s	0.7 ± 1.3	0.2 ± 0.2	3.0 ± 5.1	3.2 ± 5.6
A 2	l/min	0.4 ± 0.1	0.9 ± 0.2$^{*\lambda}$	0.4 ± 0.1	0.9 ± 0.1$^{*\lambda}$
τ 2	s	18 ± 5$^{\S\lambda}$	24 ± 5	26 ± 6	37 ± 6$^{*\S\lambda}$
TD 2	s	21 ± 7	16 ± 5	21 ± 4	17 ± 4
A TOT	l/min	1.0 ± 0.1	1.6 ± 0.1*	1.3 ± 0.1*§	1.9 ± 0.1$^{*\S\lambda}$
MRT	s	23 ± 3	30 ± 6*	34 ± 10*	46 ± 9$^{*\S\lambda}$
GAIN	ml·min^{-1}·W^{-1}	7.2 ± 1.3	8.8 ± 1.7	7.4 ± 2.3	9.0 ± 1.0*

Values are means ± SD. A is the amplitude; τ is the time constant of response; TD is time delay; A tot is the total amplitude; MRT is the mean response time and gain is the ($\Delta\dot{V}O_2/\Delta$WR); 1 indicates the phase I and 2 indicates the phase II. * significantly different from 0-50 step exercise (P<0.05); § significantly different from 0-100 step exercise (P<0.05); λ significantly different from 25-75 step exercise (P<0.05).

A



B

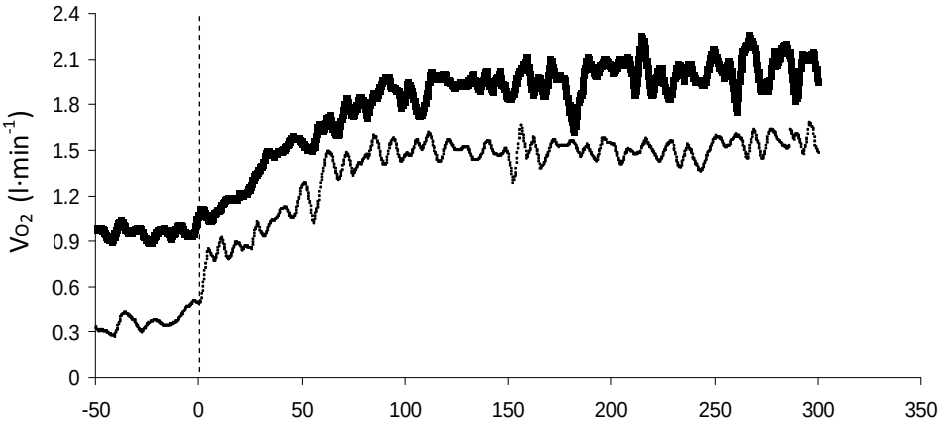


Figure 2. changes in $\dot{V}O_2$ response to step exercise. A: representative subject for – 0-50 condition and ▪ 25-75 condition; B representative subject for – 0-100 condition and ▪ 25-125 condition.

When a 50 W work rate increment was imposed from a resting baseline (0-50), the Phase II τ was faster compared to when the same work rate increment was imposed from a 25 W baseline (25-75) during cycle ergometry (0-50: 18.2 ± 4.5, 25-75: 25.8 ± 6.3 s; *P*<0.05; figure 2). Likewise, the Phase II τ was faster when a 100 W work rate increment was imposed from a resting baseline (0-100) rather than a 25 W baseline (25-125) (0-100: 23.7 ± 5.1, 25-125: 36.8 ± 5.8 s; *P*<0.01; figure 2). The Phase II τ was also faster in the 0-50 compared to the 0-100 and 25-125 conditions and in the 25-75 compared to the 25-125 condition (*P*<0.01 for all comparisons; table 2). The between-condition changes to $\dot{V}_{O_2}$ MRT was similar to those reported above for the Phase II $\dot{V}_{O_2}$ τ (*P*<0.01 for all comparisons; table 2). The $\dot{V}_{O_2}$ amplitude of the fundamental Phase was not significantly different between the 0-50 and 25-75 conditions (0-50: 0.4 ± 0.1, 25-75: 0.4 ± 0.1 L·min^{-1}; *P*>0.05; figure 2) and also was also not significantly different between the 0-100 and 25-125 conditions (0-100: 0.9 ± 0.2, 25-125: 0.9 ± 0.1 L·min^{-1}; *P*>0.05; figure 2). Conversely the Phase I amplitude was significantly different between the 0-50 and 25-75 conditions (0-50: 0.3 ± 0.1, 25-75: 0.2 ± 0.1 L·min^{-1} ; *P*<0.05; table 2) and also between the 0-100 and 25-125 conditions (0-100: 0.3 ± 0.1, 25-125: 0.2 ± 0.1 L·min^{-1} ; *P*<0.05; table 2) but not between the rest to work conditions and between the work to work conditions (*P*>0.05). The total Amplitude was different (*P*<0.05) for each condition, increasing proportionally with the work rate intensity (table 2).

The gain ($\Delta\dot{V}_{O_2}$/ΔWR) in $\dot{V}_{O_2}$ over the primary region of the response was similarly unaffected by elevating the metabolic rate before step increments in work rate within the moderate-intensity exercise domain (*P*>0.05 for all comparisons; table 2) .

Comparing the different changes between the 2 transitions from rest to work (0-50, 0-100) with the different changes between the 2 transitions from 25 W to work (25-75,

25-125) a *t*-tests showed a significant difference for Phase II $\dot{V}_{O_2}\tau$ (rest-work: 5.5 ± 2.7, work-work: 11 ± 3.5; *P*<0.01).

Table 3. the cardiovascular kinetics parameters for every step exercise condition

		Step 0-50	Step 0-100	Step 25-75	Step 25-125
$\dot{Q}$ **A 1**	l/min	3.8 ± 1.4	4.4 ± 1.1	1.7 ± 0.6*§	2.8 ± 0.6 $^{\S\lambda}$
$\dot{Q}$ **τ 1**	s	2 ± 1	2 ± 1	6 ± 3*§	5 ± 2*§
$\dot{Q}$ **TD 1**	s	2.8 ± 2.7	0.5 ± 0.6	1.4 ± 1.0	1.0 ± 1.5
$\dot{Q}$ **A 2**	l/min	0.7 ± 0. 2	2.2 ± 0.6$^{*\lambda}$	1.0 ± 0.4	2.3 ± 0.9$^{*\lambda}$
$\dot{Q}$ **τ 2**	s	4 ± 1$^{\S\lambda}$	14 ± 7	11 ± 5	24 ± 6$^{*\S\lambda}$
$\dot{Q}$ **TD 2**	s	17 ± 2	15 ± 3	27 ± 13	28 ± 7*§
$\dot{Q}$ **A TOT**	l/min	10 ± 1$^{\S\lambda}$	13 ± 1	12 ± 2	15 ± 2$^{*\lambda}$
$\dot{Q}$ **MRT**	s	8 ± 3$^{\S\lambda}$	12 ± 5	18 ± 7	26 ± 10$^{*\S\lambda}$
$\dot{Q}$ **GAIN**	ml·min^{-1}·W^{-1}	83 ± 33	66 ± 10	54 ± 13*	51 ± 10*§
HR A TOT	bpm	98 ±11	121 ±13*	112 ±11*§	138 ±11$^{*\S\lambda}$
HR MRT	**s**	3 ±3$^{\S\lambda}$	23 ±16	26 ±18	43 ±16$^{*\S\lambda}$
HR GAIN	bpm·W^{-1}	0.4 ±0.1	0.4 ±0.1	0.4 ±0.1	0.4 ±0.1
SV A TOT	ml/min	103 ±11	105 ±14	109 ±15	108 ±13
SV MRT	**s**	14 ±15	7 ±5	6 ±8	8 ±16
SV GAIN	ml·min^{-1}·W^{-1}	0.6 ±0.3	0.7 ±0.2	0.1 ±0.1*§	0.2 ±0.2*§

Values of cardiac output ($\dot{Q}$), heart rate (HR) and stroke volume (SV) are means ± SD. A is the amplitude; τ is the time constant of response; TD is time delay; A tot is the total amplitude; MRT is the mean response time and gain is the ($\Delta\dot{V}O_2/\Delta WR$); 1 indicates the Phase I and 2 indicates the Phase II. * significantly different from 0-50 step exercise (*P*<0.05); § significantly different from 0-100 step exercise (*P*<0.05); λ significantly different from 25-75 step exercise (*P*<0.05).

Cardiovascular parameters

The parameters assessed of cardiac output ($\dot{Q}$), heart rate (HR) and stroke volume (SV) are presented in table 3 and the responses for a representative individual are shown in figure 3, 4 and 5 respectively.

The Phase I $\dot{Q}$ τ was faster either in the 0-50 or 0-100 condition compared to the transitions initiated from an elevate metabolic rate (25-75 and 25-125) (0-50: 2 ± 0.8, 0-100: 2 ± 0.7, 25-75: 5.5 ± 3.1, 25-125: 4.8 ± 1.7 s; *P*<0.05; table3) with changes neither between the step transitions imposed from a resting baseline (0-50, 0-100) nor between the step transitions imposed from a 25 W work rate (25-75, 25-125) *(P*>0.05). Comparing the same increase in work rate (Δ 50 or Δ 100) the Phase II $\dot{Q}$ τ was faster when the step transitions started from a resting baseline (0-50, 0-100) rather than a 25 W baseline (25- 75, 25-125) (0-50: 4.3 ± 1.3, 25-75: 11 ± 4.7, 0-100: 14.3 ± 7.2, 25-125: 24 ± 5.8 s; *P*<0.05; table 3). The Phase II τ was also faster in the 0-50 compared to the 0-100 and 25-125 conditions and in the 25-75 compared to the 25-125 condition (*P*<0.01 for all comparisons, table 3). The Phase II time delay of the $\dot{Q}$ was shorter when a 50 W or 100 W work rate increment was imposed from a resting baseline (0-50, 0-100) rather than the 25-125 condition (0-50: 16.5 ± 2, 0-100: 15.3 ± 2.7, 25-125: 27.8 ± 6.6 s; *P*<0.05; table 3). The changes in MRT were similar to those reported above for the Phase II $\dot{Q}$ τ (*P*<0.05 for all comparisons; Table 3). When a 50 W work rate increment was imposed from rest (0-50), the Phase I $\dot{Q}$ amplitude was grater compared to when the same work rate increment began from 25 W (25-75) (0-50: 3.8 ± 1.4, 25-75: 1.7 ± 0.6 $L \cdot min^{-1}$; *P*<0.01; table 3) , equally when an increment of 100 W was required from rest (0-100) the Phase I $\dot{Q}$ amplitude was grater compared to the

transition from a 25 W baseline (25-125) (0-100: 4.4 ± 1.1, 25-125: 2.8 ± 0.6 L·min^{-1};*P*<0.01; table 3). The Phase II $\dot{Q}$ amplitude was significantly different (*P*<0.05) across the four conditions, increasing proportionally to the work rate (table 3), except between the transitions with the same work rate gain: 0-50 compared to 25-75 and 0-100 compared to 25-125 (0-50: 0.7 ± 0.2, 25-75: 1 ± 0.4, 0-100: 2.2 ± 0.6, 25-125: 2.3 ± 0.9 L·min^{-1}; *P*>0.05. Total amplitude was changed (*P*<0.05) for all conditions but not for the 0-100 condition compared to 25-75 and 25-125 condition (0-100: 12.5 ± 0.5, 25-75 12.2 ± 1.9, 25-125: 14.8 ± 2.4 L·min^{-1}; *P*<0.05; table 3). For a required work rate of 50 W the $\dot{Q}$ functional gain ($\Delta\dot{Q}$/ΔWR) was higher when the step exercise was started from rest (0-50) compared to the step exercise from a 25 W baseline (25-75) (0-50: 82.7 ± 32.7, 25-75: 53.8 ± 13.4 ml·min^{-1}·W^{-1}; *P*<0.05; table 3); likewise for an increase of 100 W the gain was higher during the transition 0-100 compared to 25-125 (0-100: 66.4 ± 9.3, 25-125: 50.7 ± 10.2 ml·min^{-1}·W^{-1}; *P*<0.05; table 3).

The value assessed for the HR total amplitude was different (*P*<0.05) for each condition (table 3) and proportional to the increase in work rate. The changes in HR MRT were significantly different (*P*<0.05) in all the step transitions but not for the 25-75 condition compared to 0-100 transition.

No changes in SV total amplitude and MRT have been detected. The SV gain (ΔSV/ΔWR) was not significantly different only between 0-50 and 0-100 condition and between 25-75 and 25-125 condition (0-50: 0.6 ± 0.3, 0-100: 0.7 ± 0.2, 25-75: 0.1 ± 0. 1, 25-125: 0.2 ± 0.2, ml·min-1; P<0.05; table 3).

For the 0-50 square-wave exercise an overshoot was present in 4 and 5 subjects for SV and HR respectively. Particularly the HR showed an overshoot which achieved ~100bpm before reaching a lower steady state (in the other 2 subjects was not

present as their steady state were around 100bpm: 92.49 ± 2.29 and 117.98 ± 1.81 bpm) while for SV an increase in the blood ejected from the heart is present only during the rest-to-work step transitions whereas in the W-W form of exercise is almost absent (R-W: 32.6 ± 12, W-W: 8.8 ± 7.9 ml·min^{-1}).

Therefore in the present study SV and HR data were not fitted because of different responses among the required bouts (mono- or bi-exponential).

T-test reported a significant difference (*P*<0.05) between $\dot{Q}$ and $\dot{V}_{O_2}$ Phase II τ throughout all the transients : $\dot{Q}$ showed a faster response for each condition (table 2, 3; figure 2, 3).

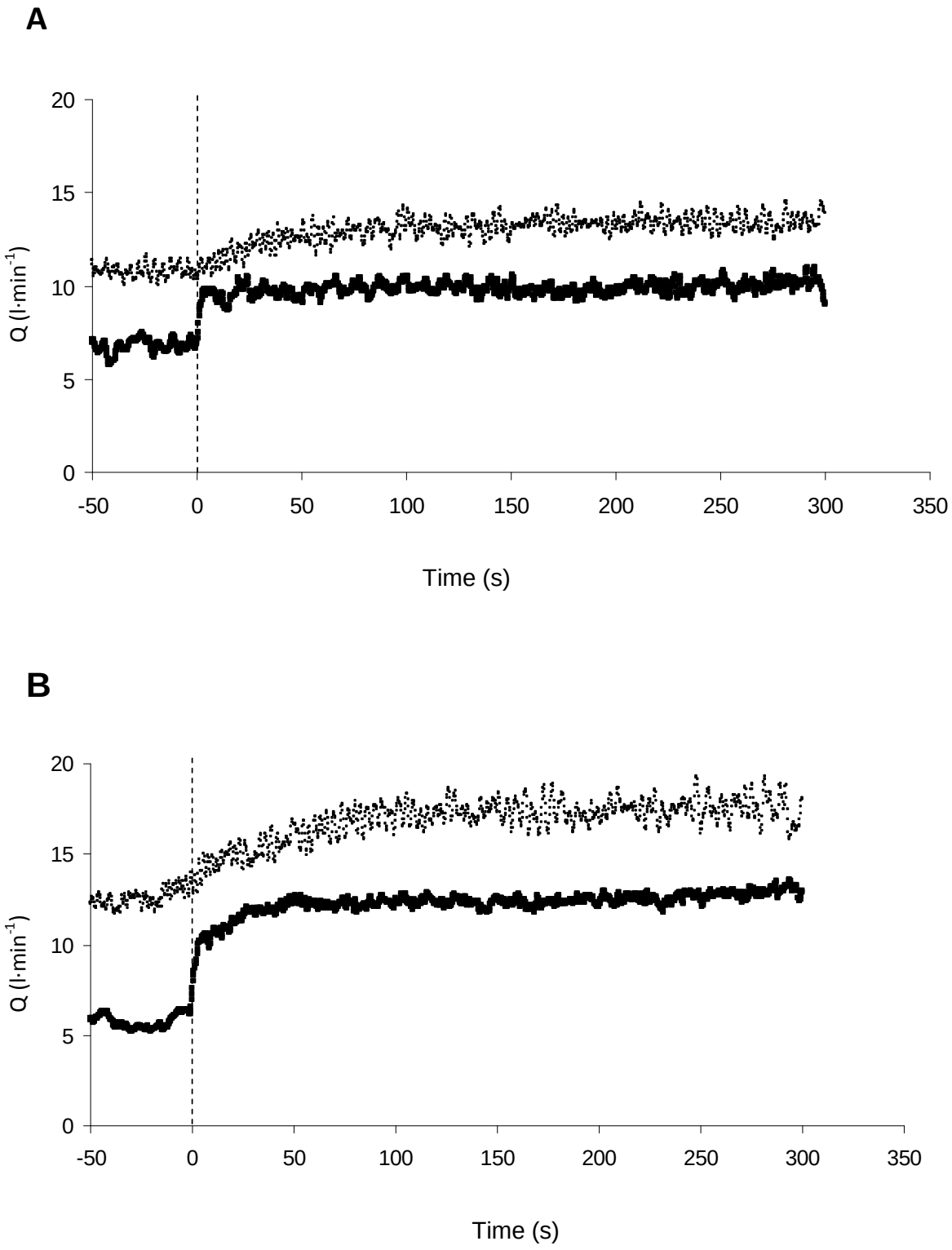


Figure 3. Changes in $\dot{Q}$ response to step exercise. A: representative subject for ▪ 0-50 condition and – 25-75 condition; B representative subject for ▪ 0-100 condition and – 25-125 condition.

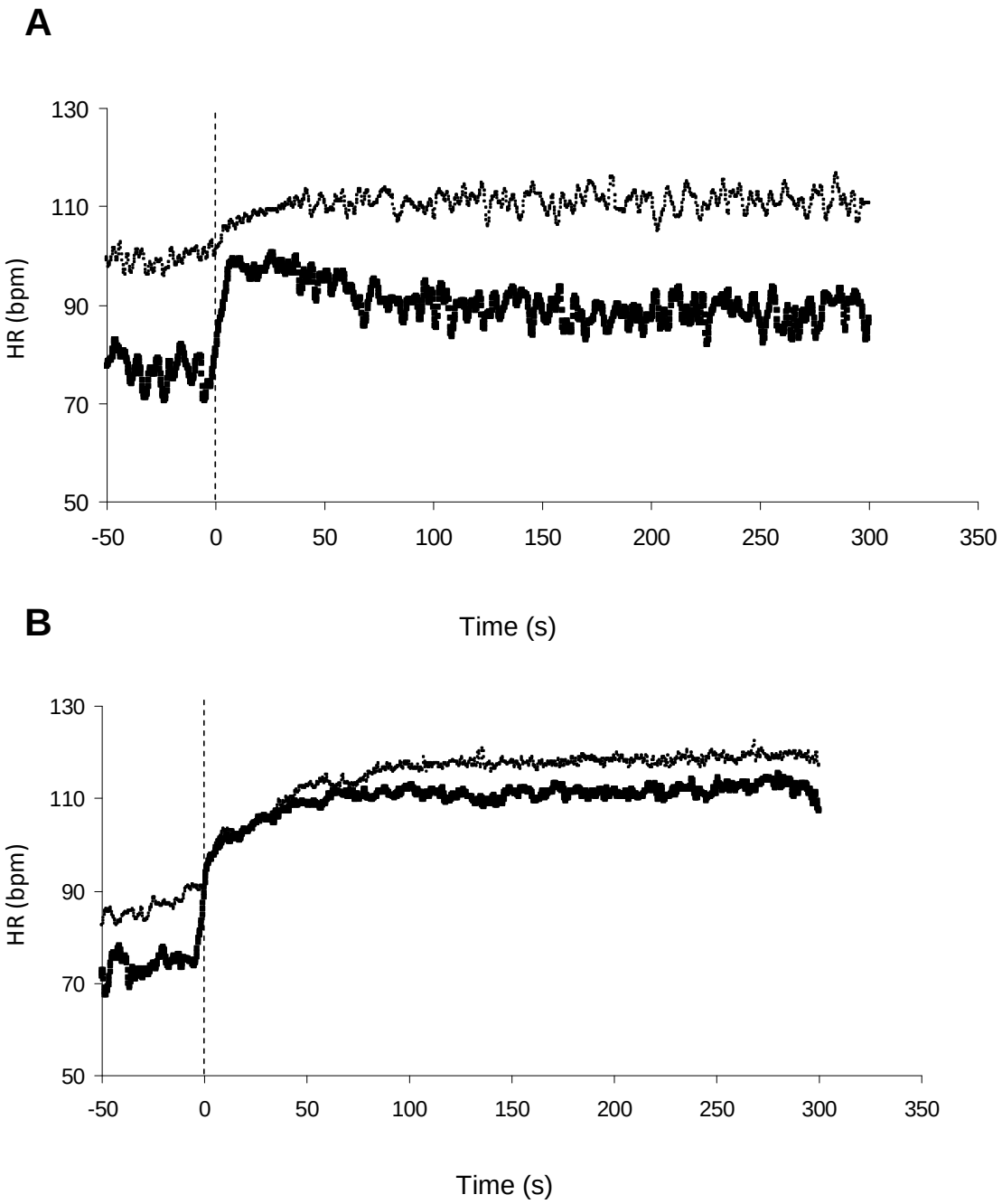


Figure 4. Changes in HR response to step exercise. A: representative subject for ▪ 0-50 condition and – 25-75 condition; B representative subject for ▪ 0-100 condition and – 25-125 condition.

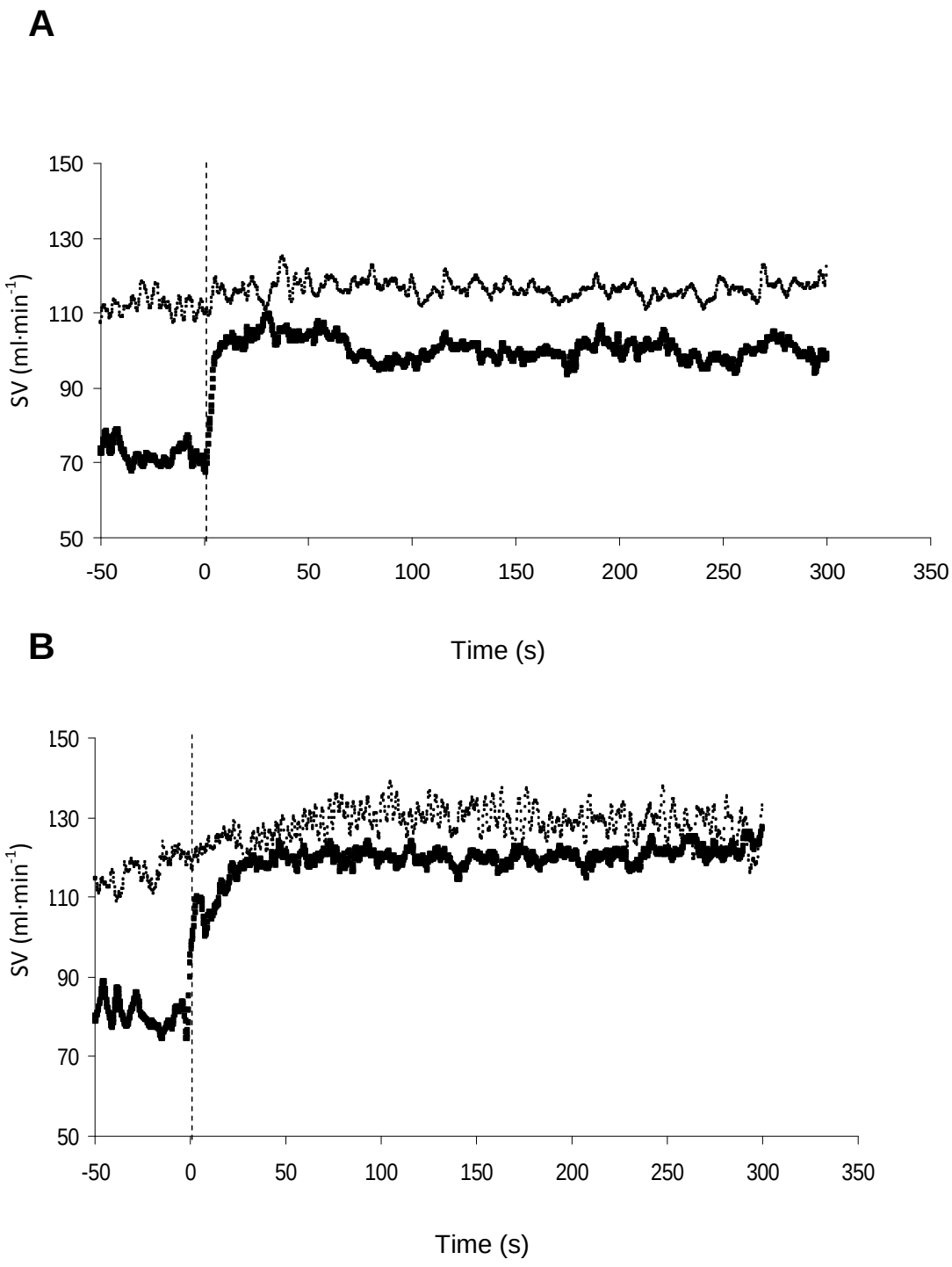


Figure 5. Changes in SV response to step exercise. A: representative subject for ▪ 0-50 condition and – 25-75 condition; B representative subject for ▪ 0-100 condition and – 25-125 condition.

DISCUSSION STUDY 1

This study variously manipulated baseline metabolic rate and the magnitude of work rate imposition within the moderate-intensity exercise domain and investigated the effects of these permutations on the kinetic adjustments of oxygen uptake and cardiovascular parameters in young healthy subjects. A novel aspect of this study was to impose an absolute rather than relative work rate within the moderate intensity domain. This was selected to test the extent to which pulmonary $\dot{V}O_2$ τ obeys the law of superposition, i.e., that $\dot{V}O_2$ τ should be similar within the moderate-intensity exercise domain irrespective of the work rate imposed. The main findings of this study were as follows: 1) $\dot{V}_{O_2}$ τ and the functional gain ($\Delta\dot{V}_{O_2}/\Delta WR$) were grater during transitions at the upper compared to the lower regions of the moderate exercise domain; 2) τ of the cardiac output was also slower during transitions at the upper region of the moderate exercise domain, but was faster than $\dot{V}_{O_2}$ τ for each exercise step; 3) $\dot{Q}$ increased more abruptly when the exercise was initiated from a resting baseline.

Consistent with our first experimental hypothesis, the Phase II $\dot{V}_{O_2}$ τ during cycle ergometry became progressively slower as work rate was increased within the moderate-intensity exercise domain. This result is in agreement with previous studies which reported a Phase II $\dot{V}_{O_2}$ τ slower in the upper regions of the moderate domain (Brittain et al. 2001, MacPhee et al. 2005, Bowen et al. 2011). This was particularly evident at work rates approaching the GET and this response was observed independent of the metabolic rate prior to the step increment in work rate (rest or 25 W). Moreover, we observed that the gain for $\dot{V}_{O_2}$ was not statistically significant for all

baseline metabolic rate-work rate increment permutations, apart from the higher $\dot{V}_{O_2}$ gain in 25-125 compared to 0-50. A higher $\dot{V}_{O_2}$ gain value at a high intensity exercise of the moderate domain has been reported by other researchers (Brittain et al. 2001, MacPhee et al. 2005, Bowen et al. 2011, Spencer et al. 2011). While it could be argued that this difference was consequent to the fact that both baseline metabolic rate and the work rate increment were different, it is also true that they are similar to the other 2 conditions: one with a the same gain in work rate and the other with the same basal state.

The results from this study suggest that $\dot{V}_{O_2}$ τ does abide by the law of superposition (Fujihara et al. 1973); that is, the law of superposition is violated since the G and τ of the $\dot{V}_{O_2}$ response dynamics varied as a function of the work rate imposition, as reported in previous studies (Brittain et al. 2001, MacPhee et al. 2005, Bowen et al. 2011, Spencer et al. 2011). The fact that $\dot{V}_{O_2}$ τ does not demonstrate linear behaviour throughout the continuum of exercise conflicts with the invariant τ presented by other researchers (Linnarsson et al. 1974, Casaburi et al. 1977, Diamond et al. 1977, Whipp et al. 1982); however the contrast finding reported in these studies might be due to a not well defined intensity level of exercise (moderate or heavy domain) and/or mathematical modelling procedures (Morton RH 1987). Thus they should be taken carefully into consideration.

Further support for a dependency of the $\dot{V}_{O_2}$ τ upon the region in which the exercise is undertaken is provided by the $\dot{V}_{O_2}$ τ comparisons between the 0-50, 0-100, 25-75 and 25-125 step exercise tests. If the law of superposition holds true, then the between-condition $\dot{V}_{O_2}$ τ values should not differ significantly since transitions with the same delta work rate (Δ50) and/or the same basal (rest or 25W) were investigated.

However, we observed a significant slowing in $\dot{V}_{O_2}$ τ in work-to-work conditions where a higher work rate was reached. Hughson and Morrissey (1982) suggested that the slower τ may be due to a sluggish adjustment of bulk O_2 delivery, likely because of a slower HR kinetics response. Support for this hypothesis was provided in a more recent study by MacPhee et al. (2005). Specifically, they proposed that the rapid HR adjustment, during transitions from rest to work, might be explained by the rapid withdrawal of parasympathetic neural activity, whereas a slower sympathetic activation, during work to work transitions might underlie the slower HR kinetics. Conversely, in this study we found that for step exercise from rest to 100W and from 25W to 75W the HR MRT was similar (P>0.05) and it was not slower than $\dot{V}_{O_2}$ MRT for each bout of exercise, thus these data challenge the hypothesis proposed by Hughson and co-workers. In addition, the HR gain and time constant have been reported to be invariant across the moderate-intensity step exercise transitions (Bowen et al. 2011, Spencer et al. 2011).

To better clarify the central contribution of O_2 delivery, we also assessed the cardiac output response during the different step exercise transitions. The $\dot{Q}$ time constant and the MRT were appreciably faster than $\dot{V}_{O_2}$ τ for each condition, consistent with other findings (De Cort et al. 1991, Yoshida et al. 1992, Whipp et al. 1994). These data suggest that bulk O_2 delivery is in excess O_2 demand during moderate intensity exercise. However, in contrast with these data, MacPhee et al. (2005) have reported slowed leg blood flow kinetics in the upper compared to the lower region of the moderate-intensity exercise domain, suggesting that bulk O_2 delivery might be involved in slowing the $\dot{V}_{O_2}$ τ in the upper region of the moderate-intensity exercise domain.

Recent research has assessed the kinetics of muscle deoxygenation (Δ[HHb]) during exercise by using near infra-red spectroscopy (NIRS). The NIRS-derived [HHb] signal offers important insights into the mechanisms for an altered $\dot{V}_{O_2}$ τ since it reflects the dynamic balance between O_2 delivery and O_2 utilisation within the muscle microvasculature. If a slower $\dot{V}_{O_2}$ τ is observed in the presence of a slower [HHb] τ, this would suggest that $\dot{V}_{O_2}$ τ is slowed owing to slower muscle O_2 extraction, whereas a slower $\dot{V}_{O_2}$ τ in the presence of a faster [HHb] τ would suggest that $\dot{V}_{O_2}$ τ is slowed owing to slower muscle O_2 delivery. Studies using NIRS have shown a slower Δ[HHb] time constant and MRT in the upper region of moderate domain compared to either step transitions in the lower region or full transitions (MacPhee et al. 2005, Spencer et al. 2011). These studies show a similar ratio between [HHb] and pulmonary $\dot{V}_{O_2}$ amplitude (Δ[HHb]/Δ$\dot{V}_{O_2}$), among all conditions of moderate-exercise (Spencer et al. 2011), suggestive of a close agreement between O_2 delivery and O_2 utilization. This assumption is supported by another observation by Bowen et al. (2011) who reported that, despite an increased basal level of total oxygenation index (TOI= [HbO_2]/ [HbO_2] + [HHb]) before the commencement of exercise and a permanent high value during the bout, the pulmonary $\dot{V}_{O_2}$ remained slow. Taken together, these results appear to support the notion that O_2 delivery to the exercising muscle is expected to be adequate pointing to a metabolic inertia to the increase in $\dot{V}_{O_2}$ during transitions in the upper region of the moderate-intensity exercise domain.

An alternative explanation to O_2 limitation is the hierarchical recruitment of muscle fibres. The importance of muscle fibre composition on $\dot{V}_{O_2}$ kinetics is reflected by the positive correlation between the speed of fundamental component $\dot{V}_{O_2}$ and the percentage of slow fibers in humans (Barstow et al. 1996). In line with the hierarchical

recruitment of muscle fibres, it follows that at lower work rates most efficient oxidative fibres (type I), which enhance vasodilatation to increase O_2 supply to meet the required O_2 utilization, are recruited resulting in a small $\dot{V}_{O_2}$ gain as well as a fast $\dot{V}_{O_2}$ and HHb time constant. However, at higher work rates an increasing proportion of less efficient fibres (type II) are recruited, which have lower mitochondrial density and capillarisation and show a slow $\dot{V}_{O_2}$ and HHb time constant, reflective of a local mismatching of O_2 delivery relative to O_2 demand, to complete the higher work rate within the moderate-intensity exercise domain (Brittain et al 2001, Behnke et al. 2003, Spencer et al. 2011). This mechanism appears to support our findings of an increasingly grater $\dot{V}_{O_2}$ τ across the moderate domain and in particular, the limited modification of the $\dot{V}_{O_2}$ τ between the 27-75 and 0-100 transitions, despite the differences in the work rate gain required and in the baseline preceding the bout. Moreover, for the transitions at the lowest intensity (0-50) it is likely that the majority of fibres recruited were slow oxidative fibres (where the fastest $\dot{V}_{O_2}$ τ was observed), and the highest intensity transition (25-125) recruited a greater proportion of fast fibres (where the slowest $\dot{V}_{O_2}$ τ was observed). For the remaining two bouts (0-100 and 25-75), an unchanged $\dot{V}_{O_2}$ τ (intermediate with respect to the $\dot{V}_{O_2}$ τ values in 0-50 and 25-125) would imply an engagement of muscle fibres from both the slow and fast twitch pools, as proposed by Brittain et al. (2001) for a full step transition compared to a 2 step transition.

It is well known that $\dot{Q}$ demonstrates a more pronounced Phase I response during transitions started from rest compared to step transitions initiated from a higher basal metabolic rate (Hughson and Morrissey 1982, De Cort et al. 1991, Yoshida et al. 1992). The results reported in this study are entirely consistent with the finding that $\dot{Q}$ has a

greater amplitude and faster time constant during rest to work exercise, relative to work-to-work transitions. It has been proposed that most of the increase in $\dot{Q}$ is principally influenced by an increased HR rather than SV (Whipp et al. 1982, Yoshida et al. 1992), since the SV increases bi-phasically during rest to work step exercise bouts, whereas it did not changed in step transitions started from a higher baseline as reported in the present study. Thus, when exercise starts from rest $\dot{Q}$ is regulated by both SV and HR, whereas when exercise starts from a higher metabolic baseline $\dot{Q}$ is principally under HR control (Yoshida et al. 1992). In order to explain this characteristic behaviour, it has been proposed that the underlying mechanism might be an immediate increase in HR due to the withdrawal of parasympathetic nervous activity (vagal tone) at the onset of exercise (Hughson and Morrissey 1982). On the other hand, Lador et al. (2008) found that even during exercise performed in acute normobaric hypoxia, where vagal activity is reduced, Phase I was reduced but still detectable. Taken together, it seems that $\dot{Q}$ is regulated more by HR than SV, nevertheless a dual origin of the Phase I, a neural (vagal) and mechanical (increase in venous return by muscle pump action), cannot be ruled out (Lador et al. 2008).

This is evident also in our results where Phase I of $\dot{V}_{O_2}$ amplitude was grater from rest to work, confirming the concept of cardio-dynamic phase, while τ was invariant for all types of transitions. It means that even though $\dot{Q}$ τ is slower in work to work since the vagal withdrawal is not present, this would not reflect on $\dot{V}_{O_2}$ which remained constant likely due to a further increase in venous return.

In conclusion, this study has shown that: 1) $\dot{V}_{O_2}$ does not conform to first order linear behaviour within the moderate domain; 2) $\dot{Q}$ does not limit $\dot{V}_{O_2}$ kinetics during step exercise tests within the moderate-intensity exercise domain; 3)$\dot{Q}$ determines a more marked increase in Phase I during rest to work than work-to-work step exercise. The observation that Phase II $\dot{V}_{O_2}$ kinetics does not demonstrate linear behaviour and that $\dot{Q}$ does not appear to regulate $\dot{V}_{O_2}$ kinetics suggests that factors residing within the contracting muscles are responsible for these responses, possibly involving the hierarchical muscle fibres recruitment from more efficient toward less efficient fibres. In addition, Phase I $\dot{Q}$ represents a greater proportion of the response, due to a vagal tone withdrawal and venous return to the heart, at the onset of rest-to-work compared to work-to-work exercise, confirming the cardio-dynamic phase.

RESULTS STUDY 2

Subject characteristics are shown in Table 1. After training a significant improvement in aerobic parameters, assessed during incremental test, has been detected ($\dot{V}_{O_2\ max}$: 7.5 ± 5% *P*<0.001; GET: 19 ± 8.3% *P*<0.001).

Table 1 . Subject characteristics and peak exercise responses

Subject	**Age (yr)**	**Height (cm)**	**Body Mass (Kg)**	$\dot{V}_{O_2\ max}$ **(ml·kg^{-1}·min^{-1})**	**Power (Watt)**
1	24	641	55	41	170
2	23	183	80	51	315
3	27	189	87	43	285
4	23	181	74	52	155
5	25	165	57	35	250
6	33	180	86	39	195
7	23	168	61	32	220
8	30	165	65	44	260
9	20	181	78	41	200
10	20	173	63	33	320
Mean	25	175	71	41	229
SD	4	9	12	7	62

On the contrary the WR_{max} reached after training was 5 ± 12 W insignificantly higher compared to the pre-training result (P>0.05). For each training session the exercise time was 40 minutes and the energy expenditure was ~1,655 KJ after the 4 week training period (12 training sessions) the total exercise time was 480 minutes and the total exercise volume was ~24,823 KJ.

Table 2. the kinetics parameters of $\dot{V}O_2$

		T0	t1	T2	t3	T4	t5
A 1	l/min	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.2
τ 1	s	1.9 ± 2.4	2.5 ± 4.4	1.3 ± 0.8	2.1 ± 1.4	1.9 ± 1.3	1.5 ± 0.9
TD 1	s	0.8 ± 1.5	0.7 ± 1.2	0.3 ± 0.5	1.9 ± 3.4	1.3 ± 2.3	1.3 ± 2.3
A 2	l/min	1.1 ± 0.1	1.1 ± 0.2	1.1 ± 0.1	1 ± 0.1	1 ± 0.2	1.1 ± 0.2
τ 2	s	27 ± 5	26 ± 4 *	25 ± 4 *§	24 ± 4 *§†	22 ± 4 *§†‡	21 ± 4 *§†‡
TD 2	s	12 ± 4	11 ± 3	12 ± 4	14 ± 5	14 ± 4	14 ± 6
A TOT	l/min	1.7 ± 0.1	1.7 ± 0.2	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.2	1.6 ± 0.1
MRT	s	31 ± 6	30 ± 5	31 ± 6	30 ± 7	29 ± 8	29 ± 4
GAIN	$ml \cdot min^{-1} \cdot W^{-1}$	0.013 ± 0.002	0.013 ± 0.002	0.013 ± 0.001	0.014 ± 0.001	0.013 ± 0.001	0.013 ± 0.001

Values are means ± SD. A is the amplitude; τ is the time constant of response; TD is time delay; A tot is the total amplitude; MRT is the mean response time and gain is the ($\Delta\dot{V}O_2/\Delta WR$); 1 indicates the phase I and 2 indicates the phase II. * significantly different from T0 (*P*<0.05); § significantly different from T1 (*P*<0.05); † significantly different from T2 (*P*<0.05); ‡ significantly different from T3 (*P*<0.05).

Pulmonary oxygen uptake kinetics

The parameters estimated for pulmonary $\dot{V}_{O_2}$ kinetics during the step transitions are presented in table 2. Figure 1 presents the $\dot{V}_{O_2}$ response during the transition from rest to 100 W for a representative individual.

A comparison among the step transition tests performed pre (T0), post (T5) and over the period of training (T1, T2, T3, T4) showed for the $\dot{V}_{O_2}$ uptake kinetics significant differences only in the time constant which decrease (22%) progressively throughout the 4 weeks of training (before 27 ± 4.7 after 21.4 ± 4 s, *P* <0.05).

The $\dot{V}_{O_2}$ Phase II τ was slowest at T0 compared to all the other step tests (T0: 27 ± 4.7; T1: 26 ± 4.2; T2: 25 ± 4.3 ; T3: 24.4 ± 4.2; T4: 22.3 ± 4.3 ; T5 21.4 ± 4; *P*<0.05; table 2). Moreover τ was significantly different between a step test and the subsequent one (table2) except between T4 and T5 (*P*>0.05; table2). The weekly rate of decrease of τ $\dot{V}_{O_2}$ was related to GET absolute variation (y = -0.0024x - 0.4603 R2 = 0.8479).

Neither changes in functional gain nor total amplitude has been detected.

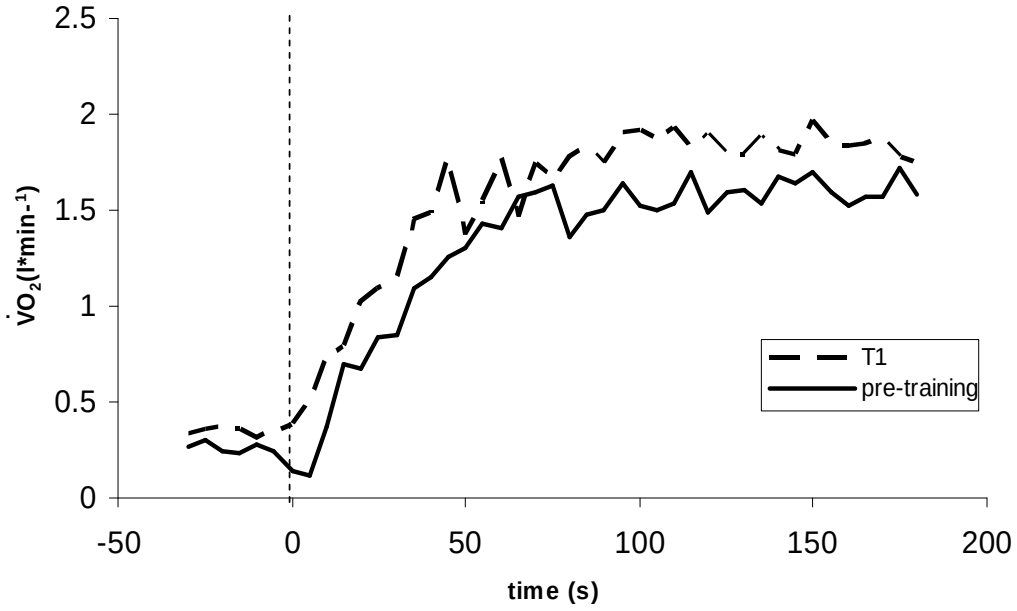


Figure 1. Changes in $\dot{V}O_2$ response to step exercise for a representative individual before and after one session of endurance training.

Cardiac output kinetics

In table 3 are illustrated the parameters assessed for cardiac output ($\dot{Q}$), and the response during the transition from rest to 100 W for a representative individual is shown in figure 2.

Table 3. the kinetics parameters of $\dot{Q}$

		T0	t1	T2	t3	T4	t5
A 1	l/min	2.8 ± 1.4	3.1 ± 1.2	4.4 ± 1.6	4.3 ± 1.3	4.6 ± 1.6	4 .3 ± 1.9
τ 1	s	1.9 ± 0.9	1.6 ± 0.7	2.0 ± 1.2	1.7 ± 0.9	1.8 ± 0.7	1 .6 ± 1.1
TD 1	s	0.4 ± 0.5	0.6 ± 0.6	2.1 ± 0.6	1.3 ± 1	0.7 ± 0.7	0.7 ± 1
A 2	l/min	4 ± 1	4 ± 1	5 ± 1	5 ± 1	5 ± 1	5 ± 1
τ 2	s	15 ± 6	15 ± 5	14 ± 5	14 ± 5	14 ± 5	14 ± 5 *§
TD 2	s	8 ± 5	9 ± 4	10 ± 5	10 ± 5	10 ± 5	11 ± 7
A TOT	l/min	13 ± 2	14 ± 2	13 ± 2	13 ± 2	13 ± 2	13 ± 1
MRT	s	14 ± 5	14 ± 5	14 ± 5	14 ± 3	14 ± 5	14 ± 5
GAIN	$ml \cdot min^{-1} \cdot W^{-1}$	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01

Values are means ± SD. A is the amplitude; τ is the time constant of response; TD is time delay; A tot is the total amplitude; MRT is the mean response time and gain is the ($\Delta\dot{V}O_2/\Delta WR$); 1 indicates the Phase I and 2 indicates the Phase II. * significantly different from T0 (P<0.05); § significantly different from T1 (P<0.05).

The time constant for the $\dot{Q}$ was the only value which changed from pre-training throughout post-training: at T5 $\dot{Q}$ τ was faster compared T0 and T1 (T5: 13.6 ± 5.2; T0: 14.6 ± 5.6; T1: 14.8 ± 5.4; *P*<0.05; table 3). No significant modifications have been found for the other parameters (*P*>0.05; table 3). Phase II $\dot{Q}$ time constant was significantly faster than $\dot{V}_{O_2}$ τ throughout the whole training period (*P*<0.05).

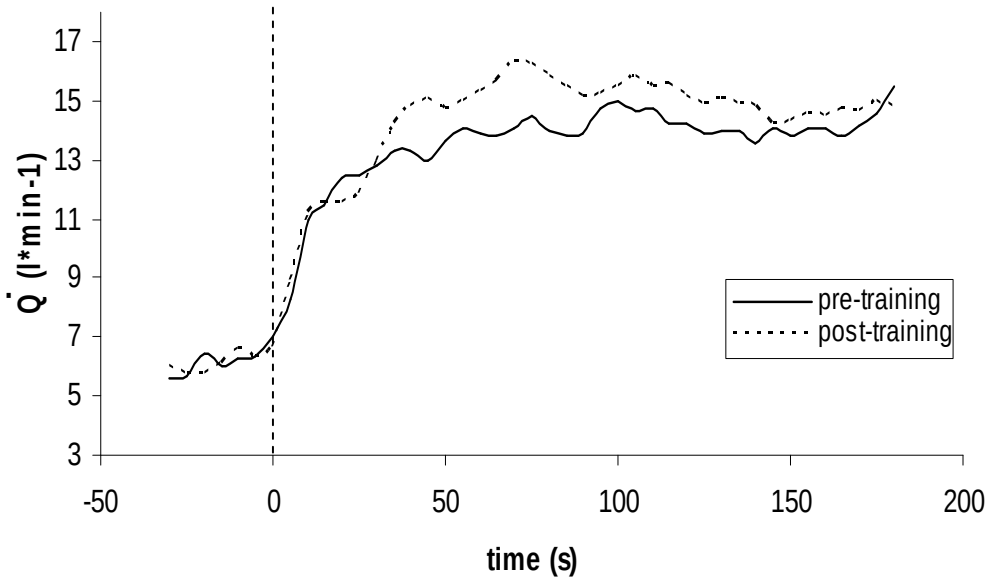


Figure 2. Changes in $\dot{Q}$ response to step exercise for a representative individual before and after the endurance training period.

Deoxyhaemoglobin kinetics

Table 4 presents the results assessed for deoxyhaemoglobin (Δ[HHb]) kinetics all over the course of training. The Δ[HHb] response for a representative individual is shown in figure 3.

Table 4. the kinetics parameters of Δ [HHb]

		T0	t1	T2	t3	T4	t5
τ Δ [HHb]	s	7.6 ± 3.6	6.4 ± 2.7	6.6 ± 2.5	6.6 ± 3.1	6.2 ± 1.8	6 .7 ± 2.0
TD Δ [HHb]	s	8.1 ± 3.6	8.2 ± 2.9	8.1 ± 2.6	9.1 ± 2.6	8.5 ± 2.6	7 .8 ± 2.5
τ' Δ [HHb]	s	16 ± 5	15 ± 3	15 ± 3	16 ± 3	15 ± 3	15 ± 2

Values are means ± SD. τ is the time constant of response; TD is time delay and τ' is the mean response time.

There were no training-induced changes in the parameters evaluated for the deoxyheamoglobin (*P*>0.05; table 4). The calculated Δ[HHb]-to-$\Delta\dot{V}_{O_2}$ ratio gives an indication of a transient "overshoot" during the step transition exercise. Δ[HHb] /$\Delta\dot{V}_{O_2}$ was progressively attenuated during the training period (r = 0.99; figure 5) and it was associated with a decrease in $\dot{V}_{O_2}$ τ (r =0.96, *P*<0.05; figure 4).

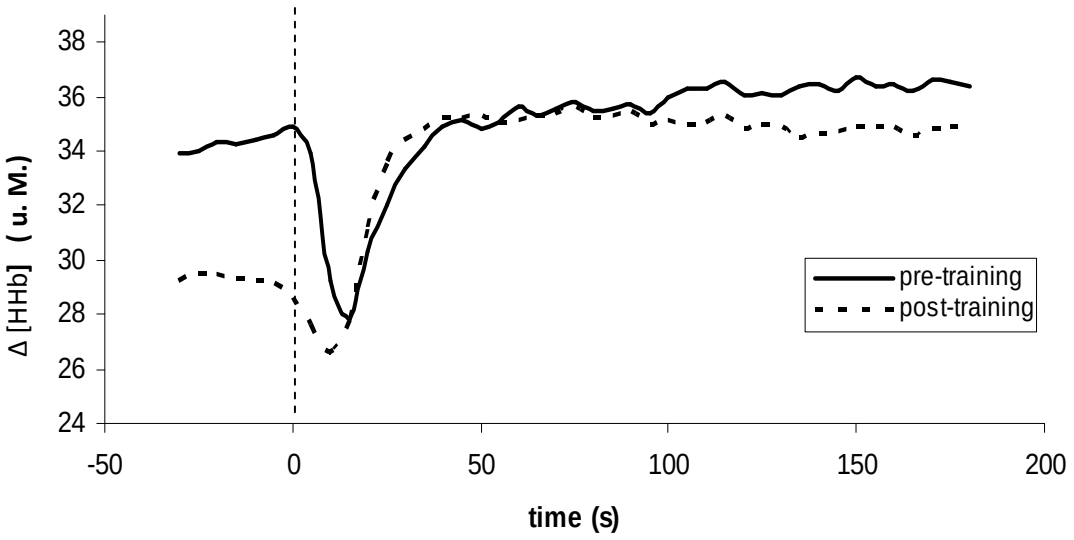


Figure 3. Changes in Δ[HHb] response to step exercise for a representative individual before and after the endurance training period.

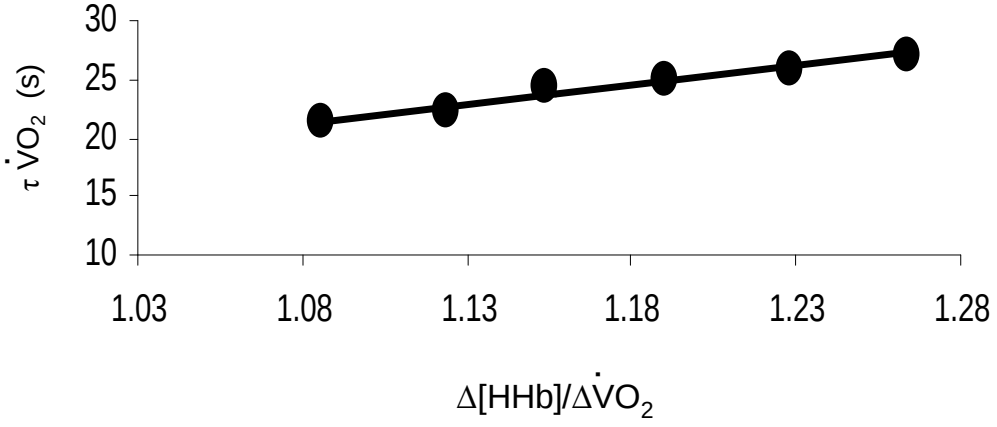


Figure 4. Correlation between Δ[HHb] /Δ$\dot{V}_{O_2}$ and $\dot{V}_{O_2}$ τ during the training period

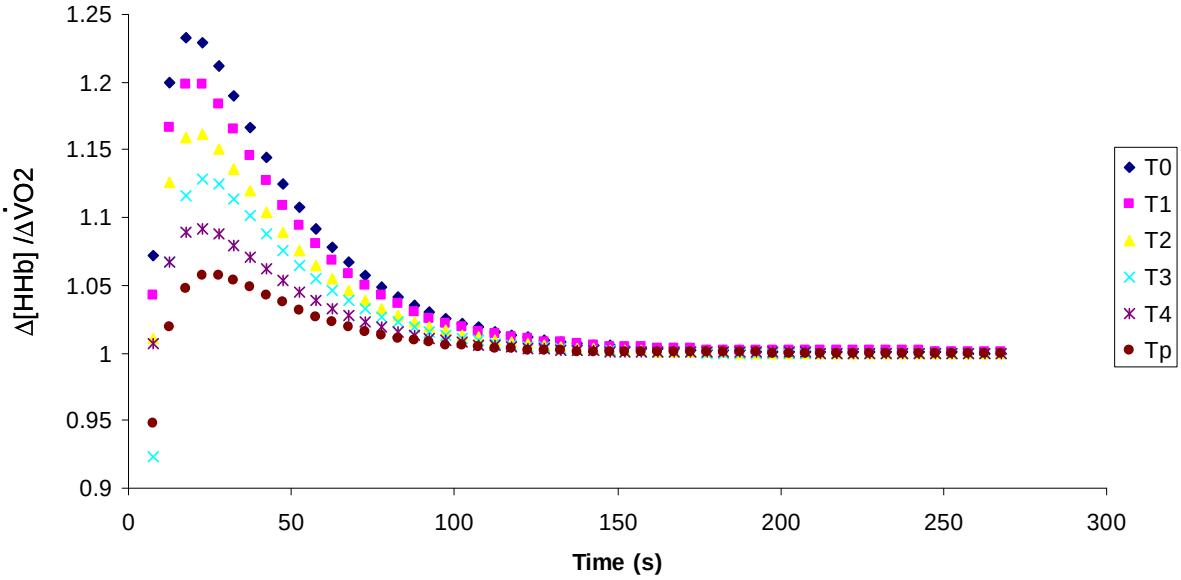


Figure 5. Gradually attenuation in $\Delta[HHb]/\Delta\dot{V}O_2$ along the period of training. Data are illustrated as 5 sec. average of the group mean response.

DISCUSSION STUDY 2

This study assessed the influence of 4 weeks of moderate-intensity endurance training on $\dot{V}_{O_2}$ kinetics, cardiac output and muscle deoxygenation adaptations during moderate exercise step transitions in young healthy subjects. Unlike most previous studies, which employ training on one specific ergometer for the duration of the training sessions, a novel aspect of this study was the utilisation of various commercially available 'cardio' fitness machines to reproduce a standard training session usually completed in a gymnasium by members of the general public. Moreover, to our knowledge, this is the first study to assess possible adaptations of $\dot{V}_{O_2}$ kinetics after only one training session and as frequently as every week over the training intervention. The main findings were as follows: 1) $\dot{V}_{O_2}$ τ became progressively faster as training progressed; 2) $\dot{V}_{O_2}$ τ was significantly faster after only one training session; 3) the cardiac output τ was speeded after 4 training sessions but remained constant thereafter; 4) Δ[HHb] kinetics did not change significantly while there was a gradually attenuation in Δ[HHb] /Δ $\dot{V}_{O_2}$ along the period of training.

Previous studies in untrained subjects have reported that 4-12 wk of endurance training results in a significant speeding of the $\dot{V}_{O_2}$ kinetics time constant (Berger et al 2006, Murias et al. 2010) and that the time course of this adaptation occurred as early as 2-4 days (Phillips et al. 1995, McKay et al. 2009). The results reported in this study are consistent with these findings since we observed an improvement in $\dot{V}_{O_2}$ time constant (25%) after the endurance training period. However, these data expand on these previous studies by showing that the $\dot{V}_{O_2}$ time constant is faster after only one training session (4%). Existing literature indicates that no further changes are

observed after 30, 60 and 90 days of training have been reported (Phillips et al. 1995, Fukuoka et al. 2002), while Murias et al. (2010) demonstrated that $\dot{V}_{O_2}$ time constant increased up to 3 wk without modifications over the remained 9 wk, even though the training was changed in interval training in the final 2 wk.

In the present study, we found progressive and significant changes in $\dot{V}_{O_2}$ kinetics over the 4 wk training program. It is known that $\dot{V}_{O_2}$ kinetics are speeded in a time dependent manner as training process and that the magnitude of the reduction in $\dot{V}_{O_2}$ τ is correlated with the pre-training value of τ (Koppo et al. 2004); that is, subjects with an initial slower $\dot{V}_{O_2}$ τ tend to show a grater speeding. Thus the diverse pre-training values of $\dot{V}_{O_2}$ τ along with a different stimulus of training performed in previous studies might influence the time course of adjustment in $\dot{V}_{O_2}$ kinetics. For example, McKay et al. (2009) reported a $\dot{V}_{O_2}$ time constant of 19 s after 2 days of training and of 14 s after 6 days with no further increases (pre- training $\dot{V}_{O_2}$ τ: 23 ± 8; post- training $\dot{V}_{O_2}$ τ: 14 ± 2); Phillips et al. (1995) reported a τ of 28 s after 4 days and of 16 s after 30 days (pre- training $\dot{V}_{O_2}$ τ: 37 ± 5; post- training $\dot{V}_{O_2}$ τ: 16 ± 1) and Murias et al. (2010) a τ of 21 s after 3 wk with no further changes (pre- training $\dot{V}_{O_2}$ τ: 34 ± 8; post- training $\dot{V}_{O_2}$ τ: 19 ± 7).

The physiological mechanisms responsible for an early improvement in $\dot{V}_{O_2}$ kinetics after endurance training are still unclear. One hypothesis is a possible limitation in O_2 delivery to the working muscle as proposed by Phillips et al. (1995). These authors argued that a faster femoral artery blood velocity after training might be responsible for the $\dot{V}_{O_2}$ τ modifications. However, the kinetics of artery blood flow has been shown to be similar or even faster than those of $\dot{V}_{O_2}$ τ also without training intervention (McPhee et al. 2005, Harper et al. 2006). Moreover, HR τ has been reported to be

always similar to $\dot{V}_{O_2}$ τ (Murias et al. 2010) and we demonstrated that, even though $\dot{Q}$ changed after 4 training session (4%), $\dot{Q}$ τ was significantly faster compared to $\dot{V}_{O_2}$ time constant over the training period and the changes in the $\dot{V}_{O_2}$ τ preceded the changes in the $\dot{Q}$ τ. Therefore the hypothesis that bulk O_2 is limiting to exercising muscles and dictates oxygen uptake kinetics is not necessarily supported by the literature and the present study, at least for young healthy humans. Alternatively, it has been proposed that a possible delay in blood distribution at the capillary level, and thus in O_2 delivery to the muscle microvsculature, might contribute towards the slow $\dot{V}_{O_2}$ kinetics in the untrained state. This is supported by the observation that capillary blood flow is slower than femoral blood flow (Harper et al. 2006).

To provide an indication of the dependency of $\dot{V}_{O_2}$ on O_2 extraction during a step increment in work rate, we calculated the Δ[HHb]-to-Δ $\dot{V}_{O_2}$ ratio using the NIRS-derived [HHb] signal and the pulmonary $\dot{V}_{O_2}$ signal. An increase in the Δ[HHb]-to-Δ $\dot{V}_{O_2}$ ratio is purported to indicate a greater increase in muscle O_2 extraction relative $\dot{V}_{O_2}$, suggestive of a mismatching between muscle O_2 delivery to O_2 utilisation. In this study, we observed a transient overshoot in the Δ[HHb]-to-Δ $\dot{V}_{O_2}$ ratio before steady state values were attained (figure 5) and that this overshoot was attenuated as training proceeded, and was accompanied by a progressive reduction in the $\dot{V}_{O_2}$ time constant. This suggests that the balance between muscle O_2 delivery and muscle O_2 utilisation was improved as training proceeds and this contributed towards the faster $\dot{V}_{O_2}$ kinetics. Similar findings were reported by Murias et al. (2010) during 3 weeks of endurance training and in another study by the same group where they reported that in a range of slow to fast $\dot{V}_{O_2}$ time constant the Δ[HHb]-to-Δ $\dot{V}_{O_2}$ overshoot was progressively attenuated as $\dot{V}_{O_2}$ τ became smaller (Murias et al. 2011). One possible

explanation for these findings and the findings of the present study is that capillary blood flow is improved with exercise training leading to a better muscle O_2 delivery and muscle O_2 utilisation and faster $\dot{V}_{O_2}$ kinetics. Indeed, it has been shown that only a single bout of exercise is able to enhance endothelium-dependent vasodilatation and blood flow as soon as 12-24 h later (Haram et al. 2006) and vascular endothelial growth factor (VEGF), which signals angiogenesis in human skeletal muscle (Gustafsson et al. 1999). These rapid adaptations would be expected to facilitate a greater distribution of O_2 to the muscle fibres.

However, Δ[HHb] dynamics, which also provide a non-invasive marker of the dynamic balance between muscle O_2 delivery and muscle O_2 utilisation, was unchanged as training progressed suggesting that muscle O_2 delivery and muscle O_2 utilisation adapted in a synchronous fashion in response to training.

While improved muscle O_2 availability after training may directly speed $\dot{V}_{O_2}$ kinetics, this may also indirectly speed $\dot{V}_{O_2}$ kinetics by increasing the proportional recruitment type I muscle fibres. These fibres have a greater sensitivity to vasodilatory substances and maximal responsiveness to endothelium-dependent compounds (Behnke et al. 2003), which might explain the reduced Δ[HHb]-to-$\Delta\dot{V}_{O_2}$ ratio as training proceeds. Moreover, this would be expected to result in a corresponding reduction in type II muscle fibre type recruitment. It is know that muscle fibre recruitment patterns have a significant influence on the kinetics of $\dot{V}_{O_2}$ (Carter et al. 2004, Krustrup et al. 2004) and as such changes in muscle fibre recruitment patterns, either independently of, or consequent to improved muscle O_2 availability, may have contributed to the faster $\dot{V}_{O_2}$ kinetics with training.

Another adaptation provoked by endurance exercise training is mitochondrial biogenesis, which has been shown to coincide with the rapid earlier modifications invoked by high intensity interval training (Daussin et al. 2008, Little et al. 2011). Moreover, recent data demonstrate that the activation of molecular signalling pathways associated with mitochondrial biogenesis was activated in a similar manner after only one session of either high intensity interval training or moderate intensity continuous training on a treadmill, matched for work done (Bartlett et al. 2012). Therefore, mitochondrial biogenesis might represent an important candidate mechanism for the rapid improvement in $\dot{V}_{O_2}$ kinetics reported in the current study. Given that type I muscle fibres have a high mitochondrial and capillary density, and that the signalling for mitochondrial biogenesis also signals for type II to type I muscle fibre type conversion (Lin et al. 2002), it is possible that muscle fibre type changes may underpin all these modifications and the rapid adaptive response in $\dot{V}_{O_2}$ kinetics after training. Further research is required to resolve the underlying mechanisms for the training-induced improvements in $\dot{V}_{O_2}$ kinetics.

The improvement in $\dot{V}_{O_2}$ kinetics after 4 wk of low intensity endurance training has important metabolic and performance implications. Moreover the data presented in this study demonstrate that undertaking a 'classical' cardiovascular training session in the gym is capable of speeding $\dot{V}_{O_2}$ kinetics after just one training session. Further research should address the minimum stimulus required to provoke adaptations in $\dot{V}_{O_2}$ kinetics since this would be expected improve exercise tolerance and would be useful to use in exercise prescription with patient and elderly populations.

In summary, the present study demonstrated that the Phase II $\dot{V}_{O_2}$ time constant was decreased during moderate-intensity exercise after 4 wk of endurance training. Importantly, $\dot{V}_{O_2}$ kinetics became significantly faster after only one training session and was progressively reduced throughout training. However, cardiac output kinetics were not improved until after 4 training sessions with no further modifications suggesting that bulk O_2 availability was not responsible for the adaptations in $\dot{V}_{O_2}$ kinetics. The time course of Δ[HHb] did not change significantly over the training intervention while the Δ[HHb] /Δ $\dot{V}O_2$ ratio was gradually reduced over the training intervention. Therefore, the acceleration of Phase II $\dot{V}_{O_2}$ time constant might be linked with an improved O_2 availability within the muscle microvasculature, however, since local factors within the muscle cells were not measured in this study, we cannot exclude the possibility the factors intrinsic to the contraction muscles, such as the muscle fibre recruitment patterns or an increase mitochondrial volume, contributed towards the faster $\dot{V}_{O_2}$ kinetics with training.

Chapter VI : GENERAL CONCLUSION

The main finding for both the studies of this thesis is that O_2 delivery to working muscle is not a limitation in $\dot{V}_{O_2}$ kinetics. In the first study has been demonstrated that cardiac output was significantly faster than $\dot{V}_{O_2}$ time constant for every bout of exercise undertook in the moderate domain; in the second study has been demonstrated that cardiac output was faster than $\dot{V}_{O_2}$ kinetics even before and throughout the training intervention.

As alternative to explain changes in oxygen consumption kinetics it has been taken into consideration the muscle fibres recruitment hypothesis. Particularly the slower response of O_2 kinetics during step exercise transitions in the upper region of moderate domain ,carried out in the first study, suggested progressively hierarchical muscle fibres recruitment from more efficient toward less efficient fibres. In the second study a likely modification in microvascular blood distribution suggests that muscle fibre type changes may underpin these modification in O_2 uptake and signalling for this adaptive response which can occur rapidly after training.

In conclusion, the present thesis has established that muscle fibres recruitment exerts a strong influence on the pulmonary O_2 response either during step transitions within the moderate domain or in modulating the adaptation following a period of endurance training.

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