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Effects of acute hypoxia on the VO_{2p} kinetics of older adults during exercise

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Abstract

This thesis investigated the effect of hypoxia in determining the rate of adjustment in pulmonary O_2 uptake (VO_{2p}) for young and older adults. The pulmonary oxygen uptake (VO_{2p}), heart rate (HR), limb blood flow (LBF), and muscle deoxygenation [HHb] were examined during the on-transient of moderate-intensity exercise in normoxia and hypoxia.

Young and older adults exhibited slower VO_{2p} kinetics in hypoxia compared to normoxia. Under hypoxic conditions, older adults showed a great mismatch between O_2 delivery and O_2 utilization within the muscle, this being responsible for the slower VO_{2p} kinetics. On the other hand, young adults did not show significant differences in the matching of O_2 delivery and O_2 utilization between normoxia and hypoxia. Thus, factors other than the microvascular O_2 delivery (i.e central O_2 delivery and/or mithocondrial respiration) are responsible for the slower VO_{2p} kinetics observed for young adults under hypoxic conditions.

For older adults, the vasodilatory response to hypoxia was found to depend on the work rate. At very low work rate, older adults were able to counterbalance the hypoxia-induced drop in CaO_2 with an increased blood flow, thus preventing the slowing of the VO_{2p} kinetics. At higher work rate however, older adults exhibited a slow VO_{2p} kinetics, with a great mismatch between O_2 delivery and O_2 utilization within the muscle.

Co Autorship

The following thesis includes material from the manuscripts which have been published, accepted for publication or submitted for publication:

1. Livio Zerbini; Alfredo Brighenti; Barbara Pellegrini; Lorenzo Bortolan; Tommaso Antonetti; Federico Schena : **Effects of acute hypoxia on the VO_{2p} kinetics of older adults during cycling exercise**. Accepted 7 march 2012 Applied Physiology, Nutrition, and Metabolism.

2. Livio Zerbini; Matthew D. Spencer ; Tyler Grey; Juan M. Murias; John M. Kowalchuk; Federico Schena ; Donald H. Paterson: **Effect of acute hypoxia on muscle blood flow, VO_{2p}, and [HHb] kinetics during leg extension exercise in older men**

These studies were designed by L.Zerbini and with input from the co-authors. The majority of the data were collected by L Zerbini with the help of Tyler M Grey for the second study and Alfredo Brighenti for the third study. All the data were analyzed by L.Zerbini.

L.Zerbini wrote the original manuscript for each of the studies and the co-authors provided financial, lab support and editorial feedback.

Ad Elena e ai miei genitori

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*p<0.05, hence significantly different from normoxia (FIO_2=20.9%)

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*p<0.05, hence significantly different from normoxia (FIO_2=20.9%)

LIST OF ABBREVIATIONS

Acetyl CoA	Acetyl co-enzyme A
ADP	Adenosine diphosphate
Amp	Amplitude
AMP	Adenosine monophosphate
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
a-v0_2diff	Arterial venous oxygen difference
BSL	Baseline
c	Velocity of sound in a tissue (1540 cm•s^{-1})
C_{95}	95% Confidence Interval
Ca^{2+}	Calcium
cm	Centimeters
CO_2	Carbon
Cr	Creatine
CSA	Cross-sectional area
d	Diameter
DCA	Dichloroacetate
e	Exponential function
EMG	Electromyography
f	Frequency
f_0	Emitted frequency
FA	Femoral artery

$FADH_2$	Flavin adenine dinucleotide
g	Gram
H^+	Hydrogen
H_2O	Water
Hb_{tot}	Total hemoglobin
HHb	Deoxygenated hemoglobin
HR	Heart Rate
KE	Knee-extension
Kg	Kilograms
L	Litre
L-NAME	Nitro-$_L$-arginine methyl ester
LBF	Leg Blood Flow
MBV	Mean blood velocity
mg	Milligrams
min	Minutes
MHz	Mega Hertz
mL	Milliliters
mM	Millimolar
mmHg	Millimiters of mercury
MRT	Mean response time
ms	Milliseconds
n	Sample size
N_2	Nitrogen
NAD^+	Nicotinamide adenine dinucleotide

NADH	Reduced form of NAD^+
NIRS	Near Infrared Spectroscopy
NO	Nitric Oxyde
O_2	Oxygen
O_2Hb	Oxygenated hemoglobin
PCO_2	Partial pressure of carbon dioxide
$P_{cap}O_2$	Partial pressure of O_2 within the capillary
PCr	Phosphocreatine
PDH	Pyruvate dehdrogenase
$P_{ET}CO_2$	End-tidal partial pressure of carbon dioxide
$P_{ET}O_2$	End-tidal partial pressure of oxygen
Pi	Inorganic phosphate
$P_{mito}O_2$	Partial pressure of O_2 within the mitochondria
PO_2	Partial pressure of oxygen
PPO	Peak Power Output
Q	Blood flow
r	Radius
RBC	Red blood cell
RER	Respiratory exchange ratio
s	Seconds
SD	Standard deviation

CHAPTER 1

INTRODUCTION

1.1 Energy production during exercise

At rest and during steady-state exercise, the amount of energy required by the human body is balanced by the aerobic energy production. In these cases, the O_2 demand and energy production systems (ATPases, mitochondrial oxidative phosphorylation) are perfectly matched with the O_2 delivery systems (lungs, heart and vascular system). However, humans rarely perform constant-load exercise in the daily life. Rather, they exhibit several, frequent changes in exercise intensity and metabolic rate. For this reason, has always been interest in studying the rate of adjustment of oxygen consumption (VO_2) during the transition from rest or basal intensity of exercise to a higher work rate. Moreover, it is during the transition from rest to a new steady state or from one steady-state to another that the control mechanisms involved in the physiological response can be studied.

Because the body cannot easily store ATP, [only ~5 mM wet/kg (Hultman, et al. 1967) and what is stored gets used up within a few seconds], it is necessary to create ATP continually during the exercise. There are three major ways used by the body to convert nutrients into energy: the anaerobic alactic system, the anaerobic lactic system and the aerobic oxidative phosphorylation.

The anaerobic alactic system is based on the breakdown of stored phosphocreatine (PCr) by creatine kinase to rephosphorylate adenosine diphosphate (ADP) resulting in the formation of ATP and creatine (Cr):

$$PCr + ADP + H^+ \leftrightarrow Cr + ATP \qquad (1.1)$$

The energy supplied by the breakdown of PCr is large, and it can be produced instantaneously. However, the time during which this high energy can be supplied never exceeds 10 seconds because of the limited amount of PCr stored in tissues (Tschakovsky and Hughson, 1999).

The anaerobic lactic system produces ATP via oxidation of carbohydrates by glycolysis resulting in lactate and H^+ production:

$$\text{Glucose} + 2P_i + 2ADP \rightarrow pyruvate \leftrightarrow lactate + 2H_2O + 2ATP \quad (1.2.)$$

The disadvantage of this energy pathway is the production of H^+ ions, which may alter the pH of the contracting cells. For these reasons, the role of O_2 as final acceptor in the phosphorylation chain becomes crucial for the synthesis of new ATP.

In fact, the majority of the ATP supplied to working muscles is derived from the re-synthesis of ADP via oxidative phosphorylation (OxPhos) in the mitochondria. The acetyl coenzyme A (acetyl CoA) produced from pyruvate, enters the Krebs cycle within the mitochondria to produce the reducing equivalents nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$). These substrates are then available to the electron transport chain starting a series of redox reactions with O_2 being the final electron acceptor in the cytochrome-c complex.

$$\text{NADH} \rightarrow \text{Complex I} \rightarrow \text{Ubiquinone} \rightarrow \text{Complex III} \rightarrow \text{Cytochrome-c} \rightarrow \text{Complex IV} \quad (1.3)$$

Complexes I and III both cause a net efflux of 4 protons from the mitochondrial matrix into the intermembrane space (IMS) and Complex IV causes a net efflux of 2 protons from the mithocondrial matrix into the IMS. This “proton gradient”, outside of the mitochondria, then drives the synthesis of ATP from ADP and Pi via Complex V.

This proton flux results in the formation of ATP from ADP and Pi from the following reaction:

$$\text{NADH} + 3\text{Pi} + \text{H}^+ + 3\text{ADP} + ½\,\text{H}_2\text{O} \rightarrow 3\text{ATP} + \text{NAD}+ + \text{H}_2\text{O} \quad (1.4)$$

Even if the majority of ATP is derived by the OxPhos, it is well-known that when energy demand is increased in a square wave fashion, a delay is observed in matching the ATP supply from oxidative phosphorilation (OxPhos) to this ATP demand (see Fig.1.1). It is apparent that the availability of all substrates on the left-hand side of equation 1.4 is necessary to support ongoing ATP synthesis. Thus, delay in providing any of the required substrates as ATP requirements change, may limit or constrain the

full activation of oxidative phosphorylation, thus requiring alactic and lactic energy sources to support the deficit in oxidative ATP synthesis.

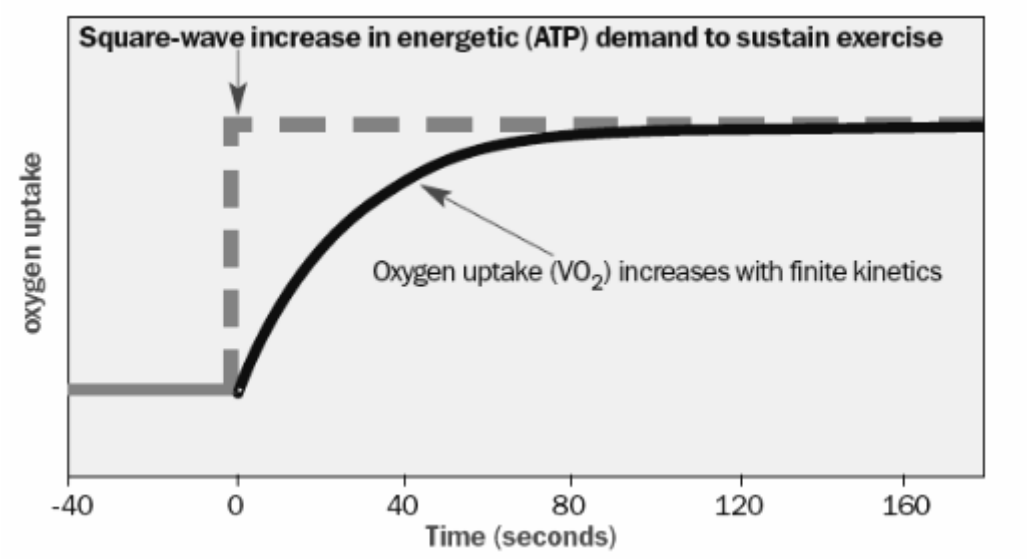

Fig 1.1

Schematic representation of the immediate increase in ATP requirements at the onset of exercise and the delay in the response of the VO_2

1.2 Pulmonary Oxygen Uptake and Muscle Oxygen Consumption

Rest-to-work transitions or an increase in generated power output require a rapid adjustment in the ATP supply. However, it takes 2-4 min for the pulmonary O_2 uptake (VO_{2p}) to reach the steady-state level necessary to fully synthesize the ATP via oxidative phosphorylation. This kinetic response is best approximated by an exponential function (Barstow, et al. 1994) and it can be divided into 3 distinct phases.

Phase 1 (known as the *cardio-dynamic phase*) is characterized by a rapid increase in VO_{2p} within the first 20 s from the onset of muscular contractions. It represents the initial increase in cardiac output and thus in pulmonary blood flow. Within this phase, there is little change in $PETCO_2$ and $PETO_2$, because blood is still not affected by changes in the metabolic rate (Whipp, et al. 1982), so that the venous O_2 content in

the muscles is unchanged. Phase 2 (or *fundamental phase*) is characterized by an exponential increase in VO_{2p} reflecting the change in O_2 content of mixed venous blood of the exercising muscle. The issue on the relation between pulmonary and muscle oxygen uptake was addressed by studies where the leg blood flow and the leg VO_2 (the latter calculated via the Fick's equation: $VO_2 = Q[CaO_2 - CvO_2]$) were simultaneously measured during moderate-intensity (Grassi, et al. 1996) and heavy-intensity (Bangsbo 2000) exercise. It was demonstrated that both the leg VO_2 (VO_{2m}) and the pulmonary VO_2 (VO_{2p}) increase exponentially with time and that the associated time constants (τ is the time constant defined as the duration of the time for VO_2 to increase to 63% of the steady state increase) differ by less than 10%. Moreover, similar time constants were found (Rossiter, et al. 1999) to characterize the exponential decay in PCr and the exponential increase in VO_{2p} (provided the Phase 1 is excluded in order to correct for the venous transport delay between muscles and lungs). These evidences support the idea that the kinetics of phase 2 VO_{2p} reflects the kinetics of VO_{2m}, so that the VO_{2m} kinetics can be more easily obtained by measuring the VO_{2p} kinetics (Whipp, et al. 2002).

Phase 3 in the VO_{2p} response occurs when a steady-state is reached, where the ATP demand is met by aerobic ATP production (exercise intensity below the estimated lactate threshold). When the VO_{2p} increases above the steady-state level characteristic of phase 3, a so-called VO_{2p} "slow component" appears (exercise intensity above the estimated lactate).

1.3 Control of muscle O_2 uptake kinetics

Whether the VO_{2p} kinetics is limited by insufficient O_2 delivery to the muscle or by slow activation of factors governing O_2 utilization within the muscle or by some combination of the two is still under debate. Two main hypotheses have been suggested in order to explain the mechanisms that limit the rate at which the VO_2 rises at the onset of the exercise:

1) the *feed-forward* or *O_2 delivery* hypothesis which includes:

- the activation in the cytosolic ATP hydrolysis through Ca^{2+}

- the limitation in the NADH delivery to the electron transport chain
- the level of intra-mitochondrial PO_2 and the competition between nitric oxide and O_2 for the binding site at cytochrome c oxydase
- the terminal electron acceptor in the electron transport chain (Kindig, et al. 2001).

2) the *feed-back* or *metabolic inertia* hypothesis which includes:

- the alteration in the concentration of ATP, ADP, Pi, PCr and Cr (Rossiter, et al. 1999).

These two hypotheses are briefly examined below.

1.3.1 Limitation in the O_2 Delivery

It is well known that patients exhibiting a slower VO_{2p} kinetics (because they are affected by various pathological diseases) also exhibit an altered O_2 delivery to the working muscles (Jones, et al. 2005). For instance, in addition to a slower VO_{2p} kinetics, oxygen delivery is impaired in chronic respiratory disease patients (Nery, et al. 1982), in COPD patients (Palange, et al. 1995), in chronic heart failure patients (Arena, et al. 2001), and in diabetics (Regensteiner, et al. 1998). It is still unknown whether the slower VO_{2p} kinetics observed for these diseases is due to a metabolic impairment or to an impaired ability to adequately deliver O_2 or to both of them.

If O_2 delivery exceeds the O_2 need at the onset of contractions, the capillary and the intracellular PO_2 increase transiently. On the other hand, if O_2 delivery is not enough to meet the O_2 need at the onset of contractions, then the intracellular PO_2 declines and the capillary PO_2 decreases accordingly (Behnke, et al. 2001).

A good amount of studies on muscle fibers preparation showed that the intracellular PO_2 does not fall immediately at the onset of an exercise (Hogan 2001; Hogan, et al. 1999). Combined with findings that the blood flow rapidly increases at the onset of contractions (Delp 1999), these results suggest that the increase in O_2 delivery and the increase in O_2 demand are well matched in the first seconds of the contractions, in a single muscle fiber preparation.

Moreover, several studies suggest that the rate of muscle O_2 consumption becomes slower when less O_2 is available. This fact reveals that a possible constraint in the VO_2 kinetics resides in the delivery of O_2 to the working muscles. Supporting this idea, a slower VO_{2p} kinetics was measured as a result of an impaired cardiac output due to pharmacological 3-blockade (Hughson, et al. 1991), as a result of a slower femoral artery limb blood flow on moving from upright to supine (MacDonald, et al. 1998) and as a result of a reduction in inspired O_2 content (Hughson, et al. 1995). However, compared to "normal healthy" conditions, an increased O_2 delivery (convective and diffusive) alone, does not appear to make the VO_{2p} kinetics faster during moderate intensity exercise. In fact, the use of hyperoxic gas mixtures (Hughson, et al. 1995) and EPO (Wilkerson, et al. 2005) to improve the O_2 diffusion and O_2 transport in human subjects performing a moderate-intensity exercise did not result in a faster adjustment of VO_{2p}.

Thus it appears that reducing the O_2 delivery makes the VO_{2p} kinetics slower, whereas increasing the O_2 delivery does not make the VO_{2p} kinetics faster compared to normal conditions.

It is therefore likely that within populations where O_2 delivery is impaired, the VO_{2p} kinetic response is constrained by the inability to increase the O_2 delivery while exercising

1.3.2 Animal models: metabolic inertia or oxygen delivery?

Studies of intracellular metabolic processes and their coupling to VO_{2p} provide evidence that the control of muscle O_2 consumption resides within the muscle.

For instance, a canine gastrocnemius muscle preparation was used for a series of experiments (Grassi, et al. 1998) devoted to study whether an increase in O_2 delivery to the muscle was able to produce a faster muscle VO_2 kinetics or not. All these studies used a mechanically constant pump and electrically stimulated dog muscles. The first experiment studied the effect of increasing the *convective O_2 delivery* (increase in cardiac output via a mechanical pump, with vasodilatation sustained via adenosine) during the transition from rest to an electrically induced isometric titanic contraction at 60% and 100% of VO_{2max}. No speeding up of muscle O_2 consumption was observed during the transition to 60% of VO_{2max}, thus

suggesting that control of muscle O_2 consumption was regulated by factors other than O_2 delivery in this case. On the contrary, a faster VO_2 kinetics compared to the control condition was observed during the transition to 100% of VO_{2max}, thus suggesting that O_2 delivery could play an important role in the limitation of the VO_2 kinetics for transitions to high intensity exercises (Grassi, et al. 2000). Utilizing the same model, the authors (Grassi, et al. 1998) evaluated the effect of *peripheral O_2 diffusion* on the kinetics of muscle O_2 consumption via elevated inspired O_2 content and pharmacological intervention. Similar adjustments of muscle O_2 consumption were observed under control conditions, as well as during conditions of hyperoxia (100% inspired O_2) and hyperoxia plus pharmacological intervention (RSR-13 induces a significant rightward shift of the oxy-hemoglobin dissociation curve), suggesting that improvements in O_2 diffusing capacity do not improve muscle O_2 consumption. However, caution in interpretation and transfer of these results to human models must be exercised. The isolated *in-situ* canine gastrocnemius muscle model uses highly oxidative muscle fibers, it is artificially exposed to high levels of blood flow (three times more than the blood flow of humans at the same exercise intensity) and exhibits fast blood flow kinetics even under control conditions. Moreover, the non-physiological muscle stimulation protocol may be difficult to apply to human models.

1.3.3 Limitation due to metabolic inertia (cellular respiration)

If part of the literature focused on the effect of the modification of O_2 delivery on the VO_2 kinetics, trying to confirm or disconfirm the O_2 delivery hypothesis, other studies concentrated their attention on the potential rate-limiting steps in oxidative phosphorylation that may constrain the adjustment of VO_{2m}. Nitric oxide (NO) came to the attention of the researchers as a possible mechanism in the control of the adjustment of VO_2. NO is involved in a wide range of physiological functions, including neurotransmission, platelet aggregation and the regulation of vasodilatation (it is a strong vasodilator) (Joyner, et al. 1997). NO could also impair mitochondrial respiration by competing with O_2 at the final electron acceptor at the cytochrome c oxidase (Brown 2000), potentially limiting the VO_{2p}. Thus, a

reduction in NO via inhibition of NO synthesis by nitro-L-arginine methyl ester (L-NAME) may improve the mitochondrial respiration favoring O_2 in the competition to be the final acceptor at the end of the phosphorilation oxidative chain. In fact, a faster VO_{2p} kinetics was observed during moderate-intensity exercise in humans (Jones, et al. 2003) after use of L-NAME.

On the contrary, no improvement (speeding up) in the adjustment of VO_2 associated with L-NAME was observed in the canine model (Grassi, et al. 2005). However, this study was criticized because of some bias in the attempt to extend the results of dog preparations to the human model (McDonough, et al. 2006).

An area that has recently received much attention is the mitochondrial pyruvate dehydrogenase (PDH) complex. PDH catalyzes the rate-limiting step for the oxidative decarboxylation of carbohydrate-derived pyruvate (a product of glycolysis) to acetyl-CoA. As the Krebs cycle utilizes acetyl groups to generate reduced co-enzymes (i.e., NADH and $FADH_2$) for oxidative phosphorylation, it is crucial that PDH supplies a sufficient amount of acetyl-CoA. Any imbalance between pyruvate production and conversion to acetyl CoA (as regulated by PDH activity) would result in the reduction of pyruvate to lactate, catalyzed by lactate dehydrogenase (LDH) the high activity equilibrium enzyme. Use of dichloroacetate (DCA) infusion to increase the PDH activity before contractions in a couple of experiments resulted in less phosphocreatine [PCr] breakdown (Howlett, et al. 1999) (Timmons, et al. 1998) and reduced lactate accumulation (Howlett, et al. 1999). These finding suggest a smaller (compared to normal conditions) contribution of the alactic and lactic anaerobic energy sources, potentially resulting in a faster VO_{2m} kinetics. Furthermore, DCA infusion caused a drop in intracellular PO_2 (a proxy measure for VO_{2m}) in isolated Xenopus single muscle fibers (Howlett and Hogan, 2003). However, despite the compelling evidence for promoting faster oxidative phosphorylation, DCA infusion was not observed to speed up the VO_2 kinetics in dogs (Grassi, et al. 2002) or the VO_{2p} kinetics in humans (Rossiter, et al. 2003). Of interest, recent experimental data (Gurd, et al. 2006) has shown that, following a bout of heavy-intensity

warm-up exercise, PDH activity was increased before a subsequent moderate-intensity exercise bout. In the study of Gurd et al (2006) however, no correlation between PDH activity and the VO_{2p} characteristic time constant τ was found, so that no evidence exists for a PDH rate-limiting role on the VO_{2p} kinetics.

A recent paper (Grassi, et al. 2011) demonstrated a mechanism which is likely to limit the VO_2 kinetics at the onset of an exercise, once O_2 delivery limitations were removed using a pump-perfused canine skeletal muscle preparation. The study showed a significantly faster VO_2 kinetics after acute creatine kinase (CK) inihibition, thus suggesting that the CK-catalyzed breakdown of phosphocreatine (PCr) at the onset of exercise attenuates the rise in ADP concentration, so that the activation of oxidative phosphorylation is slowed.

1.4 Towards a unifying concept

It is likely that factors related to both metabolic substrate provision and O_2 transport are interacting to regulate the rate of adjustment of VO_2. In this context, Poole et al. (2009) noticed that, when plotting the VO_2 time constant as a function of the muscle O_2 delivery (Fig 1.2), a "tipping point" exists which separates two regimes in the VO_2 kinetics, namely the O_2 delivery dependent and the O_2 delivery independent zone. Furthermore, a recent study demonstrated that, for a time constant τVO_{2p} (time needed to reach 63% of the VO_2 steady state response) greater than 20 seconds, the VO_2 rate of adjustment is primarily limited by O_2 provision for young healthy humans (Spencer, et al. 2011a). So it is likely that τVO_{2p} = 20 s represents a sort of threshold above which the VO_2 kinetics is primarily limited by O_2 provision and below which the VO_2 kinetics is primarily limited by O_2 utilization

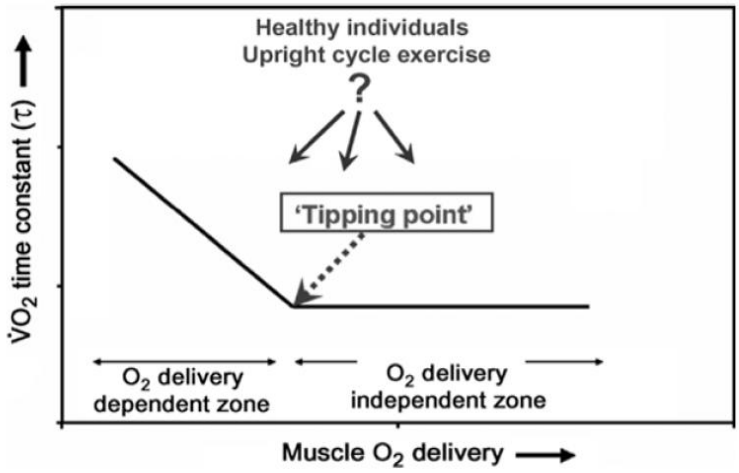

Fig 1.2.

Schematic that explains the dependence of VO_2 kinetics on muscle O_2 delivery in some, but not all, circumstances (Poole-Jones 2005)

1.5 Aging and VO_2 kinetics

Beyond maturity, exercise tolerance declines with advancing age, as proven by the well-established age-related reduction in the maximal capacity for O_2 utilization (i.e. VO_{2max}) (Betik, et al. 2008) and by the slowing in the VO_2 kinetics at the onset of exercise (Babcock, et al. 1994), (Bell, et al. 1999), (Scheuermann, et al. 2002).

Whereas older adults rarely spend time close to their VO_{2max}, all their everyday tasks involve a VO_2 transient. So, the ability of older adults to perform everyday tasks is greatly dependent upon the age-related slowing in the VO_2 kinetics. A slowed VO_2 kinetics compromises exercise tolerance causing a greater O_2 deficit and thus perturbing the intramyocyte milieu (i.e., Δ phosphocreatine, [ADP], H^+, inorganic phosphate, [glycogen]).

The slowing in the VO_2 kinetics with advancing age is generally ascribed to a potential impairment of O_2 delivery at several levels. Limitations are reported to occur at the *central level*, i.e. slower HR kinetics and reduced submaximal cardiac output for a given VO_2, which could lead to potential central impairment in muscle O_2 delivery, slowing the muscle VO_2 kinetics (Bell, et al. 1999), (Chilibeck, et al. 1996), (Scheuermann, et al. 2002). Concerning *upstream limitations*, a reduced artery blood flow was measured for older adults (Proctor, et al. 1998). However, Bell et al. (1999) observed a faster limb blood flow kinetics (mean blood velocity measured via Doppler ultrasound) compared to the VO_{2p} kinetics for older adults, suggesting that O_2 delivery was sufficient throughout the entire exercise on transient. Thus, a general consensus does not exist and some studies failed at showing aging related changes in muscle blood flow (Magnusson, et al. 1994), (Proctor., et al. 2003), (Richardson, et al. 1980). This upstream impairment in O_2 delivery may, in turn, alter downstream conduit artery and local muscle O_2 delivery response (DuManoir, et al. 2010c), leading to focus on *downstream limitations* (microvascular O_2 delivery impairment) as the primary limitation for the VO_2 kinetics in older adults (Murias *et al.*,2011).

In this regard, Behnke et al. (2005) observed a lower microvascular PO_2 in old compared to younger rats at rest using phosphorescene quenching techniques to monitor microvascular PO_2 in isolated spinotrapezius muscles. During the exercise on-transient, in the same experiment, microvascular PO_2 fell transiently to levels below those observed at the steady-state for older but not for younger animals. These data suggest that older animals maintain the required VO_{2p} response in the presence of reduced microvascular O_2 delivery, so that microvascular PO_2 falls to low levels, which may constrain the kinetics of O_2 consumption. In terms of near-infrared spectroscopy (NIRS) which reflects the matching of O_2 delivery and O_2 utilization within the exercising muscle microvasculature, DeLorey et al. (2004), observed that the microvascular deoxygenated hemoglobin increases more for old than for younger adults, for a given increase in VO_{2p} ($\Delta HHb/\Delta VO_{2p}$), thus reflecting a greater local muscle O_2 extraction. Beside a slower VO_{2p} kinetics, the adjustment of microvascular O_2 extraction (ΔHHb) was faster in older adults, suggesting that local muscle O_2 delivery was lower or that the adjustment of microvascular O_2 delivery was slower in older compared to younger adults. Again, this greater reliance on O_2 extraction in older adults may result in a greater fall in microvascular PO_2 in older adults, so that diffusive O_2 delivery may be impaired constraining the muscle O_2 consumption kinetics.

It has been further demonstrated that, irrespectively of bulk blood flow and O_2 delivery to the exercising limbs, older adults experience a redistribution of O_2 delivery away from oxidative muscle fibers and without an increased capillary red blood cell flux (as observed in their younger counterparts) leading to hinder the dynamic balance between O_2 delivery and VO_2. As a consequence, the microvascular O_2 pressure falls to extremely low levels, thus impairing the blood-myocyte O_2 flux, as dictated by Fick's law (Poole, et al. 2009).

Other factors that could affect the VO_2 with advancing age are the reduction in capillary density, in the capillary-to-fiber ratio, in the mitochondrial oxidative capacity and in the mitochondrial volume density (Poole-Jones 2005), (Betik, et al. 2008). However, the mitochondrial theory of aging which attributes the

age-related decline in muscle performance to a decreased mitochondrial function was experimentally disconfirmed (Rasmussen, et al. 2003a). The authors of this study concluded that, despite a muscle mass reduction with age, the mitochondrial energy producing system remains unaffected. It is well known that the reduction in mitochondrial content is related to physical inactivity (Waters, et al. 2009). To prove this, Waters et al (2009) selected 2 groups of older adults (one composed of sarcopenic people and one of normal people) with similar background and level of physical activity, to study the effect of sarcopenia (loss of muscle mass) on the mitochondrial function of active older adults (Waters, et al. 2009). They demonstrated that oxidative phosphorylation capacity during recovery from exercise was similar between the normal and sarcopenic participants, whereas energy production during rest and during exercise was impaired in the older adults-sarcopenic-group. The older-adults-sarcopenic-group showed a significantly lower absolute oxygen uptake, as compared to the non sarcopenic group, in a walking ramp test at VT_1,VT_2 and at the maximal effort, while the relative oxygen uptake was lower only at the maximum effort (de Oliveira, et al. 2009).

Some studies, however, support the idea that intracellular control (the concentrations of cellular metabolic controllers and/or mitochondrial enzyme activation) may be an important limitation for the slowing of the VO_{2p} kinetics with advancing age (Gurd, et al. 2008).

Nevertheless, the decline in the mitochondrial function (Conley, et al. 2007), is less pronounced than the decline in the O_2 delivery while the VO_2 kinetics is slowing with age, at least for young/middle older people (Betik, et al. 2008), thus indicating a primary role of O_2 delivery over the mithocondrial function in the determination of the VO_{2p} kinetics with advancing of age.

It is worth noting that, in all these studies, it was not possible to distinguish between the effect of aging *per se* and the effect of decreased activity (*detraining effect*). In this regard, a crucial study was performed by Stathokostas et al (2004). Taking changes in physical activity into account, these authors found that a significant part of VO_{2max} decline with aging is not just due to a lower physical activity.

As a last point on the detraining effect for older adults, it is worth mentioning that the VO_2 kinetics for plantar flexion (based on muscles used for walking) was significantly faster than the VO_2 kinetics for cycling (an activity to which older adults were unaccustomed) (Jones, et al. 2005).

1.6 Aging and Hypoxia

The maximal oxygen uptake (VO_{2max}) decreases by 5 $mL \cdot kg^{-1} \cdot min^{-1}$ per decade in sedentary individuals at sea level, starting at the age of 20 years (Astrand PO. 1986) and it decreases by 1% every 100 meters of altitude gain (Levine, et al. 1997a).

Thus, both aging and hypoxia (caused by altitude, by a poor oxygen environment or by a disease) challenge the O_2 delivery system, constraining the VO_2.

A brief literature survey on what is known about the physiological response of older adults (>65yr) to hypoxia is given in the following.

1.6.1 Respiratory changes of older adults in hypoxia

Some studies have shown that aging is associated with a reduced ventilator (V_E) response to hypoxia (Garcia-Rio, et al. 2007) (Kronenberg, et al. 1973). It was demonstrated that the chemosensitivity to hypoxia decreases as a function of age until 70-75 years (Garcia-Rio, et al. 2007) and that the response to an hypoxic stimulus is impaired in older sedentary adults (ventilator rates of 15.4 $l \cdot min^{-1}$) as compared to young subjects (ventilator rates of 29.5 $l \cdot min^{-1}$) (Kronenberg, et al. 1973). This reduction can be due to the different cellular response to hypoxia between young and older adults, as suggested by a study where a poor expression of VEGF and HIF-1 in the carotid bodies during hypoxia was found for older rats as compared to young rats (Giulio, et al. 2005).

In contrast to the above findings, Smith et al (2001) found a greater ventilator response to hypoxia in older adults (24.6 $l \cdot min^{-1}$) as compared to young people (22.6 $l \cdot min^{-1}$). We should note however, that the older adults of this study were all physically active and with a high vital capacity, as compared to subjects of the same age, so that smaller ventilator response measured for older subjects in the study of

Kronenberg et al (1973) could be associated with their lack of fitness and hence potentially smaller lung capacity.

Burtscher et al (2001) investigated the trend of the arterial oxygen saturation (SaO_2) in a group of older adults (67±7) during a week spent at 2000 meters. The authors found a decrease in S_aO_2 at rest in the first days of hypoxia-altitude exposure. However, after the 2^{nd} day, the increase in pulmonary ventilation helped to compensate the low inspiratory oxygen partial pressure. The decrease in the first day is likely due to an aging related ventilation-perfusion inequality and/or to a limitation in lung diffusion during the exercise (Burtscher, et al. 2001).

The SaO_2 response to hypoxia, was also studied longitudinally in a group of native 61 Sherpa (29 men, 32 women) who had lived at 3450–3850 m in Nepal from adolescence through old age (Arai, et al. 2002). The authors demonstrated that baseline SaO_2 during sleep decreased with age in Sherpa living at high altitude.

It is known that arterial hypoxemia develops in senile age as a result of increased alveolar-arterial PO_2 gradient which in turn reflects a lack of coordination between pulmonary ventilation and perfusion distribution (the presence of insufficiently ventilated regions in the lungs) (Korkushko, et al. 2009). The presence of insufficiently ventilated regions in the lungs with increased $PaCO_2$ and decreased of PaO_2 determines the phenomenon of intrapulmonary functional shunting that leads to the arterial hypoxemia characteristic of senility. Although it has been demonstrated that the artero-venous difference in the oxygen content increases with age (Korkushko, et al. 2009), the rightward shift of the oxyhemoglobin dissociation curve does not compensate completely for the hypoxic shifts occurring with aging, which is confirmed by the decreased PvO_2.

1.6.2 Cardiovascular and VO_2 changes of older adults in hypoxia

Some studies have shown that aging is associated with a reduced heart response (HR) to hypoxia (Garcia-Rio, et al. 2007)-(Kronenberg, et al. 1973). In fact, according to some authors, no change in heart rate

between normoxia (600m) and hypoxia (2000m) was measured at rest for 2 groups of older adults. During an exercise step test on the contrary, the heart rate was higher in hypoxia than in normoxia (Burtscher, et al. 2001). The same authors did not find any significant difference in the mean oxygen consumption (VO_2) in response to 3min step exercise test between the 2 groups.

1.6.3 Vasodilatation response of older adults to hypoxia

An increased vasodilatation in response to hypoxia is referred to as compensatory vasodilatation. This compensatory vasodilatation allows for an adequate amount of oxygen to be delivered to the working musculature and it is, in part, nitric oxide (NO) dependent (Casey, et al. 2011).

Casey et al (2011) reported an attenuated hypoxic vasodilatation in a group of older adults (55-70 years) performing a handgrip exercise while breathing an hypoxic gas that caused a decreased ~~in the~~ arterial saturation up to 80%. The authors ascribed the decrease in compensatory vasodilatation to diminished nitric oxide (NO) signaling (Casey, et al. 2011). Any change in the regulation of skeletal muscle blood flow may limit the functional capacity and impair the ability to perform exercise with aging.

1.7 Doppler Ultrasound and Conduit Artery Blood Flow

Doppler Ultrasound is a method for detecting the direction and velocity of moving blood within the vessels and the heart. It is based on the frequency shift that occurs when sound or light is emitted from a moving source (Eden 1984).

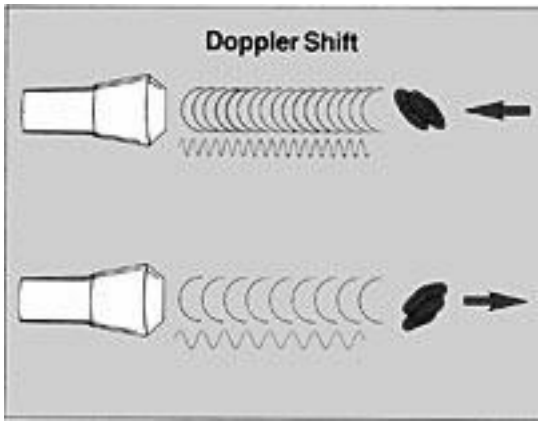

Fig 1.3
Representation of the Doppler Shift effect

Doppler systems are totally dependent on the changes in the frequency of the transmitted ultrasound that result from the encounter of the wave-front with moving red blood cells (RBC).

Fig 1.3 shows a transducer on the left that is emitting a given frequency of ultrasound toward the right and into the tissues. When the transmitted sound waves encounter a group of red cells are reflected back at a frequency higher or lower than that transmitted, depending on the velocity vector of the RBC in relation to the probe (Gill 1985). If the RDC are moving toward the transducer, the transmitted sound waves are reflected back at a frequency higher than that at which they were sent, producing a positive return frequency. If the RDC are moving away from the transducers, the transmitted sound waves are reflected back at a frequency lower than at which they were sent, producing a negative return frequency. This returned frequency is also called the "frequency shift" or "Doppler shift" and is highly dependent upon the angle between the beam of ultrasound transmitted from the transducer and the moving red blood cells.

The 'Doppler effect' of moving red blood cells (RBC) in human blood vessels can be described mathematically via equation 1.5:

$$f = \frac{2(v \cdot \cos\theta \cdot t_o)}{c} \qquad (1.5)$$

where **f** is the change in frequency or the Doppler shift, $\mathbf{t_o}$ is the transmitted frequency, **v** is the velocity of the RBC, **θ** is the angle between the ultrasound beam and the direction of the movement of red blood cells and **c** is the velocity of sound in tissue (~1540 cm/s) (see fig 1.4).

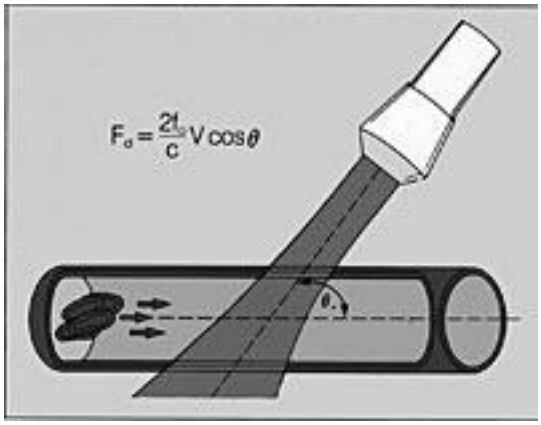

Fig 1.4
Representation of the Doppler effect

The calculation of velocity is possible by rearrangement of the Doppler equation to:

$$v = \frac{f \cdot c}{(2t_o \cdot \cos\theta)} \qquad (1.6)$$

The velocity of sound in blood is constant (**c**) and is an important part of the Doppler equation. The Doppler shift is proportional to the velocity of the RBC and it is calculated by the Doppler system comparing the transmitted waveform with the received waveform for a change in frequency as shown in Fig 1.5. As mentioned before if there is a higher returning frequency (+AP) then the flow is called a "positive Doppler shift" and represented as moving toward the transducer (Gill 1985). If there is a lower returning frequency (-AP) then the flow is called a "negative Doppler shift" and represented as moving

away from the transducer. All components of the Doppler equation, except velocity, are readily measured by the Doppler instrument.

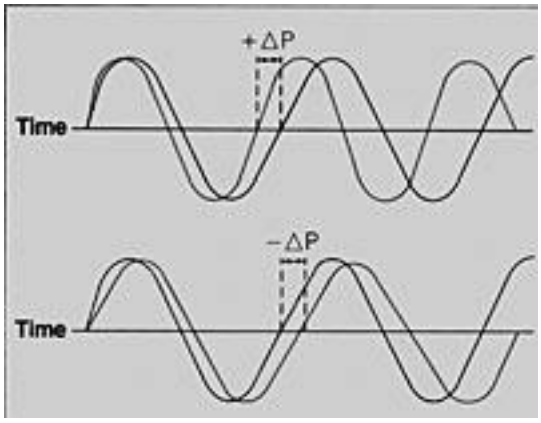

Fig 1.5
Transmitted and Received waveform

Flow velocity toward the transducer is displayed as a *positive*, or *upward shift in velocities*, while flow velocity away from the transducer is displayed as *negative*, or *downward shift in velocities*. (see Fig. 1.6). Time is on the horizontal axis.

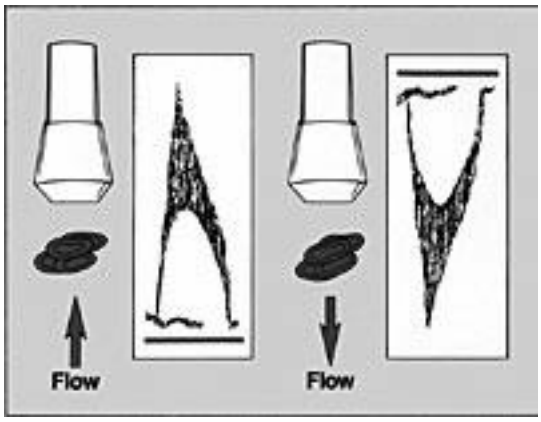

Fig 1.6
Display of the flow velocity toward the transducer

The ultrasound transducer, that is a key component in the measure of the “Doppler Shift” is composed of one or more piezoelectric crystals which are capable of converting (modulating) an alternating electrical current into emitted sound waveforms as the crystal is deformed and rebounds in a characteristic manner proportional to the input current. The crystal is also capable of receiving reflected ultrasound waveforms and converting (demodulating) them into an electrical current proportional to the frequency of the received waveforms.

1.7.1 Doppler Ultrasound Transducers

There are two main types of Doppler Ultrasound Transducers in common use today, *continuous wave* and *pulsed wave*. They differ in transducer design and operating features, signal processing procedures and in the types of information provided. Each has important advantages and disadvantages.

Continuous wave (CW) Doppler ultrasound devices emit the ultrasound in a fixed direction from a single piezoelectric crystal and the reflected ultrasound waveform is detected by a second crystal adjacent to the emitting crystal (Gill 1985). The main advantage of CW Doppler is its ability to measure high blood velocities accurately. The main disadvantage of CW Doppler is its lack of selectivity or depth discrimination. The sample volume in this instance is dictated by ultrasound penetration and the baseline frequency emission and is created by the region of overlap between the transmitted and reflected ultrasound waveforms. Thus the reflected waveforms include low velocities from arterial wall motion and valve artifacts in veins as well as the higher velocities of RBCs, which may result in a decreased calculated mean velocity and a lower signal to noise ratio or special resolution (Gill 1985).

Pulsed wave Doppler ultrasound devices consist of a single piezoelectric crystal in which a pulse of transmitted ultrasound is followed by a period of no waveforms being emitted. At a specific time interval the elements are activated in the 'receive' mode. The length of the period when the piezoelectric crystal is in the transmission mode is dependent on the depth of the sample gate. This 'gating' of the returned phase shift from the desired depth allows an operator to modify the depth of insonation within the limits of

emitted ultrasound frequency and attention of this signal in the desired tissue (Gill 1985). Thus, the pulsed wave Doppler transducer provides velocity information from a specific point along the ultrasound beam and provides improved special resolution as the sampling volume can be modified with this gating procedure. The main disadvantage of pulse wave Doppler is its inability to accurately measure high blood flow velocities.

Doppler ultrasound transducers emit at a specific frequency or over a range of frequencies. Higher ultrasound frequencies produce shorter wavelengths of emitted waveforms, which provide higher resolutions. However, the shorter wavelengths are attenuated to a greater extent and do not penetrate as deeply into tissues resulting in low signal to noise ratios. Therefore, deeper structures and vessels require lower ultrasound frequencies resulting in a loss of resolution.

1.7.2 Measurement of Flow in Arteries

With the Doppler technique above described, the information is limited to the velocity of RBCs traveling through the vessel of interest. However, the flow through a specific vessel represents the most interesting variable. Knowing the cross-sectional area of a given vessel it is possible to calculate the flow through it. The cross-sectional area can be calculated from the measurement of with the following equation:

$$CSA = \pi\left(\frac{d}{2}\right)^2 \tag{1.7}$$

where **CSA** is cross-sectional area, and **d** is the diameter of the vessel in cm (calculated using an on-line caliper on the images obtained by a duplex ultrasound system). The greatest source of error lies within the calculation of the vessel cross-sectional area.

One of the problems that occured with the use of continuous wave and earlier pulsed wave Doppler ultrasound instruments was the inability to display simultaneously the vessel of interrogation and the velocity within the vessel. To overcome this problem, the measurement of the diameter of the vessel has usually taken via M-mode or two dimensional ultrasound imaging at separate time points from the measurement of velocity, at the start and at the end of the measurement. This is adequate for large vessels

such as the femoral artery where the lumen diameter does not appear to change with the alterations in systolic and diastolic pressure as well as over periods of time (MacPhee, et al. 2005).

Smaller vessels may instead have diameter changes throughout an exercise period. For this reason recently the technology developed the possibility to have two dimensional images and Doppler velocity measures simultaneously utilizing duplex scanning. This new tool has allowed investigators to display the vessel and determine diameter measures while measuring flow.

It is extremely important obtain precise measures of the vessel diameter. The CSA is proportional to the square of the diameter (eq.1.7), thus, any error in this dimension will be squared as well, resulting in a multiplication of the error in the measurement of the area. For example, a 11 mm vessel will have a cross-sectional area of 9.49 cm^2; however, if a 2 mm measurement error is utilized (9 mm diameter) the cross-sectional area would be calculated as 6.35 cm^2, a 34% decrease in the calculated cross-sectional area. Further, vessels are assumed to be circular and hold a constant shape, allowing the use of the diameter to calculate cross-sectional area, and while this may be the case in large vessels, this may not hold true for smaller ones.

The calculation of flow in the vessel of interest is derived from the following equation:

$$\mathrm{LBF}\,(\mathrm{ml}\cdot\mathrm{min}^{-1}) = MBV\,(cm\cdot s^{-1})\cdot\pi\cdot r^{2}\cdot 60 \qquad (1.8)$$

The use of ultrasound for the measurement of conduit artery blood flow was validated with measures obtained from venous occlusion plethysmography (Tschakovsky, et al. 1995) and thermodilution techniques (Radegran 1997).

The use of this technique allowed some author to calculate the kinetics of the blood flow at the onset of exercise. For instance faster blood flow kinetics respect to VO_{2p} kinetics have been reported during the on-transient of exercise (MacDonald, et al. 1999).

Nevertheless, it is important to note that the measures of conduit artery limb blood flow do not allow having an idea of the distribution and kinetics of blood flow within the exercising muscle. For this reason

in order to study the possible constrain to the adjustment of VO_{2m}, some authors began to use the Near-Infrared-Spectroscopy (NIRS) in order to study the micro-vascular O_2 delivery and its relationship with the VO_{2m} (DeLorey, et al. 2003).

1.8 Near Infrared Spectroscopy and Microvascular O_2 delivery

Near-infrared spectroscopy (NIRS) is a spectroscopic method that uses the near-infrared region of the electromagnetic spectrum (from about 800 nm to 2500 nm). It is a non-invasive tool that allows for the continuous measure of changes in concentration of local muscle oxy-(ΔO_2Hb), deoxy-(ΔHHb) and total-(ΔHb_{tot}) hemoglobin-myoglobin within the exercising muscle (Elwell 1995).

When placed over the muscle of interest, the emitted near-infrared light from the source probe passes through the tissue of interest into the microvasculature of that muscle, to be picked up by the detector probe. The NIRS signal is derived from the absorption of light by hemoglobin within the microvasculature as oxygenated and deoxygenated hemoglobin have specific absorption characteristics for different wavelengths of NIR light and exist in sufficient quantities so as not to absorb all transmitted light (Boushel, et al. 2001). Even if the NIRS is not able to distinguish between the contributions of haemoglobin and myoglobin, it is believed that mostly haemoglobin is being detected, (Boushel, et al. 2001) as myoglobin is considered to contribute less than 10 % to the NIRS signal (Boushel, et al. 2001).

As HHb and O_2Hb have different absorption characteristics, the reflected light will vary based on oxygenation status (Boushel, et al. 2001; Elwell 1995).

During exercise on-transients, the measurement of the adjustment of ΔHHb in concert with VO_2 exemplifies the profile of microvascular oxygenation that is reflective of the dynamic balance between O_2 delivery and O_2 utilization within the NIRS field of interrogation (De Blasi, et al. 1993; DeLorey, et al. 2003). The ΔHHb, that is essentially insensitive to blood volume changes, has a temporal profile similar to the changes in muscle intracellular PO_2, microvascular PO_2, and (a-v) O_2 difference, resulting in a good indicator of fractional O_2 extraction (Jones, et al. 2009).

A recent investigation has shown a spatial heterogeneity of quadriceps muscle deoxygenation kinetics suggesting that varying degrees of perfusion/metabolism matching/mismatching are present within and between muscles during cycling exercise transients (Koga, et al. 2007).

For this reason caution in the interpretation of the NIRS results has to be performed because the NIRS activity is limited to a relatively small area of muscle and may not be indicative of the heterogeneity of blood flow that has been observed between and within exercising muscle (Kalliokoski, et al. 2000).

Principle Aims and Hypothesis:

The overall aim of this thesis is to examine the relationship among VO_{2p} kinetics (reflecting muscle O_2 consumption), bulk O_2 delivery (as measured by conduit artery limb blood flow at the femoral artery) and NIRS-derived measures of local muscle deoxygenation (reflecting the balance between microvascular O_2 delivery and O_2 utilization within the muscle) during moderate intensity exercise on-transient in young and older adults. Hypoxia was used to stress the O_2 delivery system. Combining use of Doppler ultrasonography, breath-by-breath VO_{2p} and NIRS, we can determine the (bulk) blood flow response to the exercising limb during exercise and the relationship between O_2 delivery and O_2 utilization within the muscle. The approach helps to elucidate potential bottlenecks in the O_2 delivery cascade (central to peripheral) which may constrain the O_2 uptake kinetics in older adults.

The overall hypothesis of this thesis is that hypoxia would slow the VO_{2p} kinetics at the onset of moderate-intensity exercise to a greater extent in older adults as compared to younger adults. This greater slowing, due to the aging process, would be caused by a greater impairment in the microvascular O_2 delivery system. If this hypothesis is confirmed it will corroborate the idea that the major limitation to VO_2 in older people resides in the microvascular O_2 delivery system.

The initial study (**Chapter 2**) evaluates the adjustment of VO_{2p} and NIRS-derived measures during the on-transient of moderate-intensity cycling exercise for older adults in normoxia and hypoxia. In addition,

the distribution of the balance between microvascular O_2 delivery and O_2 utilization within the quadriceps muscle group (vastus lateralis) is investigated.

Chapter 3 evaluates the effects of hypoxia on the kinetics of VO_{2p}, conduit artery limb blood flow and NIRS-derived measures of muscle deoxygenation during the on-transient of moderate-intensity knee-extension exercise within a group of older adults. Taking into account that the compensatory vasodilatation was shown to be preserved for older adults exercising at 10% of the maximal effort of a handgrip exercise whereas it is reduced when they are exercising at 20% of the maximal effort (Casey, et al. 2011), we hypothesize that exercising at a low work rate would prevent the VO_2 kinetics to get slower in hypoxia.

The final study of this thesis (**Chapter 4**) seeks to determine the effect of aging on the response to exercise under hypoxic conditions. It is hypothesized that, due to the reduced response of Heart Rate and Ventilation to hypoxia with advancing of age (Garcia-Rio, et al. 2007; Kronenberg, et al. 1973), the major limiting factor of the slowing in the VO_{2p} kinetics on going from normoxia to hypoxia would be peripheral in older adults and central in younger adults.

Significance of the study

Since this study helps to understand the physiological mechanisms involved in situations of natural hypoxia, it could be relevant to the large amount of older adults who spend some of their free time in a mountain environment. It is also relevant to all the clinical situations were hypoxia is a consequence of a disease.

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CHAPTER 2
EFFECTS OF ACUTE HYPOXIA ON THE VO_2 KINETICS OF OLDER ADULTS DURING CYCLING EXERCISE

2.1 Introduction

It is well-known that, during moderate intensity exercise, the Phase 2 pulmonary O_2 uptake (τVO_2), reflecting muscle O_2 consumption (D. C. Poole, et al. 2008), (Grassi, et al. 1996), (Rossiter, et al. 1999) slows with advancing age (Babcock, et al. 1994; Poole, et al. 2009; 2009), (Bell, et al. 1999), (Scheuermann, et al. 2002). Both O_2 provision (Murias, et al. 2010),(Tschakovsky, et al. 1999)and intracellular control (Grassi, et al. 1998) were proposed to play a role in this respect.

In addition to the slower VO_{2p} kinetics, other effects associated with aging, are: slower HR kinetics in response to exercise (Bell, et al. 1999), (Scheuermann, et al. 2002), a reduced submaximal cardiac output for a given oxygen uptake (VO_2) (Proctor., et al. 1998), a redistribution of the blood flow from the more oxidative toward the more glycolytic muscles (Eklund, et al. 2005) and a reduction of muscle blood flow in trained older subjects during steady-state dynamic exercise (Proctor, et al. 1998). On this last point however, a general consensus does not exist and other studies failed to show aging related changes in muscle blood flow (Proctor., et al. 2003), (Richardson, et al. 1980).

Some studies support the idea that intracellular control is an important limitation of the VO_{2p} kinetics at the onset of exercise. It was shown that skeletal muscle mitochondrial oxidative capacity declines with aging (Betik, et al. 2008) and that there is, in vitro, an inverse relationship between the mitochondrial content and the rate of adjustment in VO_2 (Glancy, et al. 2008). Thus a reduced functionality of mitochondria could lead to a decreased rate of adjustment of VO_2.

On the contrary, other studies conclude that, despite muscle mass reduction with age, the mitochondrial energy producing system seems unaffected (Rasmussen, et al. 2003b).

A recent investigation by (Koga, et al. (2007)) showed a spatial heterogeneity of quadriceps muscle deoxygenation kinetics, suggesting varying degrees of perfusion/metabolism, matching/mismatching are present within and between muscles during cycling exercise transients.

About this topic the work of Murias et al (2010) underlined the importance of O_2 distribution within the muscles as a key component of the slowing in VO_{2p} kinetics with aging.

Acute hypoxia has been used in previous investigations as a way to challenge the O_2 transport system. It is known that, in young adults, hypoxia causes a reduction in the maximal oxygen consumption (VO_{2max}) and lactate threshold (Lawler, et al. 1988), together with a slowing in the Phase 2 VO_{2p} kinetics (Engelen, et al. 1996), (Hughson, et al. 1995), (Linnarsson, et al. 1974), (Springer, et al. 1991) Yet, other authors did not find any difference in phase 2 VO_{2p} during a leg extension exercise performed in hypoxia and normoxia (MacDonald, et al. 1999).

Concerning older adults on the other hand, very few studies performed under acute hypoxia exist. These studies were focused on the long (Arai, et al. 2002), (Levine, et al. 1997b) and short (Burtscher, et al. 2001) term response to mild hypoxia. More recently, a further study has shown that aging reduces the compensatory vasodilatation during hypoxic forearm exercise, through a blunted nitric oxide signaling (Casey, et al. 2011).

However the influence of hypoxia on the VO_{2p} kinetic response to constant-work-rate exercise of moderate intensity in older adults remains to be determined. Studies of this kind would be important to understand the physiological mechanisms in situations of hypoxia caused both by pathological diseases and/or by the natural environment.

The aim of this study is to characterize the kinetics of VO_{2p}, HR and muscle deoxygenation for a group of older adults, during a moderate-intensity step change in cycling exercise, performed in normoxia and in mild acute hypoxia. According to the literature, one would expect for older adults, in hypoxia, a greater slowing of the phase 2 VO_{2p} kinetics.

2.2 Methods

2.2.1 Subjects

Fourteen older male subjects (age, 66±6 yrs; height 173±7 cm; body mass 75±7 kg) agreed to participate in this study after giving written informed consent. All subjects were nonsmoking and free of cardiac, metabolic and pulmonary diseases. This study was carried out according to the Declaration of Helsinki. The protocol was approved by the Ethics Committee for Research on Human Subjects of the University of Verona.

2.2.2 Protocol

A randomized cross-over design was used. After medical screening, subjects were reported to the laboratory on four separate occasions. The order in which the 2 conditions (normoxia and hypoxia) were assigned was randomized.

A normobaric hypoxic chamber (B-Cat Netherlands) that uses a nitrogen pump system to lower the level of oxygen inside the chamber was used. The mean barometric pressure at the altitude of the chamber (204 meters above sea level) was 750 mmHg. An automatic system kept the temperature (22°-24° Celsius) and the humidity (40-50%) in a constant range. A CO_2 scrubber ran continuously in order to remove the carbon dioxide expelled by the body while exercising in the chamber. Tests conducted in hypoxia were preceded by 30 min of rest in the chamber before each trial in order to achieve a stabilization of resting metabolic and ventilator parameters ((Easton, et al. 1986)). One incremental test was performed with subjects breathing normoxic room-air (FIO_2 =20.9%) and a second (randomized order) with subjects breathing hypoxic room-air (FIO_2 =15%) to establish the FIO_2-specific first ventilatory threshold (VT_1) and the maximum oxygen uptake (VO_{2max}). After a standardized warm-up of 15 minutes unloaded pedaling the ramp incremental test started with an increment of 15 watt per minute. The work rate increase was selected so as to reach the maximum-tolerated work rate in ~ 12 min. VT_1 was defined as the VO_2 at which CO_2 output (VCO_2) began to increase out of proportion in relation to VO_2 with a systematic rise in

minute ventilation-to-VO_2 ratio (V_E/ VO_2) and end-tidal PO_2 , whereas minute ventilation-to-VCO_2 ratio (V_E/ VCO_2) and end-tidal PCO_2 were stable ((Beaver, et al. 1986)). Subsequently, the subjects repeated constant work rate (WR) cycling exercises for 3 times and for each of the 2 different FIO_2 conditions. Each single test was separated by two days. All the subjects performed a transition in work rate from unloaded cycling to a moderate intensity work rate and back to unloaded cycling. The work rate was selected to elicit a VO_2 corresponding to 80% of the specific VT_1. The subjects completed 30 min of accommodation at rest (sitting on a chair in the chamber) before the start of the test. Each loaded and free-wheel unloaded period lasted 6 min. Rather than using the same absolute mechanical power, we used the same metabolic power to avoid early lactate production in the hypoxic situation (Hogan, et al. 1999), and to maximize the amplitude in both conditions, in order to have a better signal to noise ratio (Jones, et al. 2005). When the rest to work transitions are performed within the moderate intensity domain (below the lactate threshold), the τVO_2 follows a linear first order response kinetic (DiMenna, et al. 2009). In fact a part the work to work transition within the moderate intensity domain (Brittain, et al. 2001) and the intensity above the lactate threshold (DiMenna, et al. 2009) the τVO_2 does not change with the variation of the work load. Moreover the use of similar relative intensities (80% of the specific VT_1) allows comparison of the subjects's VO_2 responses, to different conditions (normoxia and hypoxia), avoiding, for instance, that one subject exercises in normoxia below the lactate threshold (with the VO_2 following a first-order response kinetic) and in hypoxia above the lactate threshold (with a possible VO_2 slow component).

2.2.3 Metabolic Measurements

Values of minute ventilation (V_E), breathing frequency (Fb), tidal volume (VT), end tidal partial pressure of oxygen and carbon dioxide ($PETO_2$ and $PETCO_2$), carbon dioxide output (VCO_2) and oxygen uptake (VO_2) were continuously measured by a breath-by-breath gas exchange measurement system (Cosmed Quark b², Rome, Italy). Gas analysers were calibrated before each test in each condition. [Normoxia:

ambient air (O_2: 20.93 % and CO_2: 0.03 %) and a gas mixture of known composition (O_2: 16.00 % and CO_2: 5.00 %); hypoxia: ambient air (O_2: 15.00 % and CO_2: 0.03 %) and a gas mixture of known composition (O_2: 10.08 % and CO_2: 4.00 %)]. The low dead space (70-mL) face-mask was equipped with a low-resistance, bidirectional digital turbine (28-mm diameter). This turbine was calibrated before each test with a 3-L syringe (Cosmed, Rome, Italy). Face-masks allowed subjects to simultaneously breathe with mouth and nose, for more comfort. Heart rate was continuously measured via a wireless Polar-monitoring system (Polar Electro Oy, Kempele, Finland) and synchronized with the Cosmed system. Before each test, ambient conditions were measured and the gas analyzer and flow-meter were calibrated.

2.2.4 NIRS measurements

Local muscle oxygenation of the vastus lateralis muscle was measured with Near-Infrared-Spectroscopy (NIRS, Nimo-Nirox, Brescia Italy). The natural transparency of the skin and the muscular tissue to near-infrared light (from 650 to 1000 nm) allowed measuring the change in oxy and deoxy-hemoglobin concentration in the muscle tissue. The interaction of radiation-tissue is regulated by two physical characteristics: the first is the absorption, which is the attenuation of the intensity of the NIR signal through the tissue and it is due to water, lipids, oxyhemoglobin (HbO_2) and deoxy-hemoglobin (HHb); the second is the scattering that consisted of a deviation from straight trajectory by the non-uniformity characteristic of tissue. This technique, furthermore, performs accurate optical absorption measurements at specific wavelengths that are converted into quantitative hemoglobin concentration (HbO_2 and HHb) using a proprietary algorithm. The probe was an active emitter unit comprised of a laser diode source (wavelengths from 670 to 980 nm) and a multiplexer (optomechanical switch). The sampling frequency was 40 Hz. The skin was carefully shaved and cleaned before each test. A skinfold thickness at the site of application of the NIRS probe was determined before the warm-up using a caliper and the value of the subcutaneous adipose tissue was inserted into the NIRS software. The probe was firmly attached to the skin overlying the distal third of vastus lateralis muscle (~ 10-12 cm above the knee joint) of the right

limb, parallel to the major axis of the thigh, by a biadhesive tape ((Quaresima, et al. 2004)). A bandage was used to cover the probe from the external light sources.

2.2.5 O_2 arterial saturation measurement

SaO_2 was measured by portable pulsoximeter (Intermed SAT-500). Measurements were made on the index finger. SaO_2 was monitored for the duration of the test and the values used were relative to the average of last minute of constant work rate exercise.

The arterial oxygen content (CaO_2) was estimated as the product of SaO_2 from pulse oxymetry and the O_2 content of Hb, assuming an arterial [Hb] of 15.0 g · 100 ml^{-1} and an O_2 carrying capacity of 1.34 ml · g^{-1} Hb.

2.2.6 Heart rate measurements

Heart rate (HR) was continuously measured via a wireless Polar-monitoring system (Polar Electro Oy, Kempele, Finland) and synchronized with the Cosmed system.

2.2.7 Analysis

Breath-by-breath VO_2 signals of each of the 3 constant-power tests were filtered by removing aberrant data points that lay outside 4 standard deviations of the local mean, then were interpolated to derive a sec-by-sec profile; the 3 transitions were then time-aligned to the onset of exercise and averaged to provide a single response for each subject in each condition. The on-transient response for VO_2 was fitted with a mono-exponential model of the form: $Y(t) = Y_{bas} + Y_{amp} \cdot (1 - e^{-(t-TD)/\tau})$.

Y_{bas} is the baseline VO_2 value during the unloaded cycling, Y_{amp} is the difference between the steady state value of VO_2 reached after the transient and the baseline value of VO_2 in the 30 sec before the transient, τ is the time constant defined as the duration of the time for VO_2 to increase to 63% of the steady state increase.

The phase 1-phase 2 transition was determined with an “experimental” fitting strategy. The end of phase 1 or the start of phase 2 for VO_2 was determined as the breath before the sudden fall in respiratory exchange

ratio (RER). This coincides with the inflection point during the first 15-30 sec of the rise of VO_2 ((Mole, et al. 1999)). Subsequent phases 2 and 3 were fitted using the mono-exponential model described previously. The VO_2 data were modeled from the Phase 1- Phase 2 transition to 240 sec.

Data for HR were re-sampled with a 1-s sampling rate, averaged together and modeled with the mono-exponential model described above. The on-transient HR responses were modeled from the onset of exercise to 240 sec.

The 3 repetitions of HHb were time aligned, interpolated with a 1-s sampling rate and averaged together to calculate the rate of adjustment of the HHb. The calculated time delay (TD-HHb) was determined visually from the onset of exercise to the first rapid increase in the HHb signal. The HHb was fitted with a mono-exponential fit from the calculated time delay of the exercise to 90 sec ((DeLorey, et al. 2003)). The effective time constant, τ', for the HHb signal was calculated as $\tau' = TD + \tau$.

To investigate the relationship between the HHb and the VO_{2p} kinetics, data on the time evolution of HHb and VO_{2p} were normalized, for each subject, in such a way that the response varies from 0 (unloaded condition) to 1 (loaded condition). The normalized VO_{2p} curve was left-shifted taking the duration of phase 1, as calculated for each subject, into account. Thus the onset of exercise (t=0) coincides, for the VO_{2p} curve, with the beginning of phase 2, which is known to reflect closely the muscle oxygen uptake (Grassi, et al. 1996). Curves obtained in this way are referred to as HHb^{nor} and $VO_{2p}{}^{nor}$. They are shown, for a representative subject, in Fig.2.2 for normoxia (left panel) and for hypoxia (right panel). Similar curves are obtained for all subjects.

Subsequently, the ratio $HHb^{nor}/VO_{2p}{}^{nor}$ was evaluated for each subject. The resulting curve is shown in Fig.2.3 for the same subject of Fig.2.2. From this curve, an overall HHb/VO_{2p} ratio, was derived, (Murias *et al*.2010) by calculating the time-average of $HHb^{nor}/VO_{2p}{}^{nor}$ from t=10 s (beyond the physiological TD-HHb) to t=120 s (at the steady state for both HHb^{nor} and $VO_{2p}{}^{nor}$), i.e.: $\frac{1}{N}\sum_{n=1}^{N}\left(\frac{HHb^{nor}}{VO_{2p}^{nor}}\right)_n$, where n=1

corresponds to t=10s and n=N corresponds to t=120s. The two time-limits are indicated by vertical dotted lines on Fig.3.

All the calculations were performed using software implemented in Matlab (Matlab.7.0 Natick, Massachusetts, U.S.A.)

2.2.7 Statistical analysis

Values presented are expressed as mean ± SD. Statistical significance was accepted at $p < 0.05$. Paired T-tests were used to analyze the 2 conditions. Data were analyzed with the software package SPSS version 3.5 (SPSS Inc., St Louis, MO, USA).

2.3 Results

Incremental Test: Maximal and first ventilatory threshold values derived from this test were given in Table 2.1 for WR, HR and VO_{2p}. Exercising in hypoxia led to a significant (i.e. $p<0.05$) decrease in the maximal and VT_1 values for the WR and VO_2. On the contrary no changes were observed for the HR (maximal values and VT_1 values).

Table 2.1

	Maximum value			First Ventilatory Threshold		
	WR (W)	HR (beats/min)	VO_{2max} (l/min)	VT_1 (watt)	VT_1 (beats/min)	VT_1 (l/min)
Normoxia	213±20	144±18	2.9±0.5	135±18	118±12	1.9±0.3
Hypoxia	196±15*	141±12	2.4±0.4*	120±13*	122±11	1.7±0.4*

Table 2.1
Maximum and ventilator threshold values (mean ± SD) for WR, HR and VO_2 in normoxia (FIO_2=20.9%) and hypoxia (FIO_2=15%)
*$p<0.05$, hence significantly different from normoxia (FIO_2=20.9%)

Square wave test: As shown in Table 2.2, the SaO_2, was always significantly lower (i.e. $p<0.05$) in hypoxia as compared to normoxia. The estimated arterial O_2 content (CaO_2) was 180 $ml \cdot l^{-1}$ in hypoxia and 196 $ml \cdot l^{-1}$ in normoxia during the unloaded period and 168 $ml \cdot l^{-1}$ in hypoxia and 194 $ml \cdot l^{-1}$ in normoxia during the loaded period.

Table 2.2

	Baseline Normoxia	Baseline Hypoxia	Steady State Normoxia	Steady State Hypoxia
Power	[Unloaded]	[Unloaded]	[102.5 watts]	[84.1 watts]
SaO_2 (%)	98.6 ± 0.7	90.7 ± 1.8 *	97.6 ± 0.8	84.1 ± 2.6 *
HHb (μM)	31.7 ± 11	33.3 ± 8	42.3 ± 18	43.2 ± 13
Total Hb (μM)	81.5 ± 0.8	85.7 ± 0.8 *	85.5 ± 0.8	93.9 ± 1 *
OxyHb (μM)	49.8 ±1	52.4 ±1.3 *	43.2 ± 0.59	50.7 ± 4 *
Hr (b/min)	72 ± 10	73 ± 12	108 ± 11	112 ± 13
VO_2 (ml/min)	622 ± 127	597 ± 110	1719 ± 225	1481 ± 180 *
V_E (l/min)	19.1 ± 3.7	20.2 ± 3.6	48.7 ± 6.4	48.5 ± 6.6

Table 2.2
Baseline and steady state values (mean ± SD) for several parameters in normoxia (FIO_2=20.9%) and hypoxia (FIO_2=15%). *$p<0.05$, hence significantly different from normoxia (FIO_2=20.9%)

VO_{2p} kinetics: Fig.2.1 shows the VO_{2p} time dependence (representative subject) during the square wave test in normoxia and hypoxia. Due to the lower absolute work rate in hypoxia (84.1 watts) compared to normoxia (102.5 watts), the VO_{2p} at steady-state was lower in hypoxia than in normoxia. The corresponding amplitudes were 884 ± 173 ml/O_2 and 1097 ± 192 ml/O_2 for hypoxia and normoxia, respectively ($p<0.05$). However the VO_{2p} amplitude normalized to the work rate amplitude, $\Delta VO_{2p}/\Delta WR$, did not show any difference between the two conditions ($p>0.05$) (see Table 2.3).

The duration of the VO_{2p} phase 1 was not different between the two conditions ($p>0.05$). On the contrary, the τ of the VO_{2p} phase 2 was greater ($p<0.05$) in hypoxia than in normoxia (see Table 2.4).

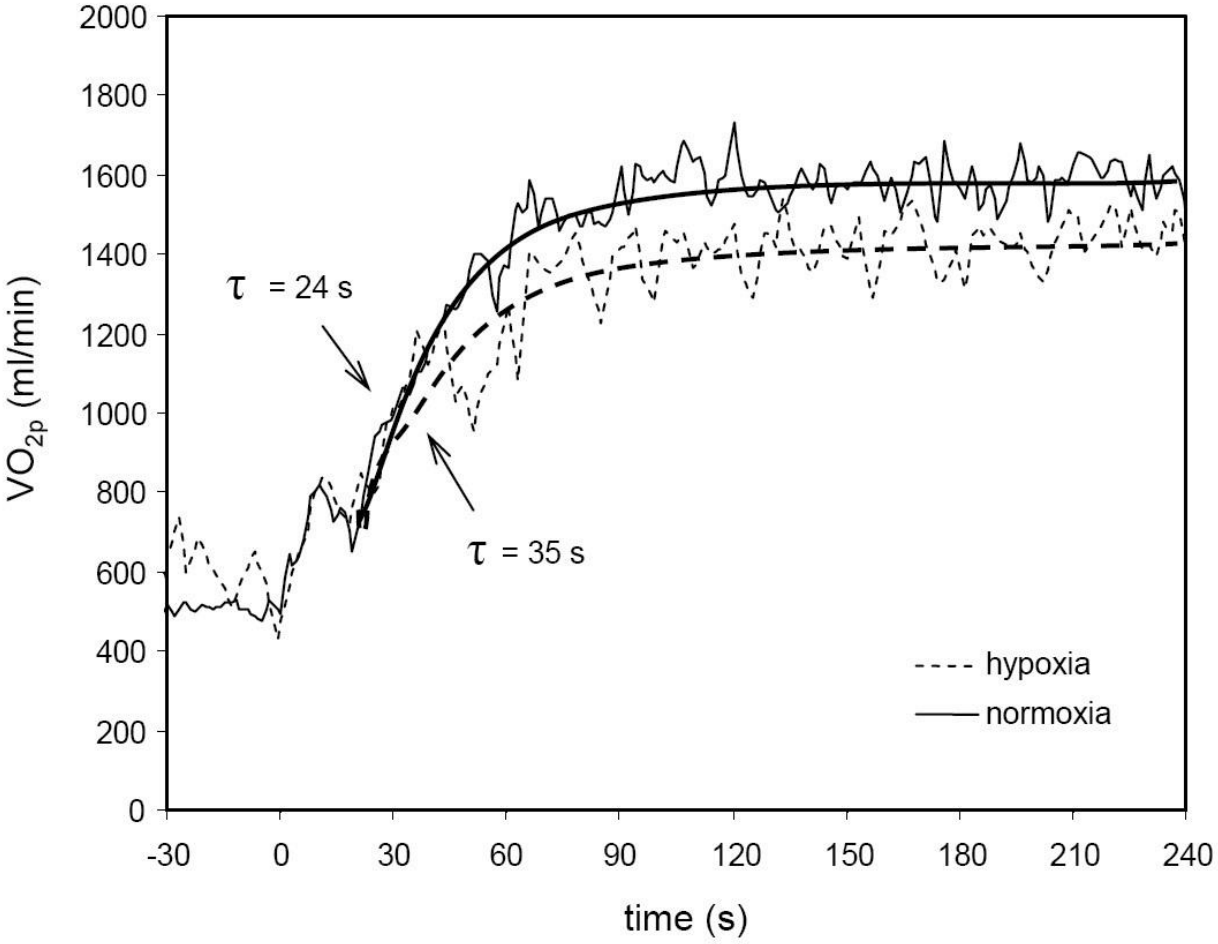

Figure 2.1
Response of pulmonary O_2 uptake during the square wave exercise in normoxia(FIO_2=20.9%) and hypoxia (FIO_2=15%). Data represents the average of 3 repetition of the square wave exercise for one representative subject.

HR-kinetics: In the loaded period, the HR measured in hypoxia and normoxia was the same ($p>0.05$) (see Table 2). However the HR amplitude normalized to the VO_2 amplitude ($\Delta HR / \Delta VO_2$) was greater in hypoxia ($p<0.05$) (see Table 2.3).

The τ HR did not change between hypoxia and normoxia (See Table 2.4).

Table 2.3

	Normoxia	Hypoxia
$\Delta HR/\Delta VO_2$	33 ± 4	44 ± 6 *
$\Delta VO_2/\Delta WR \left(\frac{ml/\min}{W}\right)$	10.7 ± 1	10.5± 1.5
$\Delta HHb/\Delta VO_2 \left(\frac{\mu M/\min}{L}\right)$	11.8 ± 6	14.3 ± 5 *
$\Delta V_E/\Delta VO_2$	26.9 ± 3	32.0 ± 5 *

Table 2.3
$\Delta VO_2/\Delta WR$, $\Delta HHb/\Delta VO_2$, $\Delta HR/\Delta VO_2$, $\Delta VE/\Delta VO_2$ values in normoxia (FIO_2=20.9%) and hypoxia (FIO_2=15%).
*p<0.05, hence significantly different from normoxia (FIO2=20.9%)

HHb kinetics: In the loaded period, the HHb measured in hypoxia and normoxia was the same (p>0.05) (see Table 2.4). The HHb amplitude normalized to the VO_{2p} amplitude ($\Delta HHb/\Delta VO_{2p}$) was therefore greater in hypoxia as compared to normoxia (p<0.05) (see Table 3). The τ of HHb and the effective time constant, $\tau' = TD + \tau$, were the same for the 2 conditions (p>0.05) (see Table 2.4).

Table 2.4

Characteristic times	Normoxia	Hypoxia
Phase1 VO_{2p} duration	20 ± 4 sec	20.8 ± 3 sec
τ Phase2 VO_{2p}	27 ± 7 sec	31 ± 9 sec *
τ HR	40 ± 10 sec	43 ± 14 sec
τ HHb	10 ± 2 sec	11 ± 4 sec
TD HHb	5.7 ± 1.5 sec	5.6 ± 3.2 sec
$\tau' = TD + \tau$ HHb	15.7 ± 3.8 sec	16.6 ± 4.3 sec
τ V_E	64 ± 15 sec	53 ± 10 sec *

Table 2.4
Characteristics times involded in the work rate transition (mean ± SD) for VO_2, HR, HHb, VE in normoxia (FIO2=20.9%) and hypoxia

HHb kinetics-VO_{2p} kinetics:

The HHb/VO_{2p} ratio used to analyze the relationship between the rate of adjustment of HHb and VO_{2p}, is greater in hypoxia (1.24 ± 0.3) compared to normoxia (1.17 ± 0.2) ($p<0.05$) (see fig 2.3)

V_E kinetics: In the loaded period, the V_E was the same in hypoxia and normoxia ($p>0.05$) (see Table 2). The V_E amplitude normalized to the VO_2 amplitude ($\Delta V_E / \Delta VO_2$) was however greater in hypoxia ($p<0.05$) (see Table 2.3). The rate of adjustment, τ, was shorter in hypoxia than in normoxia ($p<0.05$) (see Table 2.4).

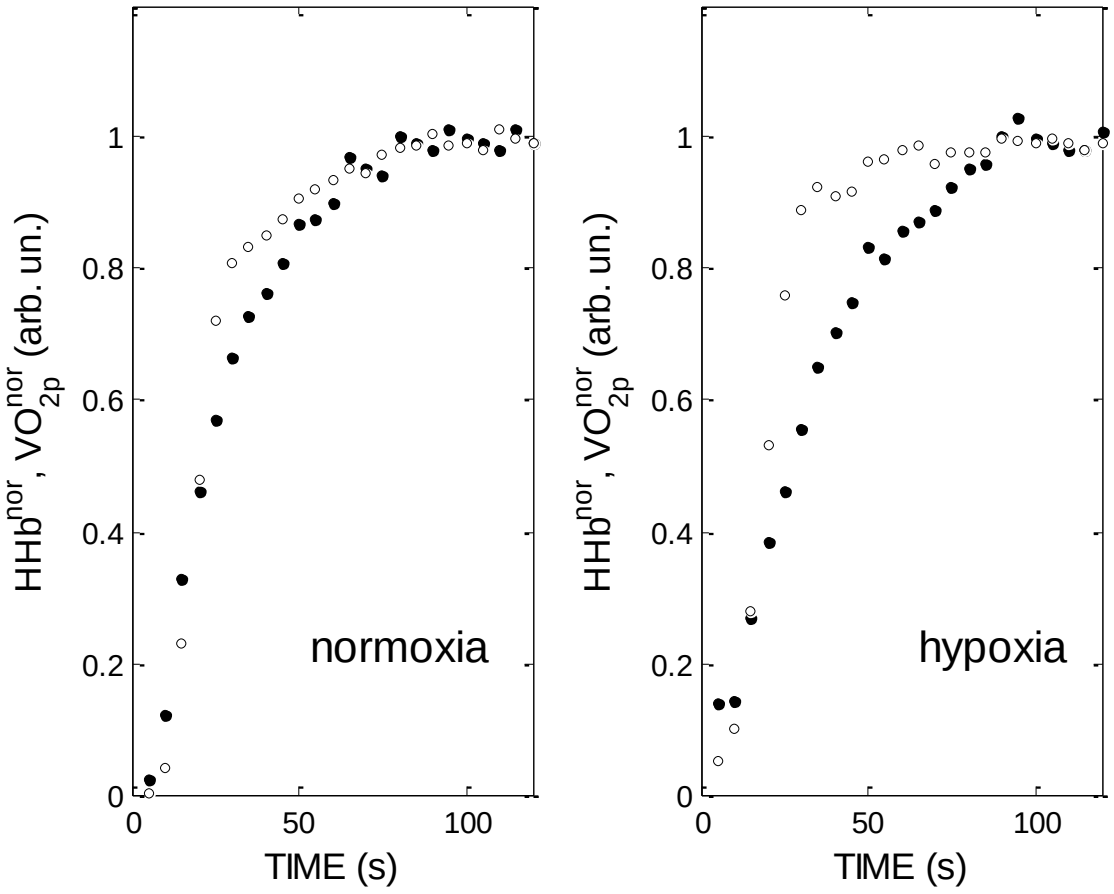

Figure 2.2
Time evolution of HHb^{nor} (open circles) and $VO_{2p}{}^{nor}$ (filled circles) for normoxia(FIO_2=20.9%), left panel, and hypoxia (FIO_2=15%), right panel. Data is shown for a single subject.

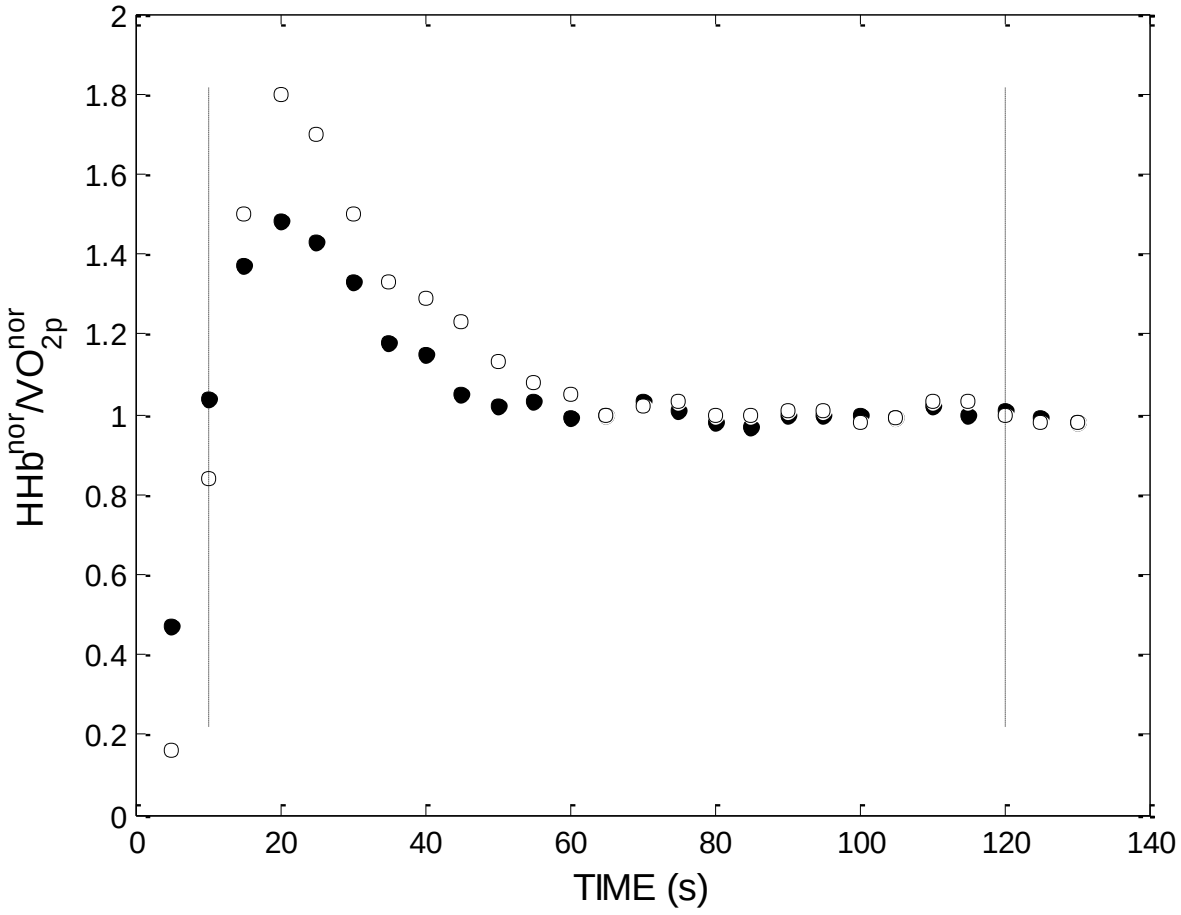

Figure 2.3
Time evolution of $HHb^{nor}/VO_{2p}{}^{nor}$ for hypoxia (open circles) and normoxia (filled circles). Data is shown for a single subject. The vertical dotted lines represent the time limits used to calculate the HHb/VO_{2p} ratio (see text).

2.4 Discussion

This study examines VO_{2p}, HR, and NIRS derived variables, together with their kinetic response, during exercise at the same relative intensity (80% VT_1), in a group of 14 older adults in normoxia (FIO_2=20.9%) and hypoxia (FIO_2=15%).

The major findings was: lower rate of adjustment of phase 2 VO_{2p} and a greater absolute $\Delta HHb/\Delta VO_2$ ratio in hypoxia compared to normoxia at the steady state, due, in hypoxia, to a poorer matching of oxygen delivery to oxygen utilization to the active tissues.

Transition between unloaded and loaded exercise:

In the literature, results on the phase 2 VO_{2p} rate of adjustment in normoxia and hypoxia are controversial. Although some authors have found no differences between normoxia and hypoxia (Griffiths, et al. 1986) (MacDonald, et al. 1999), others (Springer, et al. 1991), (DeLorey, et al. 2004), (Engelen, et al. 1996), (Hughson, et al. 1995) have found a slower VO_2 kinetics response in hypoxia. The slower rate of adjustment for VO_2 in these studies has been attributed to the fact that an increase in HR in hypoxia does not completely compensate for the reduced arterial oxygen content (CaO_2) (Engelen, et al. 1996). However, it must be emphasized that in hypoxia, even if the O_2 delivery may be fully normalized by the hypoxia-induced vasodilatation, the change in microvascular and intracellular PO_2 could independently influence the mitochondrial respiration through the following reaction:

$$NADH + 3P_i + H^+ + 3ADP + \frac{1}{2}O_2 \rightarrow 3ATP + NAD^+ + H_2O$$

In fact Haseler et al. (1998) suggests that tissue or intracellular oxygenation may play a role in the modulation of the regulators of cellular respiration and thereby suggest that cellular levels of O_2 may influence metabolic control even during submaximal exercise.

In the present study we found slower phase 2 VO_{2p} kinetics, whereas no difference in the HR and HHb kinetics. One could argue that slower VO_{2p} kinetics in hypoxia would be associated with a greater reliance

on O_2 extraction during the exercise on-transient, implying faster HHb kinetics. However we did not find faster HHb kinetics. This observation can be explained assuming that hypoxia affects the control of mitochondrial respiration, slowing the muscle O_2 extraction (estimated from changes in muscle deoxygenation) (Wilson, et al. 1988). This would prevent the HHb kinetics from getting faster in response to a reduction in the microvascular blood flow.

In the present study, for older adults, the HHb/VO_{2p} ratio was greater in hypoxia (1.24 ± 0.3) compared to normoxia (1.17 ± 0.2) ($p<0.05$) indicating a greater mismatch between O_2 delivery to O_2 utilization. The present results support previous findings, by (Russell, et al. (2003)) which found, on rats, that the distribution and hemodynamics of blood flow within the microcirculation is impacted by the aging process. Since, however, the HHb kinetics does not change between the 2 conditions ($p > 0.05$), the greater index must be due to the slower VO_{2p} kinetics. The slower VO_{2p} kinetics could reflect a slow vasodilator response to the increasing demand of blood which is however O_2 deficient. In this regard, it was shown that aging reduces the compensatory vasodilatation during hypoxic forearm exercise ((Casey, et al. 2011)).

It has been recently demonstrated that the HHb/VO_{2p} ratio is greater in older adults than in younger adults (DuManoir, et al. 2010b) and it can be reduced (i.e the matching between O_2 delivery and muscle O_2 utilization is improved) with endurance training, both in young and in older adults (Murias et al.,2010). Moreover (Behnke, et al. (2005)) demonstrate on this regard that in older rats, at the onset of muscle contractions, oxygen delivery dynamics are slowed disproportionately to those of muscle O_2 uptake (VO_2), such as microvascular PO_2 (PO_2m) was found reduced and blood–tissue O_2 transfer compromised. Many aspects related to the aging process could affect the rapid matching of O_2 delivery to the metabolic muscle requirements. For instance, aging related morphological changes such as decreased capillary density ((Coggan, et al. 1992)), thickening of vascular walls (Moreau, et al. 1998), impaired endothelial function ((MacAllister 2002)) , altered ratio of endothelial receptors (Ergul, et al. 1998) and altered

reactivity of the smooth muscle to sympathetic activity (Moreau, et al. 1998) could prevent from quickly adjusting and delivering the microvascular blood flow in response to a drop in CaO_2 during the transient. The result, that in older adults during hypoxic exercise, the HHb/VO_{2p} ratio is higher than in normoxia, hence slowing the VO_{2p} kinetics, are supported by a recent study of Spencer et al (2011) where a greater HHb/VO_{2p} ratio was found in a group of young adults exercising in hypoxia, resulting in slower phase 2 VO_{2p} kinetics. The present data in combination with those of Bell et al (1999) suggest that aged individuals reside in very close proximity to the "tipping point" (D. C. Poole, et al. 2009) where hyperoxia (that increased muscle/mitochondrial O_2 availability) did not speed VO_2 kinetics, whereas hypoxia did slow it.

Steady-state:

We found that $\Delta VO_{2p}/\Delta WR$ did not change between normoxia and hypoxia, in agreement with the study of Burtscher et al (2001). Moreover we found a significant increase in central blood flow ($\Delta HR/\Delta VO_{2p}$) ($p<0.05$) together with an increase in $\Delta HHb/\Delta VO_{2p}$ ($p<0.05$) during the steady state in hypoxia. These increases offset the decrease in CaO_2, maintaining the $\Delta VO_{2p}/\Delta WR$ constant between the 2 conditions. Since the $\Delta VO_{2p}/\Delta WR$ was constant between the 2 conditions, the simultaneous increase, under hypoxic conditions, in $\Delta HHb/\Delta VO_{2p}$ and $\Delta HR/\Delta VO_{2p}$ suggests that the delivery of O_2 across the muscles, regulated by the following version of the Fick's law [$mVO_2(t)=Q_m(t)\cdot(CaO_2-CmO_2)$; where $mVO_2(t)$ is the VO_2 of the muscle at time t, $Q_m(t)$ is muscle blood flow at time t, and CaO_2 and CmO_2 are the oxygen contents of the arterial and microvascular blood, respectively] is impaired at the microvascular level in hypoxia. The latter phenomenon usually increases with aging (DeLorey et al.,2004; DeLorey et al.,2007) and could be caused by the reduced vasodilator response found for older adults in hypoxia (Casey;, et al. 2011).

The hypoxia induced decrease in CaO_2 likely acts as a signal to increase blood flow (Roach, et al. 1999a). The hemoglobin itself could contribute, in hypoxia, to the enhancement of blood flow via vasodilatation

through the hemoglobin deoxygenation process. This process promotes in fact the release of nitric oxide (NO), thereby allowing the diffusion of the NO group to the vascular endothelium, where it stimulates vessel relaxation (Norris, et al. 1996).

It is worth noting however that these mechanisms of enhancement of blood flow in response to a drop in CaO_2 could be limited by the aging-related reduced vasodilator response (Casey, et al. 2011).

These results are in contrast to what was found for young healthy adults. In this case a higher muscle O_2 delivery and a lower O_2 extraction was reported in hypoxia compared to normoxia during knee extension exercise (Heinonen, et al. 2010). Differences were found however between trained and untrained subjects. In the former case, an increase in O_2 extraction during leg extension exercise, with no change in muscle blood flow, was observed in hypoxia (Richardson, et al. 1995). In the latter case a decrease in O_2 extraction with an increase in muscle blood flow was found in healthy adults exercising in an hypoxic condition (Rowell, et al. 1986).

Conclusion

We conclude that for older adults exercising in hypoxic conditions, the main limiting factor for the VO_2 lies in the capacity of the body to match properly the VO_2 demand to the VO_2 delivery at the microvascular level.

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CHAPTER 3
EFFECT OF ACUTE HYPOXIA ON MUSCLE BLOOD FLOW, VO_{2p} AND [HHb] DURING LEG EXTENSION EXERCISE IN OLDER MEN

3.1 Introduction:

During the on-transient to moderate-intensity exercise the Phase 2 of the pulmonary O_2 uptake (VO_{2p}) response, reflecting muscle O_2 consumption (Barstow, et al. 1987), (Grassi, et al. 1996), (Rossiter, et al. 1999), has been shown to be slower in older compared to younger adults (Babcock, et al. 1994), (Bell, et al. 1999), (Murias, et al. 2010). Although during exercise performed at different submaximal intensities bulk delivery of O_2 may (Bell, et al. 1999; Chilibeck, et al. 1996) or may not (Magnusson, et al. 1994; Proctor., et al. 2003) be affected by aging, slower HR kinetics and reduced leg blood flow have been observed in older compared to young individuals (Chilibeck, et al. 1996; Proctor, et al. 1998). More recently, microvascular O_2 delivery limitations have been proposed to be an important factor regulating the rate of adjustment of oxidative phosphorylation in both older and young adults (Murias, et al. 2010). Poole and Musch reported in their review a series of elegant papers that support the idea that for aged individuals the site of limitation of VO_2 kinetics might shift upstream from the mitochondria (O_2 utilization) to the O_2 transport and delivery (Poole, et al. 2009).

In contrast to these studies which support a role for an O_2 delivery dependent slowing of VO_{2p} kinetics with aging, the idea that intracellular control is an important contributor to the slowing of VO_{2p} kinetics with advancing age (Gurd, et al. 2008) cannot be dismissed.

Breathing hypoxic gas mixtures, which causes a decrease in arterial oxygen content (CaO_2), poses a challenge for the O_2 transport system. Studies that have used hypoxia to analyze the phase 2 VO_{2p} kinetics during the on-transient to moderate intensity exercise have found contradictory results. Investigations of changes in VO_{2p} kinetics (τVO_{2p}) during cycling exercise in young adults reported a greater phase 2 VO_{2p} time constant (reflecting a slower adjustment) under mild hypoxic conditions (FIO_2 = 14-15% O_2) (Engelen, et al. 1996), (Hughson, et al. 1995),(Linnarsson, et al. 1974),(Springer, et al. 1991). On the

other hand, studies in which a leg-extension exercise protocol was used in younger adults reported either a slower rate of adjustment for VO_{2p} throughout the exercise transient in hypoxia (FIO_2 = 12% O_2) compared to normoxia (DeLorey, et al. 2004) or no change in the phase 2 τVO_{2p} between the two conditions (MacDonald, et al. 1999). Concerning older adults, very few studies have been performed under hypoxia. These studies have focused on the long (Arai, et al. 2002), (Levine, et al. 1997b) and short (Burtscher, et al. 2001) term response to mild hypoxia and did not describe the VO_{2p} kinetics response. Recent data from our laboratory (Zerbini, et al. 2012) showed that phase 2 τVO_{2p} was greater in a group of older adults during cycling in a mild hypoxic (FIO_2 = 15% O_2) environment compared to normoxia. This was ascribed to a slow microvascular O_2 delivery within the muscle, suggested to be due to a poorer local vasodilatory response to hypoxia. These data in combination with those of Bell et al (1999) suggest that aged individuals reside in very close proximity to the "tipping point" (Poole, et al. 2009) where hyperoxia (that increased muscle/mitochondrial O_2 availability) did not speed VO_2 kinetics, whereas hypoxia did slow it. In order to further investigate whether O_2 delivery plays a major role in slowing of the VO_{2p} kinetics in older adults exercising in hypoxia, we adopted a 2- legged knee-extension model. This approach has been shown, to increase leg blood flow to compensate for the lower CaO_2 during submaximal exercise in young adults (DeLorey, et al. 2004; Koskolou, et al. 1997) so that O_2 delivery is kept constant in hypoxia (Koskolou, et al. 1997). However, since aging has been shown to reduce the compensatory vasodilatation during hypoxic exercise (Casey, et al. 2011), our goal was to evaluate whether or not older adults would be able to increase LBF to compensate for the lower CaO_2 during knee-extension exercise performed in hypoxic conditions, and to determine how this response affects the VO_2 kinetics.

Thus the aim of this study was to examine the effect of acute hypoxia on VO_{2p}, HR, conduit artery LBF and muscle deoxygenation ([HHb]) kinetics during knee-extension exercise in older adults. We hypothesized that, during a submaximal 2-legged knee-extension exercise, older adults would be able to

increase LBF to compensate for the reduction in CaO_2 under hypoxic conditions, and that would prevent the slowing of the VO_{2p} kinetics.

3.2 Methods

3.2.1 Ethical Approval

This protocol and all procedures were approved by The University of Western Ontario Ethics Committee for Research on Human Subjects, in accordance with the Declaration of Helsinki. Subjects were given verbal and written explanation of the experimental protocol, including possible risks and discomforts associated with the testing procedure. Subjects provided written consent before volunteering to participate in this study.

3.2.2 Subjects

Six older (70 ± 4 yrs) male subjects participated in this study and were all nonsmokers and free of known respiratory, cardiovascular, and metabolic disease.

3.2.3 Protocol

All tests were performed on a custom-built alternate-leg knee-extension ergometer as previously described (MacPhee, et al. 2005). This upright 2-leg knee extension model has advantages over the one-leg knee extension exercise such as a reduced O_2 cost from accessory muscles due to postural support, reduced risk of performing isometric contractions, and a larger increase in metabolic rate (power output) which favors an increase in the VO_{2p} signal-to-noise ratio.

Subjects were in a seated upright position with their legs strapped to a padded “arm” that was attached to a Monark cycle ergometer (model 814E). The ergometer allowed the contraction of quadriceps and hamstrings muscles in an alternating “kicking fashion”. Passive movement of the subject’s legs was accomplished by having an assistant pedal the cycle-ergometer, whereas during the active exercise the subjects alternately extend their legs over a 2-s duty cycle (i.e., 1-s contraction, 1-s relaxation). Both passive and active exercises resulted in a frequency of 30 extensions per minute (epm) per leg. On

separate days, subjects performed five repetitions of a moderate-intensity square wave transition protocol in each of the following two conditions. Under normoxic conditions, the protocol involved 2 minutes of passive knee-extension, 4 minutes of knee-extension at 3 W followed by an instantaneous transition to an absolute work rate of 21 W (expected to be within the moderate intensity domain for older subjects) for 6 minutes. For trials performed in acute, mild hypoxia, the protocol involved a 30 minute resting accommodation period, before starting the same sequence used for the normoxic trial. In normoxia, subjects inspired normal room air (FIO_2=21%) throughout the entire 12 minute protocol, whereas in hypoxia subjects inspired a hypoxic gas mixture (FIO_2=15%) throughout the entire 42 minute protocol (30 min accommodation and 12 min protocol). The work load of 21 Watts was chosen in order to be in the moderate intensity domain both in hypoxic and in normoxic conditions. To ensure this, a linear interpolation was made through the steady state VO_2 data for both conditions, to confirm the absence of any slow component (see Fig. 1). Given the small amplitude of the workload transition, five repetitions of the same exercise were performed. Repeat testing is required in order to reduce the random "noise" in the measured signals with such testing and thus increase the underlying signal-to-noise ratio (Lamarra, et al. 1987).

3.2.4 Gas exchange

Inspired and expired flow rates were measured throughout the exercise protocol by a low dead space (90 ml) bidirectional volume turbine (VMM-110; Alpha Technologies, Laguna Hills, CA) which was calibrated before each test using a syringe of known volume (3.0 liters; Hans Rudolph, Kansas City, MO). Inspired and expired gases were sampled continuously (every 20 ms) at the mouth and analyzed for the fractional concentrations of O_2, CO_2, and N_2 by mass spectrometry Innovision, AMIS 2000, Lindvedvej, Denmark following calibration with precision-analyzed gas mixtures. Inspired and expired volumes were time-aligned with changes in gas concentrations by measuring the time delay for a bolus of gas to travel

through a capillary line from the turbine transducer and be detected by the mass spectrometer. Breath-by-breath alveolar gas exchange was calculated by using the algorithms of (Beaver, et al. 1981).

3.2.4 Leg blood flow and heart rate

Measures of femoral artery mean blood velocity (MBV) were made from the right leg using pulsed wave Doppler ultrasound (Vingmed System FiVe; GE Medical Systems, Horten, Norway). Data were acquired continuously using a 7.5-MHz probe with a 45° angle of insonation positioned on the skin surface at 2–3 cm distal to the inguinal ligament and proximal to the femoral artery bifurcation. This location was selected to minimize the turbulence from the femoral bifurcation (MacPhee, et al. 2005). MBV was obtained from each trial by integrating the total area under the MBV profile and averaging over the 2-s duty cycle. Trials in the same condition for a given subject were averaged together to yield a single MBV profile for each subject in Normoxia and Hypoxia trials. Femoral artery (FA) diameters were measured continuously by echo-Doppler ultrasound (7.5 MHz probe) and stored digitally for subsequent analysis. Measures of FA diameters were made in triplicate using on-screen calipers included with the Doppler ultrasound and were averaged together to produce a single diameter value for each subject at each time point.

During hypoxic trials, FA diameter measures were taken at the end of the accommodation period, during passive exercise, during the baseline and at the end of the moderate exercise. During normoxic trials, FA diameters were measured at rest, during passive exercise, during the baseline and at the end of the moderate exercise.

Leg blood flow (LBF) was calculated as $\mathrm{LBF}\,(\mathrm{ml}\cdot\mathrm{min}^{-1}) = MBV\,(cm\cdot s^{-1})\cdot\pi\cdot r^{2}\cdot 60$ where r is the radius of the FA. Beat-by-beat heart rate (HR) was monitored continuously by electrocardiogram (three-lead arrangement) using PowerLab (ML132/ML880; ADInstruments, Colorado Springs, CO) and was calculated (using a 5 s rolling average) based upon the R-R interval; arterial O_2 saturation (O_{2Sat}) was

monitored by finger pulse oximetry (Nonin 8600, Plymouth, Minnesota, USA). Data were recorded using LabChart v6.1 (ADInstruments, Colorado Springs, CO) on a separate computer.

Near-infrared spectroscopy

Local muscle deoxygenated haemoglobin ([HHb]) of the quadriceps vastus lateralis muscle was monitored continuously with a frequency-domain multi-distance NIRS system (Oxiplex TS, Model 95205, ISS, Champaign, IL, USA). The arrangement for the present study included a single channel consisting of eight laser diodes operating at two wavelengths (λ = 690 and 828 nm, four at each wavelength) which were pulsed in a rapid succession (frequency modulation of laser intensity was 110 MHz) and a photomultiplier tube. The lightweight plastic NIRS probe (connected to laser diodes and photomultiplier tube by optical fibers) consisted of two parallel rows of light emitter fibers and one detector fiber bundle; the source-detector separations for this probe were 2.0, 2.5, 3.0, and 3.5 cm for both wavelengths. The probe was placed on the belly of the muscle midway between the lateral epicondyle and greater trochanter of the femur; it was secured in place with an elastic strap tightened to prevent movement. This allowed for continuous measurement of absolute concentration changes (μM) of oxyhaemoglobin ([HbO_2]) and [HHb]. The area of interrogation was covered with an optically-dense, black vinyl sheet, thus minimizing the intrusion of extraneous light. The thigh was wrapped with an elastic bandage to further minimize intrusion of extraneous light and movement of the probe. NIRS measurements were collected continuously for the entire duration of each trial. The near-infrared spectrometer was calibrated at the beginning of each testing session following a warm-up period of at least 20 min. The calibration was done with the probe placed on a calibration block (phantom) with absorption (μ_A) and reduced scattering coefficients (μ_s') previously measured; thus, correction factors were determined and were automatically implemented by the manufacturer's software for the calculation of the μ_A and μ_s' for each wavelength during the data collection. Calculation of [HHb] reflected continuous measurements of μ_s' made throughout each testing session (i.e., constant scattering value not

assumed). Data were stored online at an output frequency of 25 Hz, but were reduced to 1 s bins for all subsequent analyses within the present study. Although spatial heterogeneities of the ΔHHb signal have been shown (Koga REF) the variance in the time-constant at different sites is small; the ΔHHb signal has a temporal profile similar to the changes in muscle intracellular PO_2, microvascular PO_2, and (a-v) O_2 difference, thus resulting in a good non-invasive indicator of the temporal profile of fractional O_2 extraction (Jones, et al. 2009).

3.2.6 Analysis of Data

VO_{2p}, MBV, and HR data for each individual trial were initially filtered for erroneous data points that lay outside four standard deviations of the local mean. Data were then interpolated to 1 second intervals and time-aligned such that time zero represent the onset of the exercise. The data for each repetition within a condition were further ensemble-averaged for each subject and time-averaged into 5-s bins to yield a single response profile. Second-by-second LBF (calculated from the averaged interpolated MBV and femoral artery radius) were averaged into 10-s time bins.

The on-transient responses for VO_{2p}, LBF, and HR were modeled using a monoexponential of the form $Y(t) = Y_{bas} + Y_{amp} \cdot (1 - e^{-(t-TD)/\tau})$ where $Y_{(t)}$ represents VO_{2p}, LBF, or HR as a function of time (t) throughout the exercise transient; Y_{Bsln} is the baseline of Y during steady-state baseline exercise before the step increase in WR; *Amp* is the amplitude of the increase in Y above the baseline value; τ is the time constant (i.e., time taken to reach 63% of the steady-state response); and TD is the time delay.

LBF and HR were fit from the first data point after the start of the exercise transient until the end of the exercise bout. On the other hand, the initial 20 sec of VO_{2p} data were excluded to avoid inclusion of data points from Phase 1 VO_{2p} in the fitting of Phase 2 VO_{2p} (Murias, et al. 2011). Moreover the TD was allowed to vary freely in order to optimize the accuracy of the estimated parameters. VO_{2p} data were modeled from the Phase 1- Phase 2 transition to 240 sec using non-linear, least-squares regression procedures (Origin, OriginLab Corp.,Northampton, MA, USA).

The 95% confidence interval (CI) for the estimated time constant was determined after preliminary fit of the data with *Bsln*, *Amp* and TD constrained to the best-fit values while τ was allowed to vary.

The NIRS-derived data were time-aligned and ensemble-averaged into 5-s bins to yield a single response for each subject for both normoxia and hypoxia trials. The [HHb] was fitted with a mono-exponential fit from the calculated timed delay (TD-HHb) to 90 sec. The time delay (TD-HHb) precedes the increase in the signal with an ”exponential-like” time course and was determined visually from the onset of exercise to the first rapid increase in the [HHb] signal (D. S. DeLorey, et al. 2003). The effective time constant, τ', for the [HHb] signal was calculated as $\tau' = TD + \tau$.

HHb kinetics vs. VO_{2p} kinetics

To investigate the relationship between the [HHb] and the VO_{2p} kinetics, data on the time evolution of [HHb] and VO_{2p} were normalized, for each subject, in such a way that the response varies from 0 (unloaded condition) to 1 (loaded condition). The normalized VO_{2p} curve was left-shifted taking the duration of phase 1 into account. Thus the onset of exercise (t=0) coincides, for the VO_{2p} curve, with the beginning of phase 2, which is known to reflect closely the muscle oxygen uptake (Rossiter, et al. 1999). Curves obtained in this way are referred to as HHb^{nor} and $VO_{2p}{}^{nor}$. Subsequently, the $HHb^{nor}/VO_{2p}{}^{nor}$ ratio was evaluated for each subject. From this curve, an overall index [HHb]/VO_2 was derived, ((Murias, et al. 2010)) by calculating the time-average of $HHb^{nor}/VO_{2p}{}^{nor}$ from t=10 s (beyond the physiological TD-HHb) to t=60 s , i.e.: $\frac{1}{N}\sum_{n=1}^{N}\left(\frac{HHb^{nor}}{VO_{2p}^{nor}}\right)_n$, where n=1 corresponds to t=10s and n=N corresponds to t=60s.

Statistical Analysis

The kinetic parameter estimates for VO_{2p}, LBF, HR, and [HHb] were analyzed using Paired t-tests to determine the statistical significance. All values are means ± SD. Data were analyzed with the software package SPSS version 3.5 (SPSS Inc., St Louis, MO, USA).

Results

Subjects age, body mass and height were 70 ± 4 yr, 86 ± 15 kg, and 168 ± 11 cm respectively. Arterial oxygen saturation was significantly lower (p=0.002) in hypoxia (85%) compared to normoxia (96%).

VO_{2p}: Fig.3.1 shows the VO_{2p} response (averaged over all subjects) during the square wave moderate intensity test. The duration of the VO_{2p} phase 2 τ was not different between the two conditions (p=0.49) (Table 3.1). The VO_{2p} gain ($\Delta VO_{2p}/\Delta WR$) was similar between the two conditions (p=0.84) (Table 3.2).

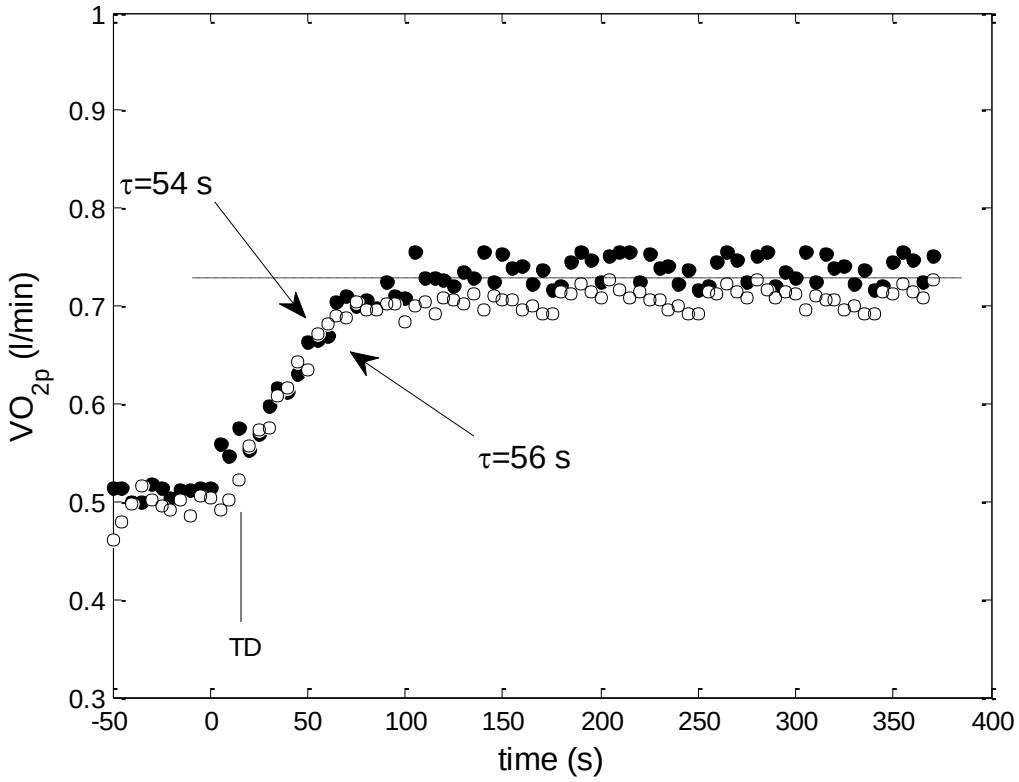

Fig. 3.1
Response of pulmonary O_2 uptake during the square wave exercise in normoxia (FIO_2=20.9%) (filled circles) and hypoxia (FIO_2=15%)(open circle) (not significantly different). Data represents the average of 5 repetitions of the square wave exercises for all the subjects. The continuous horizontal line is drawn to show that there is no evidence for the VO_2 slow component.

HR: The steady-state HR measured during the loaded period did not differ (p=0.14) between the two conditions (see Table 3.1) despite a higher unloaded HR in hypoxia compared to normoxia (p=0.03); thus,

the HR amplitude was lower in hypoxia compared to normoxia (p=0.03) (Table 3.1). The τHR was similar between hypoxia and normoxia (p=0.65) (Table 3.1).

LBF: At rest, leg blood flow was the same between normoxia and hypoxia (p=0.09) (Table 3.1), whereas during the unloaded and loaded periods, LBF was higher in hypoxia compared to normoxia (p=0.02) (Table 3.1). The rate of adjustment of LBF, was faster in hypoxia compared to normoxia (p=0.01) (see Fig. 3.2).

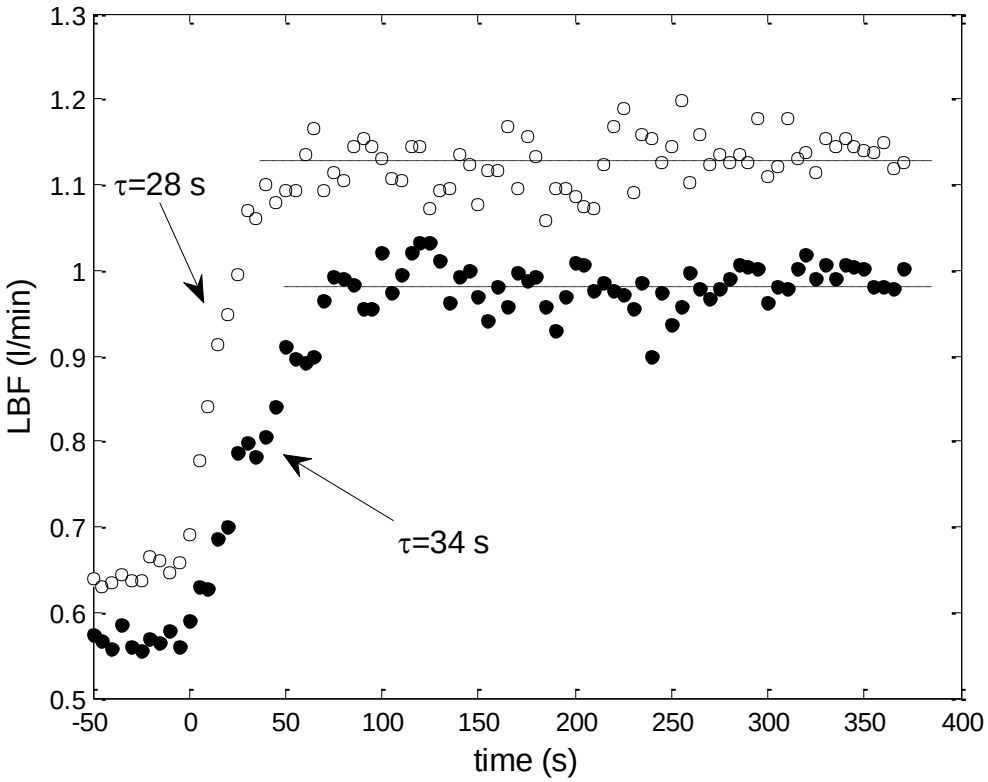

Fig. 3.2
Response of LBF during the square wave exercise in normoxia (FIO_2=20.9%) (filled circles) and hypoxia (FIO_2=15%)(open circle) (significantly different). Data represents the average of 5 repetitions of the square wave exercises for all the subjects. The continuous horizontal lines are drawn to show that there is no evidence for the LBF slow component.

Assuming an arterial hemoglobin concentration ([Hb]) of 15.0 g · 100 ml^{-1} and an O_2 carrying capacity of 1.34 ml · g^{-1} Hb, the estimated arterial O_2 content (CaO_2) was 176 mL·L^{-1} in hypoxia and 195 mL·L^{-1} in normoxia during the unloaded period (3 W) and 166 ml·L^{-1} in hypoxia and 192 ml·L^{-1} in normoxia during the loaded period (21 W). Leg O_2 delivery was calculated as the product of CaO_2 and LBF, resulting in

110 ± 4 ml·min^{-1} in normoxia and 113 ± 6 ml/min in hypoxia (p=0.08) during the unloaded period, and 189 ± 4 ml·min^{-1} in normoxia and 190 ± 6 ml·min^{-1} in hypoxia (p=0.35) during the steady state of loaded exercise.

HHb: During both the unloaded and loaded periods, the steady-state [HHb] was similar in hypoxia and normoxia (p=0.23) (Table 1) so that the [HHb] amplitude did not differ between conditions (p<0.05) (Table 3.1). The rate of adjustment of [HHb], and the effective time response $\tau' = TD + \tau$, were slower in hypoxia compared to normoxia (p=0.03) (Fig.3.3).

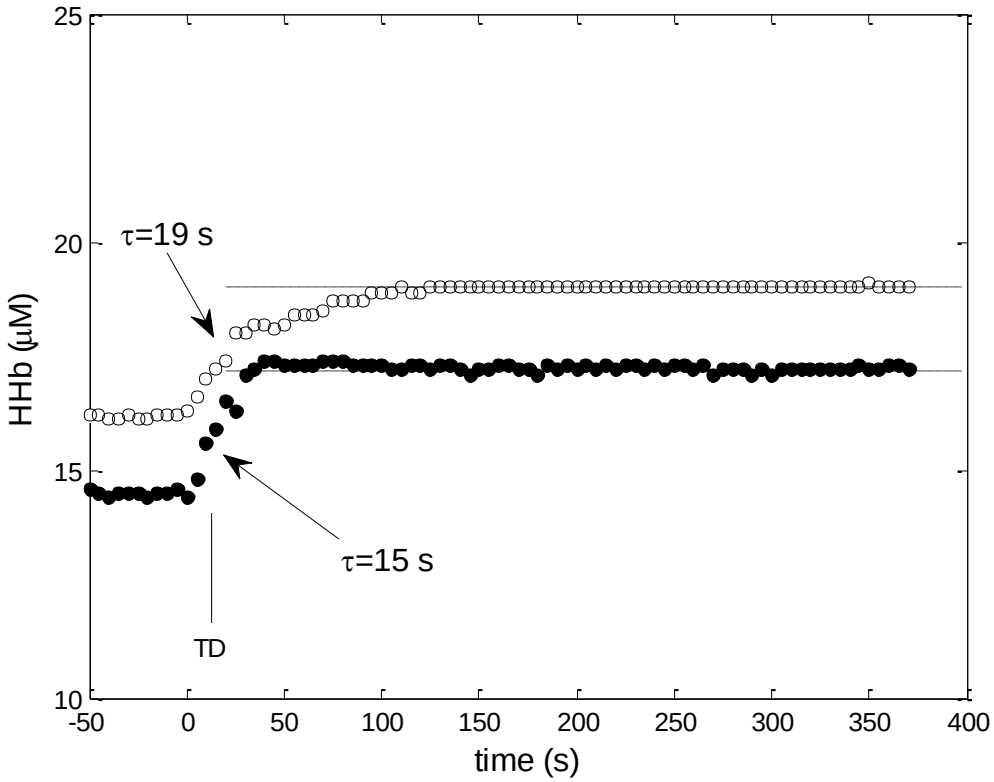

Fig.3.3
Response of [HHb] during the square wave exercise in normoxia (FIO_2=20.9%) (filled circles) and hypoxia (FIO_2=15%) (open circle). Data represents the average of 5 repetitions of the square wave exercises for all the subjects. The continuous horizontal lines are drawn to show that there is no evidence for the HHb slow component.

HHb kinetics vs. VO_{2p} kinetics:

The index [HHb]/VO_2, used to quantify the degree of possible temporal dissociations in the adjustments of normalized [HHb] and VO_{2p}, was lower in hypoxia (1.07 ± 0.06) compared to normoxia (1.21 ± 0.22) (see Fig. 3.4) (p=0.01).

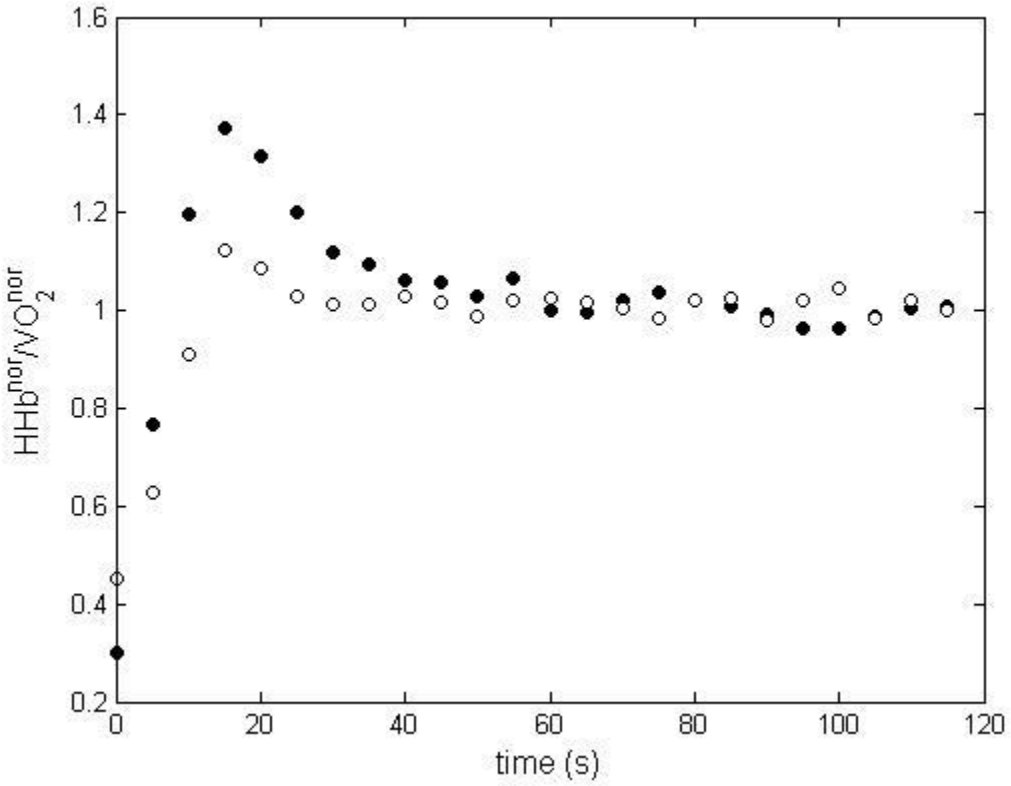

Fig. 3.4
Time evolution of $HHb^{nor}/VO_{2p}{}^{nor}$ for hypoxia (open circles) and normoxia (filled circles). Data represents the average of 5 repetitions of the square wave exercises for all the subjects.

Tab. 3.1

	Conditions	Rest	Unloaded	Loaded	Amplitude	TD (sec)	Tau (sec)	C_{95} (s)
FA (mm)	hypoxia	8.7 ± 0.1*	8.7 ± 0.4*	8.7 ± 0.5*				
	normoxia	8.2 ± 0.2	8.2 ± 0.4	8.2 ± 0.4				
LBF ($L \cdot min^{-1}$)	hypoxia	0.32 ± 0.0	0.63 ± 0.1*	1.17 ± 0.2*	0.54 ± 0.4		28 ± 2*	7 ± 3
	normoxia	0.31 ± 0.0	0.52 ± 0.1	0.96 ± 0.2	0.44 ± 0.2		34 ± 3	6 ± 4
VO_2 ($L \cdot min^{-1}$)	hypoxia	0.33 ± 0.0	0.56 ± 0.1	0.79 ± 0.2	0.23 ± 0.1	11 ± 1	56 ± 8	4 ± 1
	normoxia	0.34 ± 0.0	0.52 ± 0.1	0.76 ± 0.1	0.24 ± 0.1	13 ± 3	54 ± 6	4 ± 1
ΔHHb (μM)	hypoxia	-1.3 ± 2.1	16.2 ± 4.3	18.8 ± 3.7	2.8 ± 2	14 ± 1	19 ± 4*	2 ± 1
	normoxia	-1.4 ± 1.2	14.4 ± 3.3	17.3 ± 4.1	3.1 ± 1	13 ± 2	15 ± 4	2 ± 1
HR ($b \cdot min^{-1}$)	hypoxia	69 ± 4	84 ± 4*	93 ± 6	9 ± 3*		22 ± 12	5 ± 2
	normoxia	65 ± 6	78 ± 3	89 ± 4	11 ± 2		20 ± 7	6 ± 3
VE ($L \cdot min^{-1}$)	hypoxia	5 ± 1	14 ± 4*	25 ± 6*	11 ± 3*			
	normoxia	4 ± 1	10 ± 3	18 ± 4	8 ± 2			

Tab.3.1
Rest, unloaded, steady state, characteristic time (Tau) and time delay (TD) values (mean ± SD) for several parameters in normoxia (FIO_2=20.9%) and hypoxia (FIO_2=15%). The amplitude was calculated from the unloaded to the loaded steady state.
* $p<0.05$, significantly different from normoxia (FIO_2=20.9%)
C_{95} is the 95% confidence interval for the estimated time constant.

Tab. 3.2

	Normoxia	**Hypoxia**
$\Delta HR/\Delta VO_2$ $\left(\frac{b/\min}{L}\right)$	55 ± 6	45 ± 4 *
$\Delta VO_2/\Delta WR$ $\left(\frac{ml/\min}{W}\right)$	12.2 ± 1	11.1 ± 1.5
$\Delta HHb/\Delta VO_2$ $\left(\frac{\mu M/\min}{L}\right)$	14.5 ± 2	13 ± 2
$2\cdot\Delta LBF/\Delta VO_2$ ($L\ min^{-1}/\ L\ min^{-1}$)	4.3 ± 0.9	4.8 ± 1.3*

Tab.3.2
$\Delta VO_2/\Delta WR$, $\Delta HHb/\Delta VO_2$, $\Delta HR/\Delta VO_2$,2 $\Delta LBF/\Delta VO_2$ values in normoxia (FIO_2=20.9%) and hypoxia (FIO_2=15%).
* $p<0.05$, significantly different from normoxia (FIO_2=20.9%)

3.4 Discussion

To the best of our knowledge this the first study to simultaneously assess the rate of adjustment and steady state response for VO_{2p}, [HHb], HR and LBF in response to moderate-intensity leg-extension exercise with and without an hypoxic stimulus (FIO_2 = 15% O_2) in older adults. The main findings were: (1) LBF adjusted more rapidly during the exercise on-transient and was elevated during steady-state exercise in hypoxia compared to normoxia; (2) the kinetics of phase 2 VO_{2p} and the VO_{2p} gain were similar in normoxia and hypoxia; (3) for a given increase in VO_{2p} , the increase in LBF, ($2\cdot\Delta LBF/\Delta VO_2$) and in HHb, ($\Delta[HHb]/\Delta VO_2$) were similar between the two conditions, whereas the increase in HR, ($\Delta HR/\Delta VO_2$) was higher in hypoxia compared to normoxia; (4) The $[HHb]/VO_2$ index was smaller in hypoxia compared to normoxia, indicating a better matching between O_2 delivery to O_2 utilization at the microvascular level.

LBF-HR-VO_{2p}-HHb: *kinetics responses.*

In the present study VO_2 kinetics did not change, but LBF was faster and the [HHb]/VO_2 index was smaller in hypoxia compared to normoxia. The tau VO_{2p} in normoxia, was in line with that found by DuManoir et al. (2010) (DuManoir, et al. 2010c) for a group of older adults exercising in normoxia using the same protocol we adopted. DuManoir et al. ascribed the slower VO_{2p} and LBF kinetics of older adults, as compared to young adults, to an aging-related reduction in the blood flow distribution within the muscle microvasculature.

Recent data from our laboratory (Zerbini, et al. 2012) indicate that VO_{2p} kinetics are slower in older adults (66±6 yrs) cycling in a mild hypoxic (FIO_2 = 15% O_2) compared to normoxic environment. From these results (Zerbini, et al. 2012) and from the results of the present study, it seems that O_2 delivery plays an important role in the determination of the rate of adjustment of VO_{2p}. Indeed, in both studies, the rate of adjustment of the VO_{2p} kinetics was related to the degree of matching between O_2 delivery and O_2 utilization within the muscle. During cycling however the VO_2 kinetics was slower in hypoxia than in normoxia and [HHb] kinetics remained the same, which resulted in a greater [HHb]/VO_2 index in hypoxia compared to normoxia (Zerbini, et al. 2012). We explained these results in terms of an increased mismatch between O_2 delivery and O_2 utilization under hypoxic conditions. During knee-extension exercise, however, we ascribe the unchanged VO_2 kinetics between hypoxia and normoxia to an increase in the LBF that results in unchanged O_2 delivery. Since the difference in the muscle mass involved in cycling and 2-legged exercises is small, the different responses between exercise conditions could be explained by the different work rates in each modality (~100 watts for cycling and 21 watts for the 2-legged extension exercises). It is likely that in older adults the LBF does not increase linearly with the work rate for submaximal intensity.

For instance, Poole et al (2002) found, that LBF during cycling in normoxia is well preserved in older adults (70 yrs old) exercising at a relatively light submaximal power output (54 watt). On the contrary, when the submaximal work rate was increased from 54 to 99 watt, the LBF to work rate ratio was

significantly attenuated in older subjects (Poole, et al. 2003). As such, it would seem that older adults exercising at low work rates are able to respond to a drop in the CaO_2 by increasing the LBF in hypoxia. However, when exercising at higher work rates (even below the lactate threshold), older adults exhibit a low LBF to work rate ratio in normoxia (Poole, et al. 2003) and lose the ability to counteract the drop in CaO_2 likely because of impaired microvascular blood flow (Zerbini, et al. 2012).

Conflictive results have been reported during cycling exercise in young adults exposed to a mild acute hypoxia. Whereas some studies have shown a slowing of the phase 2 VO_{2p} kinetics in hypoxia (attributed to a reduction in the O_2 delivery to the muscles (Engelen, et al. 1996; Hughson, et al. 1995; Linnarsson, et al. 1974; Springer, et al. 1991), the present and other studies MacDonald et al 1999; (MacDonald, et al. 1999) did not find any change in the phase 2 VO_{2p} kinetics, between normoxia and hypoxia using a leg-extension exercise with younger adults. In the same population, using a one-leg extension exercise model under hypoxic conditions and with the same protocol and similar absolute work rates as presented in the current study, De Lorey et al (2003) showed a compensatory increase in LBF that was not enough to prevent VO_2 kinetics from getting slightly (but significantly) slower in hypoxia compared to normoxia. The authors argued that the slower VO_2 found in hypoxia through the transient, despite an unchanged O_2 delivery between normoxia and hypoxia, has to be ascribed to diffusion and mitochondrial aspects rather than to O_2 delivery.

In the study of Koskolu (1997) on a group of young adults exercising with a 2- legged knee-extension model, no increase in LBF was shown at FIO_2=16%, whereas a marked increase was shown at FIO_2=11%. On the contrary we have shown that for older adults, FIO_2=15% is enough to cause an increase in the blood flow to maintain the O_2 delivery to the muscle. It is therefore likely that the vasodilator compensation under hypoxic conditions occurs for older adults at a lower degree of hypoxia than for younger adults. That could be explained by the fact that older adults exhibit both in normoxia and hypoxia

a lower level of arterial oxygen saturation with regard to young adults (Koskolou, et al. 1997; Zerbini, et al. 2012).

Though we have shown that the O_2 delivery to the muscles remains constant between hypoxia and normoxia, we cannot exclude a diffusion limited mechanism. A decreased driving pressure for O_2 transport resulting in slowed VO_{2p} kinetics was in fact shown previously (Koike, et al. 1990). In contrast it has been recently demonstrated in a canine muscle preparation in acute hypoxia that keeping O_2 delivery constant by increasing the blood flow via a perfusion pump does not cause any change in phase 2 τVO_2, τHHb and MRT-HHb compared to normoxia. These observations were made despite a significantly lower mean capillary PO_2 in hypoxia that impaired peripheral O_2 diffusion (McDonald, et al. 2010). These results are in accordance with findings on young adults, that showed unchanged VO_{2p} together with an increase of LBF response in hypoxia, compared to normoxia, during leg extension exercise (Koskolou, et al. 1997). In this regard, we could speculate that during moderate exercise in hypoxia with an increased rate of blood flow through the capillaries due to vasodilatation, it is likely that the gradient of the PO_2 at the capillary level is only slightly reduced, so that it does not cause a great impairment in the peripheral diffusion of O_2. Thus, in the present study, despite the likely small reduction in the driving pressure for the O_2 transport from capillary to mitochondria, the fundamental aspect that could affect the VO_2 kinetics in hypoxia seems related more to the O_2 delivery and distribution within the muscle, than to peripheral diffusion aspects.

It has been hypothesized that the upstream limitation (conduit artery blood flow) could influence the downstream limitation (microvascular blood flow delivery) thus changing the VO_{2p} kinetics (DuManoir, et al. 2010a). Our data showed that, in hypoxia, faster LBF kinetics occurs together with a lower [HHb]/VO_2 index, slower [HHb] kinetics and unchanged VO_{2p} kinetics. The increase in LBF together with faster LBF kinetics in hypoxia, results in a tendency for the calculated leg O_2 delivery to increase during the exercise transient ($p=0.08$), thus improving the microvascular O_2 delivery and lowering the

[HHb]/VO_2 index. The kinetics of [HHb] are often associated with the capacity of the muscle to extract O_2 (DeLorey, et al. 2003; Murias, et al. 2010). Thus, the slower [HHb] kinetics found in this study could be associated with a lower O_2 extraction in hypoxia. This statement is in accordance with the study of Heinonen et al. (2010) (Heinonen, et al. 2010), where a higher muscle O_2 delivery during the steady state and a lower O_2 extraction in hypoxia, compared to normoxia, were reported during knee-extension exercise in adults. This phenomenon could be explained by the fact that during this kind of exercise in hypoxia, the blood flow increases to such an extent, that the calculated leg O_2 delivery could be even higher in hypoxia compared to normoxia (DeLorey, et al. 2004). Since we have seen that the [HHb]/VO_2 index is lower in hypoxia, which reveals a better matching between O_2 delivery to O_2 utilization, the slowing in the HHb kinetics appears to be a direct consequence of the improved microvascular blood flow delivery.

It has been demonstrated in normoxia that, for both older and young people, improving the local O_2 delivery to the muscle, through training or prior high-intensity exercise, results in a lowering of the [HHb]/VO_2 index with faster phase 2 VO_{2p} kinetics (Murias, et al. 2010),(Spencer, et al. 2011a). On the contrary when the O_2 delivery to the muscle was reduced breathing a hypoxic gas mixture, a greater [HHb]/VO_2 index, that result in slower phase 2 VO_{2p} kinetics, has been found in a group of younger adults during a cycling exercise (Spencer, et al. 2011a). During moderate intensity 2-legged knee-extension exercise in young adults the LBF kinetics were found to be several fold faster than the VO_{2p} kinetics (Koga, et al. 2005) indicating that in this kind of exercise, the O_2 delivery does not represent a limit, and that the hypoxic vasodilatation stimulus can raise LBF in order to keep the O_2 delivery constant in hypoxia (Koskolou, et al. 1997).

In the present study, the use of 2-legged knee-extension exercise with a low power output enables the body to offset the drop in CaO_2 caused by breathing a hypoxic mixture, keeping the O_2 delivery constant to the muscle, through an augmentation of the LBF thus preventing the phase 2 VO_{2p} from slowing in

hypoxia. This data are supported by a recent study of Spencer et al (2011) where the use of hypoxia after a prior heavy intensity exercise, led to a muscular O_2 delivery similar to the moderate exercise in normoxic conditions, with the increased blood flow caused by the prior heavy exercise nullified by the lower O_2 content caused by the hypoxia.

The HR kinetics were unaffected by hypoxia ($p>0.05$). This suggests that central O_2 delivery kinetics likely does not influence the phase 2 VO_{2p} kinetics when older adults exercise with the leg extension model. On the contrary, for young adults performing a cycling protocol, it was found that the HR increase during the transient and at the steady state in hypoxia (FIO_2 = 15% O_2) to keep the O_2 delivery to the exercising muscles constant, does not completely compensate for the reduced CaO_2 thereby causing a slowing of the phase 2 VO_2 (Engelen, et al. 1996; Rowell, et al. 1986).

LBF-HR-VO_{2p} –HHb: *steady state response.*

The increase in LBF during submaximal exercise in hypoxia has been ascribed to the compensatory vasodilator response due to the reduction in O_2 availability (D. P. Casey, et al. 2011). In the present study we found an increase in the LBF under hypoxia ($p<0.05$). After 30 min of accommodation, breathing a hypoxic gas mixture, the FA diameter increased with respect to normoxia; this difference persisted at rest, during the unloaded period and remained constant during the transition and the steady state of loaded exercise.

Even if it has been found that the vasodilatory signal originating from arterioles ((Segal, et al. 1989);(Welsh, et al. 1998)), capillaries (Berg, et al. 1997), or venules (Collins, et al. 1998), is capable of being transmitted through endothelial and smooth muscle cells via gap junctions (Christ, et al. 1996) and ascending up the vascular network to produce dilation of the feed artery (Welsh, et al. 1997), it is likely that the increase in FA diameter in hypoxia could be caused by the release of nitric oxide (NO). Indeed it has been demonstrated in pumped perfused rabbit, in normoxia, that the release of NO contributes to the vasodilatation of the femoral artery (Xu, et al. 2000). Moreover at the microvascular level the decrease in

CaO_2 acts as a signal to increase blood flow (Roach, et al. 1999b) and also the hemoglobin could contribute to the enhancement of blood flow in hypoxia through the hemoglobin deoxygenation process that promotes the release of nitric oxide (NO) and allows the diffusion of the NO group to the vascular endothelium, where it stimulates vessel relaxation (Norris, et al. 1996; Stamler, et al. 1997). Taking into account that the resistance of the FA is very low (Astrand 2003) it is likely that the major contribution to the enhancement of the blood flow in hypoxia originates from the increased dilatation of the resistance vessels.

Although the vasodilator response to acute hypoxia was demonstrated to be attenuated in older adults at rest and during exercise (Casey, et al. 2011), we found a higher LBF during the steady state of loaded exercise in the hypoxic with respect to the normoxic condition. This increase in LBF results in an unchanged calculated O_2 delivery between conditions (i.e., from CaO_2 and leg blood flow). Using a similar protocol applied to younger adults, the calculated O_2 delivery to the muscle was even higher in hypoxia compared to normoxia (DeLorey, et al. 2004). Another factor contributing to the increase in LBF during steady-state in hypoxia is related to the HR response. For instance, the increased HR in hypoxia results in an increase in central O_2 delivery. This is in agreement with previous findings in a group of older adults exercising in natural hypobaric hypoxia (Burtscher, et al. 2001). Although it has been demonstrated that the heart rate response to hypoxia decreases with advancing of age (Kronenberg, et al. 1973) and that the beta-adrenergic receptor density decreases to a greater extent in older compared to young rats after three weeks of hypoxia exposure (Mader, et al. 1991), the subjects in the present study were able to elevate the heart rate in response to hypoxia.

3.5 Conclusions

Microvascular O_2 delivery plays an important role during moderate exercise in hypoxia. In fact, when older adults are able to keep constant the O_2 delivery to the muscle, prevent any slowing of the phase 2 VO_{2p} kinetics in hypoxia. These data suggest that the lower CaO_2 observed in hypoxia was compensated for by an increase in LBF so that O_2 delivery to the muscle during the exercise on-transient and steady-state was maintained, resulting in unchanged VO_{2p} for the same ATP requirement as compared to normoxia.

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CHAPTER 4

PHYSIOLOGICAL RESPONSES TO EXERCISE IN HYPOXIA: YOUNG AND OLDER ADULTS

4.1 Introduction

The rate of increase in VO_{2p} was shown to be consistently slower in older as compared to young adults (Babcock, et al. 1994; Chilibeck, et al. 1996; DeLorey, et al. 2007; Murias, et al. 2010). On the contrary, NIRS measurements of muscle deoxygenation kinetics were similar between older and younger subjects (DeLorey, et al. 2004; Murias, et al. 2010) suggesting that the adjustment of muscle microvascular blood flow and O_2 delivery was slower in older adults. The vast majority of research comparing skeletal muscle haemodynmics during exercise in young and older adults was performed under normoxic conditions. Even if these studies provided novel insight into regulatory mechanisms of skeletal muscle blood flow, they leave untested questions in the case when arterial oxygen saturation may be limited by environmental conditions or medical pathologies.

Exercising in hypoxia is a great stress for the O_2 delivery and the O_2 microvascular delivery systems. Recent data from our laboratory showed a slowing in the phase 2 VO_2 in hypoxia (15% O_2) for a group of older adults with no change in the HR and HHb kinetics (Zerbini, et al. 2012). We ascribed this slowing to a mismatch between O_2 utilization and O_2 delivery within the muscle. In agreement with our results, a recent investigation by Casey et al (2010) showed an impaired vasodilatory capacity within a group of older adults performing a handgrip exercise (Casey, et al. 2011). An impaired capacity to quickly adjust the microvascular blood flow to the increased demand of O_2 utilization during an exercise in hypoxia could explain the mismatch between O_2 utilization and O_2 delivery found in our previous study.

Regarding young adults, a vast amount of literature (Engelen, et al. 1996; Springer, et al. 1991) reported slower phase 2 VO_2 and HR kinetics in hypoxia (12-15% O_2). The authors explained their results in terms of a reduction in the O_2 delivery to the muscle in hypoxia compared to normoxia. The HHb kinetics was

not measured in these studies. Recently however, Spencer et al (2011) reported slower phase-2 VO_2 kinetics with no change in the HR kinetics for a group of younger adults. The authors ascribed the results to a great mismatch between O_2 utilization and O_2 delivery within the muscle (Spencer, et al. 2011b). Since no studies, to the best of our knowledge, were performed to compare younger and older adults and their responses to acute hypoxia, we designed an experiment in this regard, considering a group of older and a group of younger healthy individuals. The aim of the study is to assess the influence of age and hypoxia on the phase 2 VO_2, HR and HHb kinetics.

We hypothesized that, due to the reduced response of heart rate, ventilation and vasodilatory capacity to hypoxia with advancing age (Casey, et al. 2011; Garcia-Rio, et al. 2007; Kronenberg, et al. 1973), the major limiting factor of the slowing in VO_{2p} kinetics in hypoxia is peripheral in older adults and central in young adults.

4.2 Methods

4.2.1 Subjects: Eight older male subjects (age, 70 ± 2 yrs; height 168 ± 7 cm; body mass 78 ± 7 kg) and eight young male subjects (age, 29 ± 4; height 181 ± 7 cm; body mass 73 ± 4 kg) agreed to participate in this study after giving written informed consent. All subjects were nonsmoking and free of cardiac, metabolic and pulmonary diseases. This study was carried out according to the Declaration of Helsinki. The protocol was approved by the Ethics Committee for Research on Human Subjects of the University of Verona.

4.2.2 Protocol: A randomized cross-over design was used. After medical screening, subjects were reported to the laboratory on four separate occasions. The order in which the 2 conditions (normoxia and hypoxia) were assigned was randomized.

A normobaric hypoxic chamber (B-Cat Netherlands) that uses a nitrogen pump system to lower the level of oxygen inside the chamber was used. The mean barometric pressure at the altitude of the chamber (204 meters above sea level) was 750 mmHg. An automatic system kept the temperature (22°-24° Celsius) and

the humidity (40-50%) in a constant range. A CO_2 scrubber ran continuously in order to remove the carbon dioxide expelled by the body while exercising in the chamber. Tests conducted in hypoxia were preceded by 30 min of rest in the chamber before each trial in order to achieve a stabilization of resting metabolic and ventilator parameters (Easton, et al. 1986). One incremental test was performed with subjects breathing normoxic room-air (FIO_2 =20.9%) and a second (randomized order) with subjects breathing hypoxic room-air (FIO_2 =15%) to establish the FIO_2-specific first ventilatory threshold (VT_1) and the maximum oxygen uptake (VO_{2max}). After a standardized warm-up of 15 minutes unloaded pedaling the ramp incremental test started with an increment of 15 watt per minute. The work rate increase was selected so as to reach the maximum-tolerated work rate in ~ 12 min. VT_1 was defined as the VO_2 at which CO_2 output (VCO_2) began to increase out of proportion in relation to VO_2 with a systematic rise in minute ventilation-to-VO_2 ratio (V_E/ VO_2) and end-tidal PO_2 , whereas minute ventilation-to-VCO_2 ratio (V_E/ VCO_2) and end-tidal PCO_2 were stable (Beaver, et al. 1986). Subsequently, the subjects repeated constant work rate (WR) cycling exercises for 3 times and for each of the 2 different FIO_2 conditions. Each single test was separated by two days. All the subjects performed a transition in work rate from unloaded cycling to a moderate intensity work rate and back to unloaded cycling. The work rate was selected to elicit a VO_2 corresponding to 80% of the specific VT_1. The subjects completed 30 min of accommodation at rest (sitting on a chair in the chamber) before the start of the test. Each loaded and free-wheel unloaded period lasted 6 min. Rather than using the same absolute mechanical power, we used the same metabolic power to avoid early lactate production in the hypoxic situation (Hogan, et al. 1999) and to maximize the amplitude in both conditions, in order to have a better signal to noise ratio (Jones, et al. 2005). When the rest to work transitions are performed within the moderate intensity domain (below the lactate threshold), the τVO_2 follows a linear first order response kinetic (DiMenna, et al. 2009). In fact a part the work to work transition within the moderate intensity domain (Brittain, et al. 2001) and the intensity above the lactate threshold (DiMenna, et al. 2009) the τVO_2 does not change with the variation

of the work load. Moreover the use of similar relative intensities (80% of the specific VT_1) allows comparison of the subjects's VO_2 responses, to different conditions (normoxia and hypoxia), and different age.

4.2.3 Metabolic Measurements: Values of minute ventilation (V_E), breathing frequency (Fb), tidal volume (VT), end tidal partial pressure of oxygen and carbon dioxide ($PETO_2$ and $PETCO_2$), carbon dioxide output (VCO_2) and oxygen uptake (VO_2) were continuously measured by a breath-by-breath gas exchange measurement system (Cosmed Quark b², Rome, Italy). Gas analysers were calibrated before each test in each condition. [Normoxia: ambient air (O_2: 20.93 % and CO_2: 0.03 %) and a gas mixture of known composition (O_2: 16.00 % and CO_2: 5.00 %); hypoxia: ambient air (O_2: 15.00 % and CO_2: 0.03 %) and a gas mixture of known composition (O_2: 10.08 % and CO_2: 4.00 %)]. The low dead space (70-mL) face-mask was equipped with a low-resistance, bidirectional digital turbine (28-mm diameter). This turbine was calibrated before each test with a 3-L syringe (Cosmed, Rome, Italy). Face-masks allowed subjects to simultaneously breathe with mouth and nose, for more comfort. Heart rate was continuously measured via a wireless Polar-monitoring system (Polar Electro Oy, Kempele, Finland) and synchronized with the Cosmed system. Before each test, ambient conditions were measured and the gas analyzer and flow-meter were calibrated.

4.2.4 NIRS measurements: Local muscle oxygenation of the vastus lateralis muscle was measured with Near-Infrared-Spectroscopy (NIRS, Nimo-Nirox, Brescia Italy). The natural transparency of the skin and the muscular tissue to near-infrared light (from 650 to 1000 nm) allowed measuring the change in oxy and deoxy-hemoglobin concentration in the muscle tissue. The interaction of radiation-tissue is regulated by two physical characteristics: the first is the absorption, which is the attenuation of the intensity of the NIR signal through the tissue and it is due to water, lipids, oxyhemoglobin (HbO_2) and deoxy-hemoglobin (HHb); the second is the scattering that consisted of a deviation from straight trajectory by the non-uniformity characteristic of tissue. This technique, furthermore, performs accurate optical absorption

measurements at specific wavelengths that are converted into quantitative hemoglobin concentration (HbO_2 and HHb) using a proprietary algorithm. The probe was an active emitter unit comprised of a laser diode source (wavelengths from 670 to 980 nm) and a multiplexer (optomechanical switch). The sampling frequency was 40 Hz. The skin was carefully shaved and cleaned before each test. A skinfold thickness at the site of application of the NIRS probe was determined before the warm-up using a caliper and the value of the subcutaneous adipose tissue was inserted into the NIRS software. The probe was firmly attached to the skin overlying the distal third of vastus lateralis muscle (~ 10-12 cm above the knee joint) of the right limb, parallel to the major axis of the thigh, by a biadhesive tape (Quaresima, et al. 2004). A bandage was used to cover the probe from the external light sources.

4.2.5 O_2 arterial saturation measurement: SaO_2 was measured by portable pulsoximeter (Intermed SAT-500). Measurements were made on the index finger. SaO_2 was monitored for the duration of the test and the values used were relative to the average of last minute of constant work rate exercise.

The arterial oxygen content (CaO_2) was estimated as the product of SaO_2 from pulse oxymetry and the O_2 content of Hb, assuming an arterial [Hb] of 15.0 g · 100 ml^{-1} and an O_2 carrying capacity of 1.34 ml · g^{-1} Hb.

4.2.6 Heart rate measurements: Heart rate (HR) was continuously measured via a wireless Polar-monitoring system (Polar Electro Oy, Kempele, Finland) and synchronized with the Cosmed system.

4.2.7 Analysis: Breath-by-breath VO_2 signals of each of the 3 constant-power tests were filtered by removing aberrant data points that lay outside 4 standard deviations of the local mean, then were interpolated to derive a sec-by-sec profile; the 3 transitions were then time-aligned to the onset of exercise and averaged to provide a single response for each subject in each condition. The on-transient response for VO_2 was fitted with a mono-exponential model of the form: $Y(t) = Y_{bas} + Y_{amp} \cdot (1 - e^{-(t-TD)/\tau})$.

Y_{bas} is the baseline VO_2 value during the unloaded cycling, Y_{amp} is the difference between the steady state value of VO_2 reached after the transient and the baseline value of VO_2 in the 30 sec before the transient, τ

is the time constant defined as the duration of the time for VO_2 to increase to 63% of the steady state increase.

The phase 1-phase 2 transition was determined with an "experimental" fitting strategy. The end of phase 1 or the start of phase 2 for VO_2 was determined as the breath before the sudden fall in respiratory exchange ratio (RER). This coincides with the inflection point during the first 15-30 sec of the rise of VO_2 (Mole, et al. 1999). Subsequent phases 2 and 3 were fitted using the mono-exponential model described previously. The VO_2 data were modeled from the Phase 1- Phase 2 transition to 240 sec.

Data for HR were re-sampled with a 1-s sampling rate, averaged together and modeled with the mono-exponential model described above. The on-transient HR responses were modeled from the onset of exercise to 240 sec.

The 3 repetitions of HHb were time aligned, interpolated with a 1-s sampling rate and averaged together to calculate the rate of adjustment of the HHb. The calculated time delay (TD-HHb) was determined visually from the onset of exercise to the first rapid increase in the HHb signal. The HHb was fitted with a mono-exponential fit from the calculated time delay of the exercise to 90 sec (DeLorey, et al. 2003). The effective time constant, τ', for the HHb signal was calculated as $\tau' = TD + \tau$.

To investigate the relationship between the HHb and the VO_{2p} kinetics, data on the time evolution of HHb and VO_{2p} were normalized, for each subject, in such a way that the response varies from 0 (unloaded condition) to 1 (loaded condition). The normalized VO_{2p} curve was left-shifted taking the duration of phase 1, as calculated for each subject, into account. Thus the onset of exercise (t=0) coincides, for the VO_{2p} curve, with the beginning of phase 2, which is known to reflect closely the muscle oxygen uptake (Grassi, et al. 1996). Curves obtained in this way are referred to as HHb^{nor} and $VO_{2p}{}^{nor}$. Similar curves are obtained for all subjects.

Subsequently, the ratio $HHb^{nor}/VO_{2p}{}^{nor}$ was evaluated for each subject. From this curve, an overall HHb/VO_{2p} ratio, was derived, (Murias *et al*.2010) by calculating the time-average of $HHb^{nor}/VO_{2p}{}^{nor}$ from

t=10 s (beyond the physiological TD-HHb) to t=120 s (at the steady state for both HHb^{nor} and VO_{2p}^{nor}), i.e.: $\frac{1}{N}\sum_{n=1}^{N}\left(\frac{HHb^{nor}}{VO_{2p}^{nor}}\right)_n$, where n=1 corresponds to t=10s and n=N corresponds to t=120s. All the calculations were performed using software implemented in Matlab (Matlab.7.0 Natick, Massachusetts, U.S.A.)

4.2.8 Statistical analysis

Values presented are expressed as mean ± SD. Statistical significance was accepted at $p < 0.05$.

Anova for repeated measurements 2 x 2 (age x oxygen level) was used to analyze the difference between the 2 groups in the 2 conditions.

Paired T-tests were used to analyze the 2 conditions. Data were analyzed with the software package SPSS version 3.5 (SPSS Inc., St Louis, MO, USA).

4.3 Results

Incremental Test: Maximal values derived from this test were given in Table 4.1 for WR and VO_{2p}. Exercising in hypoxia caused in both groups a significant (i.e. $p<0.05$) decrease in the maximal values for the WR and VO_2. The older group showed significantly lower values for VO_{2max} and peak power output (PPO) in both conditions, compared with the young group ($p<0.05$).

Table 4.1

	Older Adults		Young Adults	
	Normoxia	Hypoxia	Normoxia	Hypoxia
VO_{2max} (ml/min/kg)	39 ± 4	33 ± 3*	55 ± 7	49 ± 7*
PPO (watt)	213 ± 15	196 ± 13*	311 ± 36	278 ± 29*

Table 4.1
Maximum values (mean ± SD) for VO_2 and power output in normoxia (FIO_2=20.9%) and hypoxia (FIO_2=15%)
*$p<0.05$, hence significantly different from normoxia (FIO_2=20.9%)

Square wave test: In both groups as shown in Table 4.2, the SaO_2, was always significantly lower (i.e. $p<0.05$) in hypoxia as compared to normoxia .

Table 4.2

	Older Adults		Young Adults	
	Normoxia	Hypoxia	Normoxia	Hypoxia
Watt 80% VT_1	102 ± 8	84 ± 9*	155 ± 14	138 ± 19*
SaO_2 unloaded	97 ± 2	89 ± 3*	98.6 ± 1	93 ± 2*
SaO_2 constant load (80% VT_1)	97.6 ± 1	84 ± 4*	98.2 ± 2	90 ± 3*
HHb/VO_2	1.09 ± 0.1	1.13 ± 0.1*	1.08 ± 0.1	1.08 ± 0.1

Table 4.2
Steady state values (mean ± SD) of work rate, SaO_2 under unloaded and loaded condition, and HHb/VO_2 ratio in normoxia (FIO_2=20.9%) and hypoxia (FIO_2=15%) for older and young adults.
*$p<0.05$, hence significantly different from normoxia (FIO_2=20.9%)

The rate of adjustments of several parameters were subjected to a two-way analysis of variance having two levels of subjects age (young and old) and two levels of oxygen level content in the inhaled gas mixture (normoxia and hypoxia). All effects were statistically significant at the 0.05 significance level.

VO_{2p} kinetics: Fig.4.1 shows the VO_{2p} time dependence during the square wave test in normoxia and hypoxia. The main effect of age yielded an F ratio of $F(1, 28) = 17.04$, $p < 0.001$, indicating that the τ VO_{2p} (phase 2) was significantly greater for older (normoxia: 29 ± 6 sec; hypoxia: 34 ± 8 sec) than for young adults (normoxia: 19 ± 4 sec; hypoxia: 25 ± 4 sec). The main effect of oxygen level yielded an F ratio of $F(1,28)=8.1$, $p < 0.001$, indicating that the τ VO_{2p} (phase 2) was significantly greater in hypoxia (older: 34 ± 8 sec; young 25 ± 4 sec) than in normoxia (older: 29 ± 6 sec; young: 19 ± 4 sec)(see table 4.3). The interaction effect was non-significant $F(1,28)=0.28$, $p=0.6008$.

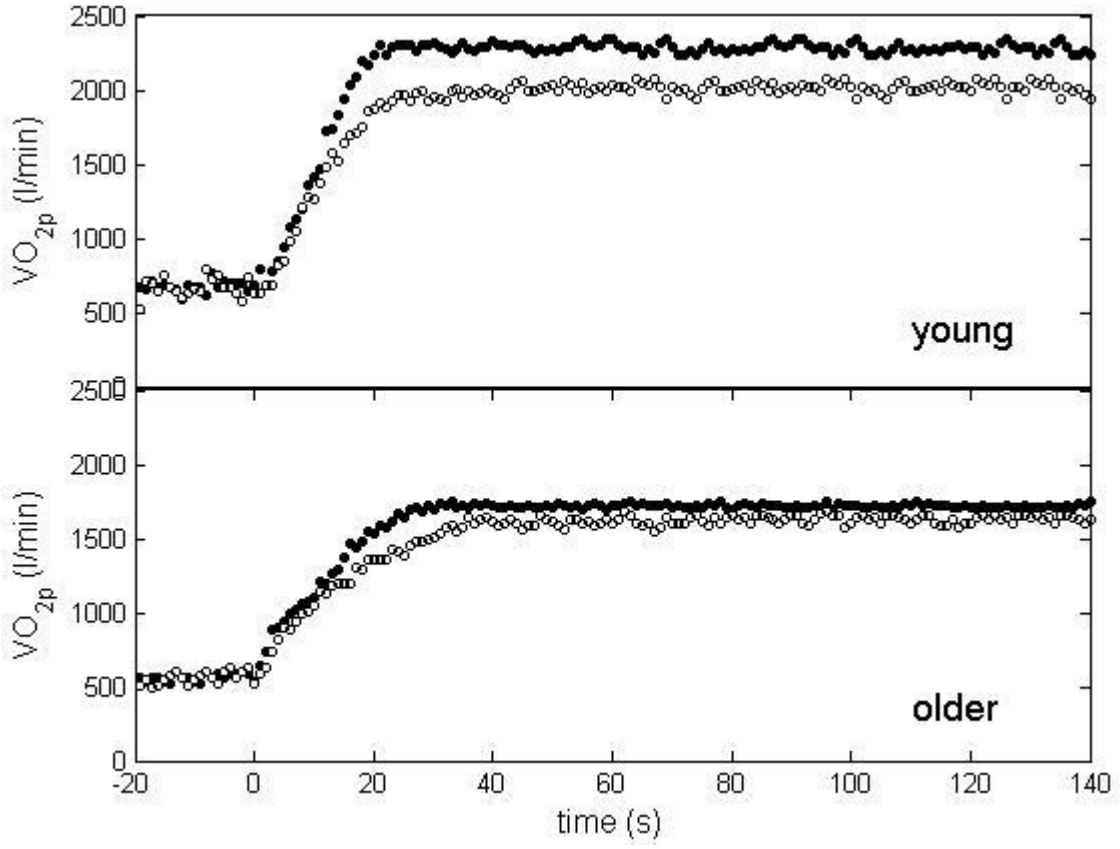

Fig. 4.1
Response of pulmonary O_2 uptake during a square wave exercise in normoxia (FIO_2=20.9%) (filled circles) and hypoxia (FIO_2=15%) (open circles). Data represents the average of 3 repetitions of a square wave exercise for all subjects. The upper panel is for the young group, the lower panel is for the older group.

The main effect of subject age yielded an F ratio of F (1, 28) = 26.97, $p < 0.001$, indicating that the duration of the VO_{2p} phase 1 was significantly greater for older (normoxia: 22 ± 5 sec; hypoxia: 23 ± 3 sec) than for young adults (normoxia: 17 ± 2 sec; hypoxia: 16 ± 2 sec). The main effect of oxygen level yielded an F ratio of F(1,28)=0.02,$p > 0.05$, indicating that the duration of the VO_{2p} phase 1 was not significantly different between hypoxia and normoxia (see table 4.3). The interaction effect was non-significant F(1,28)=0.88, p=0.3550.

HR-kinetics: The main effect of subject age yielded an F ratio of F (1, 28) = 33.43, $p < 0.0001$, indicating that the τ HR was significantly greater for older (normoxia: 42 ± 9 sec; hypoxia: 47 ± 8 sec) than for young adults (normoxia: 25 ± 7 sec; hypoxia: 33 ± 6 sec). The main effect of oxygen level yielded an F ratio of F(1,28)=5.88,$p < 0.05$, indicating that the τ HR was significantly greater in hypoxia (older: 47 ± 8

sec; young 33 ± 6 sec) than in normoxia (older: 42 ± 9 sec; young: 25 ± 7 sec). The interaction effect was non-significant F(1,28)=0.31, p = 0.5803.(see Fig 4.2).

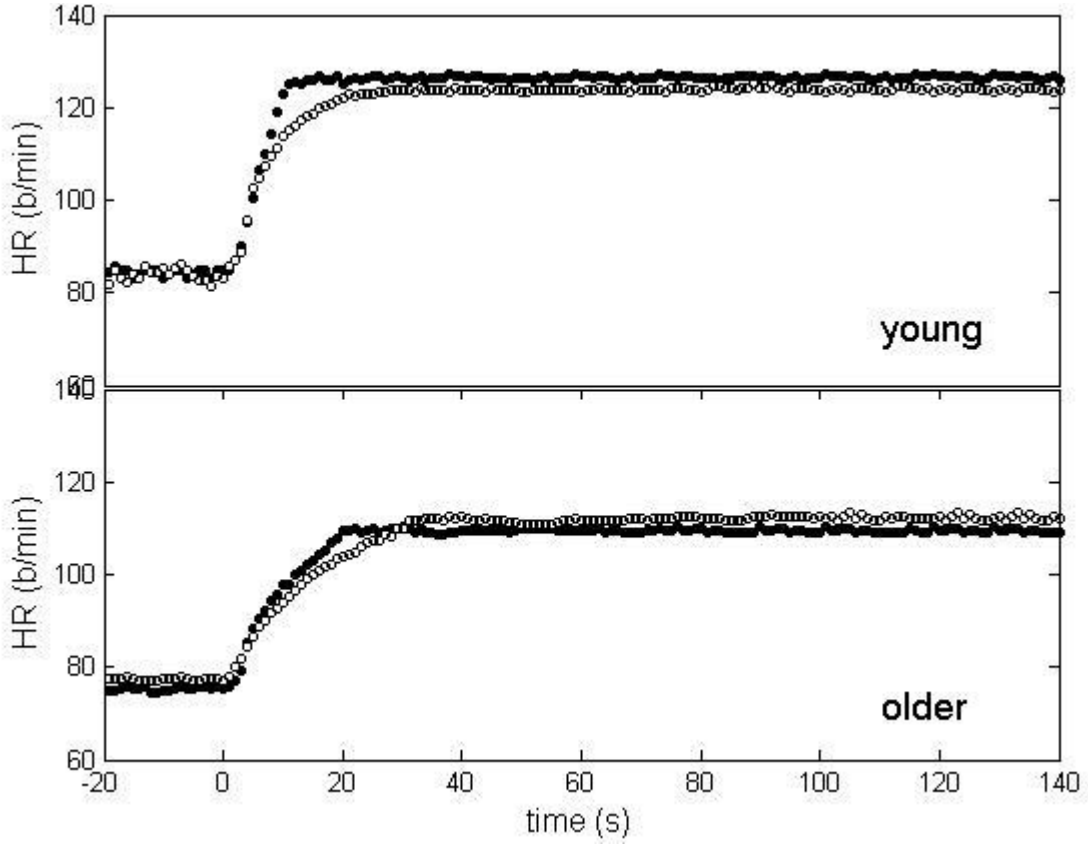

Fig. 4. 2
Response of HR during a square wave exercise in normoxia (FIO_2=20.9%) (filled circles) and hypoxia (FIO_2=15%) (open circles). Data represents the average of 3 repetitions of a square wave exercise for all subjects. The upper panel is for the young group, the lower panel is for the older group.

HHb kinetics: The main effect of subject age yielded an F ratio of F (1, 28) = 7.95, p < 0.01, indicating that the τ HHb and $\tau' = TD + \tau$ were significantly greater for young adults (normoxia: 15 ± 2 sec and 22 ± 4; hypoxia: 18 ± 3 sec and 24 ± 5 sec) than for older adults (normoxia: 10 ± 3 sec and 17 ± 4 sec; hypoxia: 12 ± 3 sec and 19 ± 5 sec). The main effect of oxygen level yielded an F ratio of F(1,28)=10.35,p < 0.01, indicating that the τ HHb and $\tau' = TD + \tau$ were significantly greater in hypoxia

(older: 12 ± 3 sec and 19 ± 5 sec ; young 18 ± 3 sec and 24 ± 5 sec) than in normoxia (older: 10 ± 3 sec and 17 ± 4 sec; young: 15 ± 2 sec and 22 ± 4sec). The interaction effect was non-significant F(1,28)=0.57, p=0.4567. (see fig 4.3).

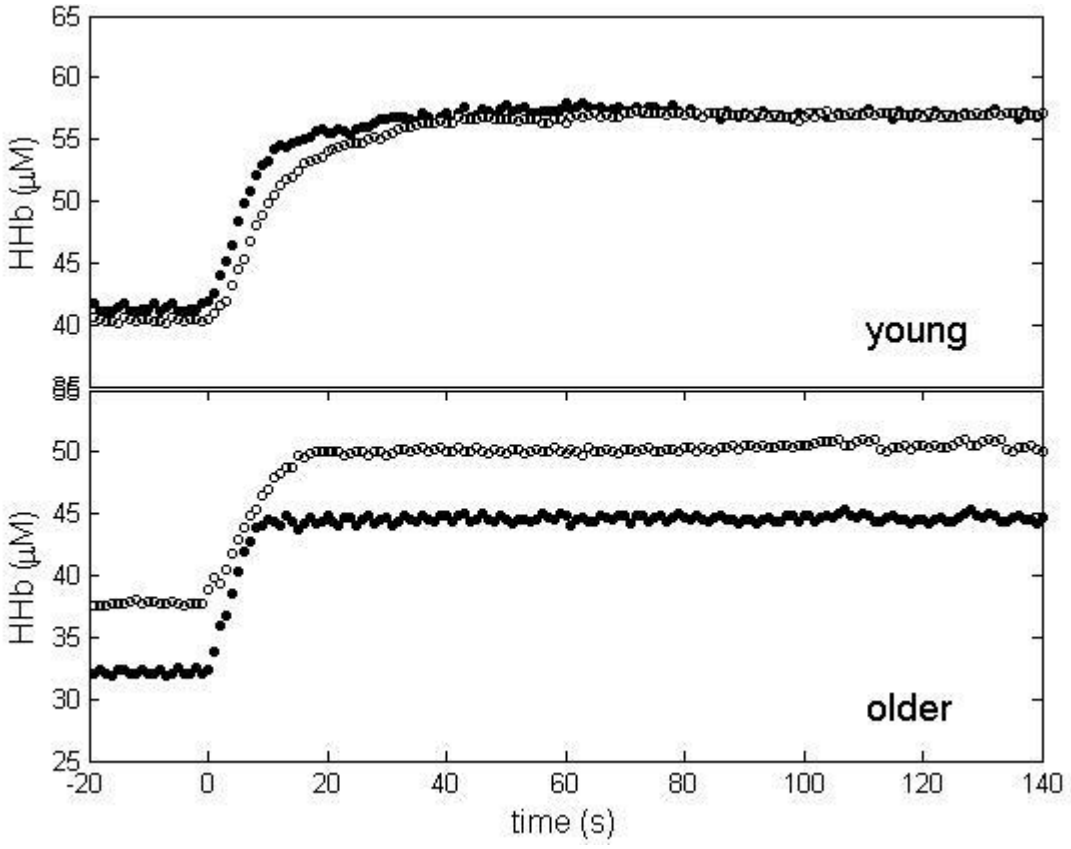

Fig. 4.3
Response of HHb during the square wave exercise in normoxia (FIO_2=20.9%) (filled circles) and hypoxia (FIO_2=15%) (open circles). Data represents the average of 3 repetitions of a square wave exercise for all subjects. The upper panel is for the young group, the lower panel is for the older group.

HHb kinetics-VO_{2p} kinetics:

The main effect of subject age yielded an F ratio of F (1, 28) = 72, $p < 0.001$, indicating that the HHb/VO_2 ratio was significantly greater for older adults (normoxia: 1.09 ± 0.02; hypoxia: 1.13 ± 0.01) than for young adults (normoxia:1.08 ± 0.01; hypoxia:1.08 ± 0.01). The main effect of oxygen level yielded an F ratio of F(1,28)=32,$p < 0.001$, indicating that the HHb/VO_2 ratio was significantly greater in hypoxia

(older: 1.13 ± 0.01 ; young 1.08 ± 0.01) than in normoxia (older: 1.09 ± 0.02; young 1.08 ± 0.01) (see Table 4.2)(see fig 4.4). The interaction effect was significant $F(1,28)=32$, $p < 0.001$.

Post-hoc Tukey's HSD tests showed that the HHb/VO_2 ratio of older adults in hypoxia was significantly higher than the HHb/VO_2 ratio of older adults in normoxia ($p<0.05$). All other comparisons were not significant.

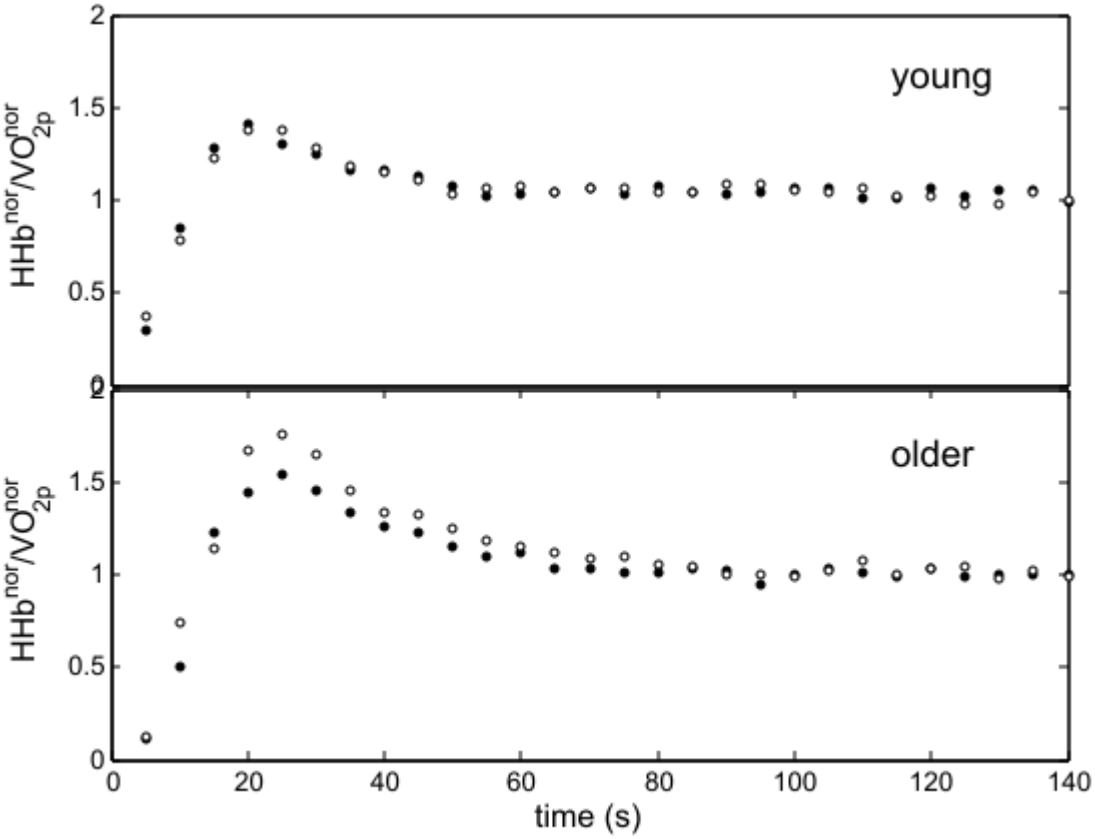

Fig. 4.4
Time evolution of $HHb^{nor}/VO_{2p}{}^{nor}$ for hypoxia (open circles) and normoxia (filled circles). Data represents the average of 3 repetitions of a square wave exercise for all subjects.

V_E kinetics:

The main effect of subject age yielded an F ratio of $F(1, 28) = 55.5$, $p < 0.001$, indicating that the τV_E was significantly greater for older adults (normoxia: 64 ± 6; hypoxia: 56 ± 6) than for young adults (normoxia: 46 ± 4; hypoxia: 46 ± 5). The main effect of oxygen level yielded an F ratio of $F(1,28)=4.53$,p

< 0.05, indicating that the τV_E was significantly greater in normoxia (older: 64 ± 6 ; young : 46 ± 4) than in hypoxia (older: 56 ± 6; young 46 ± 5) (see Table 4.3). The interaction effect was significant $F(1,28)=4.53$, $p < 0.05$.

Post-hoc Tukey's HSD tests showed that for older adults τV_E was significantly lower in hypoxia than in normoxia ($p<0.05$). All other comparisons were not significant.

Tab 4.3

Older

	Conditions	Unloaded	Loaded	Amplitude	TD (sec)	τ (sec)	C_{95} (s)
VO_2 (l·min^{-1})	hypoxia	0.6 ± 0.1	1.7 ± 0.2	1.1 ± 0.2	23 ± 3	34 ± 8*	4 ± 1
	normoxia	0.6 ± 0.1	1.6 ± 0.5	1.0 ± 0.5	22 ± 5	29 ± 6	4 ± 1
HHb (µM)	hypoxia	37 ± 10	51 ± 17	14 ± 8	7 ± 2	12 ± 3	2 ± 1
	normoxia	31 ± 10	44 ± 17	14 ± 8	7 ± 2	10 ± 3	2 ± 1
HR (b·min^{-1})	hypoxia	77 ± 11	112 ± 6	35 ± 16		47 ± 9	5 ± 2
	normoxia	74 ± 8	109 ± 16	35 ± 12		42 ± 8	6 ± 3
VE (l·min^{-1})	hypoxia	21 ± 5	55 ± 11	34 ± 12		56 ± 6*	2 ± 1
	normoxia	21 ± 3	53 ± 11	32 ± 12		64 ± 6	5 ± 2

Young

	Conditions	Unloaded	Loaded	Amplitude	TD (sec)	τ (sec)	C_{95} (s)
VO_2 (l·min^{-1})	hypoxia	0.7 ± 0.2	1.8 ± 0.2	1.3 ± 0.1	16 ± 2	25 ± 4*	3 ± 1
	normoxia	0.7 ± 0.1	2.4 ± 0.4	1.8 ± 0.2	17 ± 2	19 ± 4	3 ± 1
HHb (µM)	hypoxia	40 ± 12	61 ± 7	20 ± 7	6 ± 2	18 ± 3*	2 ± 1
	normoxia	41 ± 12	60 ± 18	19 ± 6	7 ± 3	15 ± 2	2 ± 1
HR (b·min^{-1})	hypoxia	84 ± 11	126 ± 13	42 ± 8		33 ± 6*	5 ± 2
	normoxia	85 ± 11	125 ± 15	40 ± 8		25 ± 7	4 ± 3
VE (l·min^{-1})	hypoxia	22 ± 4	57 ± 8	35 ± 9		46 ± 5	4 ± 1
	normoxia	21 ± 4	53 ± 8	32 ± 9		46 ± 4	2 ± 1

Tab 4.3

Data (baseline, steady-state, amplitude, time-delay, time constant and C_{95}) for several parameters in normoxia (FIO_2=20.9%) and hypoxia (FIO_2=15%).
*$p<0.05$, hence significantly different from normoxia (FIO_2=20.9%)

4.4 Discussion

The study examined the kinetic response of pulmonary O_2 uptake, local muscle deoxygenation, and heart rate in young and older adults at the same relative (80% VO_2 at VT_1) intensity in normoxia and hypoxia. The major findings were: 1) both groups showed slower phase 2 VO_2, HR, HHb kinetics and a greater $\tau' = TD + \tau$ in hypoxia compared to normoxia; 2) older adults exhibited a greater HHb/VO_2 ratio and a faster V_E kinetics in hypoxia compared to normoxia; 3) young adults showed similar HHb/VO_2 ratio and V_E kinetics in normoxia and hypoxia.

The results obtained for older adults are in line with our previous results (Zerbini, et al. 2012) where, for a group of older adults (66±6 yrs) cycling in hypoxia (FIO_2 = 15% O_2), the phase 2 of VO_{2p} kinetics was found to be slower and the HHb/VO_2 ratio was found to be greater as compared to results obtained for the same subjects cycling in normoxia. Based on our previous and present study, we can argue that the major limiting factor responsible for the slowing of the VO_{2p} kinetics under hypoxic conditions in the case of older adults is a mismatch between O_2 delivery and O_2 utilization within the muscle. This statement is supported by recent data about the impaired vasodilatory capacity of older adults exercising in hypoxia (Casey, et al. 2011).

Regarding young adults, the present results confirmed the slower phase 2 of VO_{2p} and HR kinetics, found in hypoxia by other studies (DeLorey, et al. 2004; Engelen, et al. 1996; Spencer, et al. 2011b; Springer, et al. 1991). However, only the study of Spencer et al (2011) provided data about muscle oxygenation based on NIRS. Both in the study of Spencer et al (2011) and in the present study, significantly slower HHb kinetics was found in hypoxia as compared to normoxia. However, if Spencer et al (2011) obtained a greater HHb/VO_2 ratio in hypoxia compared to normoxia, proposed to be responsible for the slower VO_2 kinetics, we found a similar HHb/VO_2 ratio between normoxia and hypoxia. A possible explanation for this difference could be in the different fit levels of the subjects, as revealed by differences in the rate of adjustment of the VO_2 in both conditions. In the study of Spencer et al (2011), the τ VO_2 of the phase 2

ranged from 26 ± 7 sec in normoxia to 34 ± 8 sec in hypoxia. In the present study on the other hand, the τ VO_2 of the phase 2 ranged from 19 ± 4 sec to 25 ± 4 sec. Moreover, comparing the VO_{2max} in normoxia, we can observe that subjects of the present study were slightly in better physical condition (VO_{2max} = 55 ± 7 ml/min/kg) than subjects of the Spencer et al (2011) study (VO_{2max} =51.9 ± 5.7 ml/min/kg). Yet it is likely that, in normoxia, subjects of the Spencer et al (2011) study exhibited a worst microvascular O_2 delivery system as compared to the young subjects of the present study. In this case, a worst vasodilatory responsewould be obtained in hypoxia, ultimately resulting in a higher HHb/VO_2 ratio in hypoxia as compared to normoxia. We should mention in this regard that τ VO_2 ≈ 20 s was proposed (Jones, et al. 2005; Spencer, et al. 2011b) to represent a tipping point between the O_2 delivery dependent zone (above the 20 sec) and O_2 delivery independent zone (below the 20 sec). Thus subjects whose VO_2 time constant is above 20 s-have the likely site of control of the VO_2 kinetics in the O_2 delivery system (in particular in the microvascular O_2 delivery system), whereas subjects whose VO_2 time constant is close to or below 20 s would use a metabolic intracellular control of the VO_2 kinetics.

Though we did not find any difference in the HHb/VO_2 ratio for young adults between normoxia and hypoxia in the present study, we still measure a slower VO_{2p} kinetics in hypoxia as compared to normoxia. Thus factors other than the microvascular O_2 delivery may have slowed the VO_2 kinetics in the young adults group.

One such factor could be the slow HR kinetics found in hypoxia in the present and in previous studies (Engelen, et al. 1996; Spencer, et al. 2011b; Springer, et al. 1991).

In fact, Hughson, et al. (1983) and MacPhee et al. (2005) measured a slowed HR kinetics and a slowed adjustment of femoral (conduit) artery blood flow in the transition from the lower to the upper region of the moderate-intensity domain. These findings seem to support the idea that a slow HR kinetics could be associated with a slow O_2 delivery and a slow VO_{2p} kinetics. From this study it is difficult however to discern wheter the slow HR kinetics is the cause or the consequence of the slow VO_{2p} kinetics.

Another factor that could slow the VO_{2p} kinetics in hypoxia, could be the slow HHb kinetics found in hypoxia both in the present study and in the study of Spencer et al (2011). In fact, hypoxia could affect the control of mitochondrial respiration, slowing the muscle O_2 extraction (estimated from changes in muscle deoxygenation) (Wilson, et al. 1988).

When we compare young and older adults and their physiological response to hypoxia we can observe some differences.

Young adults exhibited a greater slowing of the HR kinetics on moving from normoxia to hypoxia as compared to older adults. This fact could be explained by the reduced chemosensitivity that occurs with the aging process (Garcia-Rio, et al. 2007)and that would lead older adults to have an impaired HR response to hypoxia as compared to young adults during the transient. Another mechanism that could explain the greater slow down of the HR kinetics observed in hypoxia for young as compared to older adults, could be the fact that, under both conditions, young adults have a higher HR during the unloaded period preceding transients as compared to older adults ($p<0.05$) (see table 2). It is well known in fact that withdrawal of the parasympathetic nervous system (up to the natural frequency created by the sinoatrial node) is a faster mechanism in the increase of the HR as compared to the stimulation caused by the sympathetic nervous system (from the natural frequency created by the sinoatrial node to higher frequency) (Astrand 2003). Therefore, starting the transient at a higher HR (as for the young subjects in the present study) could imply less advantage from the quick withdrawal of the parasympathetic nervous system, thus resulting in a slower HR kinetics as compared to older adults.

The HHb and $\tau' = TD + \tau$ were slower in the young group as compared to the older group. This result could indicate a better blood flow and O_2 delivery response for young compared to older adults in both conditions. The lower HHb/VO_{2p} ratio found in both conditions for young as compared to older adults confirms the statment (see table 3). Moreover, the present results are in agreement with previous studies,

where a worst microvascular O_2 delivery and a greater reliance on O_2 extraction were observed for older adults as compared to young adults (DeLorey., et al. 2004; DuManoir, et al. 2010c; Murias, et al. 2010). Finally, contrary to our hypothesis, we found that the V_E kinetics is faster in hypoxia than in normoxia for older adults, while it does not change for young adults. The result, in contrast to the aging-related reduction in the V_E in hypoxia found by Kronenberg et al (1973) (Kronenberg, et al. 1973) could be explained by a lower arterial saturation occurring in older adults during the unloaded period in hypoxia ($p<0.05$) (see table 4.3).

4.5 Conclusions

Older adults showed an impaired microvascular O_2 delivery in hypoxia which is, according to our initial hypothesis, the main cause for the slowing in the VO_{2p} kinetics.

On the contrary, no impairment in the microvascular O_2 delivery was found for young adults in hypoxia, in spite of a slower VO_{2p} kinetics. Thus it is likely that factors other than the microvascular O_2 delivery (i.e central O_2 delivery and/or slowed mitochondrial respiration) are responsible for the slowed VO_{2p} kinetics in this case.

We interpret the result by noting that τVO_{2p} is close to the O_2 delivery independent zone for young adults, whereas it lays in the O_2 delivery dependent zone for older adults, which confirms the great influence of microvascular O_2 delivery on the VO_{2p} kinetics.

4.7 References

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GENERAL CONCLUSIONS

This thesis investigated the possible mechanisms limiting the VO_{2p} kinetics during the on-transient of moderate-intensity exercise in younger and older adults under hypoxic conditions.

While for young adults it is still not clear where the bottleneck for the VO_2 in hypoxia is, the present work demonstrated the important role of the microvascular O_2 delivery system for older adults. These results on older adults confirmed previous results showing that the vasodilatory response to hypoxia depends on the work rate. In this regard, we demonstrated that older adults exercising in hypoxia at very low work rate (i.e leg extension exercise; third chapter) were able to counterbalance the drop in CaO_2 with an increase in blood flow which is in turn able to prevent the slowing of the VO_{2p} kinetics. On the contrary, for older adults exercising in hypoxia at a higher work rate (i.e. performing a cycling exercise; second and fourth chapters), a slowing in the VO_{2p} kinetics, together with a greater HHb/VO_{2p} ratio, was observed in hypoxia, meaning a great mismatch between O_2 delivery and O_2 utilization within the muscle.

The results summarized above help to close the loop in our understanding of the mechanistic bases for the slowed VO_2 kinetics in aged individuals. In addition, they explain how, at variance with what happens for young adults, the site limiting the VO_2 kinetics shifts upstream from the mitochondria (O_2 utilization) to the O_2 transport and delivery system.

For aged individuals, the VO_2 kinetics can be markedly speeded by, for example, a single bout of prior exercise (i.e. priming) or as a result of exercise training, both in normoxia and hypoxia. Whether these interventions speed the VO_2 kinetics and improve muscle function, and hence exercise tolerance, by upregulating the dynamics of arteriolar dilatation is an important question with far-reaching therapeutic possibilities that deserves to be addressed. Further studies are however needed on the possibility to improve the microvascular O_2 delivery in older adults through training, supplementation and drugs.

Research Western

Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Donald Paterson
Review Number: 17825
Review Level: Full Board
Approved Local Adult Participants: 20
Approved Local Minor Participants: 0
Protocol Title: VO2 kinetics in acute hypoxia in older and young men: Is limb blood flow elevated?
Department & Institution: Kinesiology,University of Western Ontario
Sponsor: Natural Sciences and Engineering Research Council

Ethics Approval Date: June 09, 2011 **Expiry Date:** March 31, 2012

Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
UWO Protocol		
Letter of Information & Consent		2011/04/11

This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The UWO HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Signature

Ethics Officer to Contact for Further Information

Janice Sutherland (jsutherl@uwo.ca)	Grace Kelly (grace.kelly@uwo.ca)	Shantel Walcott (swalcott@uwo.ca)

This is an official document. Please retain the original in your files.

LETTER OF INFORMATION

VO_2 kinetics in acute hypoxia in older and young men: Is limb blood flow elevated?

Principal Investigator: Donald H Paterson, PhD

PhD Student: Livio Zerbini, MSc

Purpose of Study:

You are being invited to participate in a research study that examines the rate at which oxygen (O_2) is utilized by the body to generate energy for exercise. During the transition from rest or light-intensity exercise to higher intensities, the rate of adjustment of O_2 use (called "VO_2 kinetics") may depend on how rapidly certain enzymes in the muscle are activated or on how quickly blood flow increases to supply O_2 to the active muscle. In general, VO_2 kinetics is slower in older adults compared to young adults. It has been shown that when people breathe in ("inspire") air that contains a smaller percentage of O_2 (known as "hypoxia") than that found in "normal air" (approximately 21%) during the transition to higher exercise intensities, the VO_2 kinetics are slower (likely because less O_2 gets delivered to the muscle). In young adults, it has been shown that the body attempts to compensate for the fact that the blood is carrying less O_2 by increasing the amount and rate of blood flow going towards the exercising muscles. This study will examine the effect of hypoxia on VO_2 kinetics in older and young men to determine whether the VO_2 kinetics are slowed by approximately the same amount in both groups and to determine whether older adults also demonstrate this "compensation strategy" of increasing blood flow towards the exercising muscles.

Participation in this study involves visits to the research laboratory at the Canadian Centre for Activity and Aging (Arthur and Sonia Labatt Health Science Centre, Room 313) on a maximum of 7 different occasions (total time commitment = approximately 8.5 hours). Each exercise visit is expected to take no longer than 1 hour and 15 minutes to complete.

Up to 10 young and 10 older adult men will be invited to participate in this study. In order to participate you must be between 18-40 (young) or 60-85 (older) years of age and healthy. You will not be able to participate in the study if you have been previously diagnosed with any respiratory, cardiovascular, metabolic or musculoskeletal disease; or you are currently on medication affecting cardiovascular responses to exercise; or you are a smoker; or you respond to the exercise protocol in an irregular manner or cannot tolerate the exercise protocol. If you

are participating in another study at this time, please inform the investigator right away to determine if it is appropriate for you to participate in this study.

Research Testing Protocol:

During the first visit to the laboratory you will complete an incremental exercise test to volitional fatigue (exercise intensity increases progressively until fatigue) where you will be physically unable to continue to exercise because the intensity is too high or too uncomfortable. The exercise will consist of two-legged knee-extension (kicking motion) on a modified knee-extension ergometer while in the upright, seated position. After a few minutes the exercise intensity will gradually and continuously increase until you are unable to continue because of fatigue, or until you wish to stop. This visit should last approximately 1 hour.

In addition to this test and on 6 separate days, you will perform a series of exercise protocols on the knee-extension ergometer that involve transitions from rest to passive leg movement (where your legs are being moved, but your muscles are not responsible for any of the work) for 2 minutes, then to very light work (i.e., 3 watts; an intensity similar to slow walking) for 4 minutes, and finally to moderate intensity (exercise in the moderate domain could theoretically be performed indefinitely and should not produce signs of fatigue) for six minutes. These exercise protocols will always be presented in the following order:

Condition 1: You will complete the whole exercise protocol while inspiring normal room air, which contains approximately 21% O_2.
Condition 2: You will complete the whole exercise protocol while inspiring air that contains approximately 15% O_2.

The two conditions will both follow the same pattern of 2 minutes of passive leg movement, 4 minutes of very light exercise, and 6 minutes of moderate intensity exercise and will be separated by approximately 30 minutes of rest. During the 30 minute rest period leading up to the start of "Condition 2," you will be inspiring the hypoxic gas mixture so that your body has a chance to adjust to the lower O_2 availability. Repeat testing of each of the conditions is required in order to ensure the accuracy and reliability of the data. During the testing sessions, height and weight measurements will be taken.

Research Procedures:

During each of the exercise tests you will be required to wear a nose-clip (to prevent you from breathing through your nose) and a rubber mouthpiece (similar to breathing through a snorkel or diving mask); nose-clips and mouthpieces are disinfected before each test. This will enable us to measure the volume of air that you breathe in and out, and measure the gas concentration in that air.

During each of the exercise tests, the oxygenation of your leg muscle will be measured using near-infrared spectroscopy which projects light into a specific location of your leg muscles and measures the amount of light coming out at another location. A small piece of equipment will be placed on your leg approximately midway between your hip and your knee. It will be secured with tape, covered to prevent light from entering or leaving

the area, and bound with elastic bandage to minimize movement. You might experience a bit of discomfort by having this equipment secured to your leg during the exercise period. However, this is a non-invasive procedure. Additionally, oxygenation of your blood will be measured using infrared oximetry (a non-invasive measure similar to that performed by nurses when you go to visit a doctor at the hospital) with the probe clipped onto your earlobe or finger. This procedure is not associated with any risks or discomfort.

During each of the exercise tests, the speed of the blood in the major artery feeding the leg muscle will be measured non-invasively using a Doppler ultrasound. A probe will be placed at the level of your femoral artery (top of the mid-thigh) and will be held in place throughout the exercise test by one of the investigators.

Heart rate and rhythm will be continuously monitored by electrocardiogram. One electrode will be placed on each of the following areas: left chest, right chest, and left side under your ribs and connected to an electrocardiograph. The electrodes use adhesive tape to secure to the skin. There are no known risks or discomforts associated with this procedure.

At the end of some of the moderate-intensity tests, blood lactate and haemoglobin concentration will be measured by means of a portable analyzer. A drop of blood from one of your fingertips or one of your earlobes (approximately 25 µL) will be taken for each analysis. The tip of the finger or your earlobe will be pricked with a lancet at the end of some tests.

Possible Risks and Discomforts:

You may experience some minor discomfort from wearing the nose-clip and rubber mouthpiece, and by having the NIRS probes secured to your leg during the exercise period. These sensations often become less noticeable with time during the exercise. There may be some pain or discomfort related to the fingertip/earlobe prick.

Any exercise carries a slight risk of a heart attack (less than approximately 6:10,000) or may be uncomfortable if you are unfit or not used to exercise. There may be some minor discomfort during the exercise testing. You may experience increased awareness of breathing, muscle fatigue and soreness, increased sweating, or a general feeling of fatigue or nausea, none of which are unexpected consequences of exercise. During the moderate intensity exercise in which you are inspiring the air containing a lower percentage of O_2, some these feelings of discomfort may be more apparent, but these feelings are expected to disappear shortly after exercise is stopped.

All testing procedures will only be conducted when a lab technician or research assistant that is certified in CPR is present. In the case of an emergency, 911 will be called using the telephone located in the testing laboratory. An automatic external defibrillator is also available within the testing building. If a heavy pressure sensation or pain develops in your chest or down your left arm it is important that you discontinue the exercise immediately and report these sensations to the exercise supervisor, or seek medical attention if you have left the exercise area.

Participation in this study requires a time commitment which may be inconvenient for you at some point during the study.

Benefits of Participation:

This is a basic physiology/biochemistry study and, as such, there will be no direct benefits received as a consequence of participating in the study. If you are interested, the rational for conducting the research and theory and significance of each of the tests will be explained, as will your individual results from each of the tests. You will also have the opportunity to learn about and better understand your physiological responses to an exercise situation.

Confidentiality:

Records from this study are confidential and will be stored securely at the Canadian Centre for Activity and Aging, Sonia Arthur Labatt Health Sciences Building. Your records will be identified by a number rather than your name. The data will be available for analysis within the research group. Published reports resulting from this study will not identify you by name. We would like to keep and use your data in future, as of yet unknown analyses. There is a check box on the consent form to indicate your choice. You will be able to withdraw your data at any time by contacting the Principal Investigator, Dr, Donald H. Paterson at 519-661-1606. Representatives of the University of Western Ontario Health Sciences Research Ethics Board may contact you or require access to your study-related records to monitor the conduct of the research.

Voluntary Participation:

Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time with no effect on your academic or employment status.

You will be given a copy of this letter of information and signed consent forms. You do not waive any legal rights by signing the consent form. If you have any questions regarding this study please contact Dr. Donald Paterson (519-661-1606) at the Canadian Centre for Activity and Aging, Sonia and Arthur Labatt Health Sciences Building, The University of Western Ontario, London. If you have any question about the conduct of this study or your rights as a research subject you may contact the Director of the Office of Research Ethics, The University of Western Ontario, 519-661-3036 (ethics@uwo.ca).

LETTER OF INFORMED CONSENT

VO_2 kinetics in acute hypoxia in older and young men: Is limb blood flow elevated?

Principal Investigator: Donald H Paterson, PhD

PhD Student: Livio Zerbini, MSc

I have read the Letter of Information, have had the nature of this study explained to me and I agree to participate. All questions have been answered to my satisfaction.

❒ I consent to having my data kept for future as of yet unknown analyses.

❒ I do not consent to having my data kept for future as of yet unknown analyses.

Participant:

_____ _____

Name (please print) Signature

_____

Date

Investigator (i.e. Person Responsible for Obtaining Informed Consent):

_____ _____

Name (please print)

Signature

Date

EDUCATION AND DEGREES

Present	Doctor of Philosophy
Institution:	University of Verona, Italy
Faculty:	Faculty of Sport Science
Supervisors:	Federico Schena, Phd
	Donald H Paterson, PhD

2006-2008	Master of Sport Science
Institution:	University of Verona, Italy
Faculty:	Faculty of Sport Science

2003-2006	Bachelor of Sport Science
Institution:	University of Bologna
Faculty:	Faculty of Physical Education

REFEREED PUBLICATIONS

Tosi P., Leonardi A., **Zerbini L**., Rosponi A., Schena F. (2010). Energy cost and efficiency of ski mountaineering. A laboratory study**..** JOURNAL OF SPORTS MEDICINE AND PHYSICAL FITNESS (ISSN:0022-4707), pp. 400- 406 Vol.50

Fabre N., Bortolan L., Pellegrini B., **Zerbini L**., Mourot L., Schena F. (2012). Anaerobic threshold assessment through the ventilatory method during roller-ski skating testing: right or wrong?**.** JOURNAL OF STRENGTH AND CONDITIONING RESEARCH (ISSN:1064-8011), pp. 381- 387 Vol.26,

Zerbini L; Brighenti A; Pellegrini B; Bortolan L; Antonetti T; Schena F (2012) Effects of acute hypoxia on the VO_2 kinetics of older adults during cycling exercise. APPLIED PHYSIOLOGY, NUTRITION AND METABOLISM (Epub ahead of print).

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Zerbini Livio;Fabre Nicolas;Angius Luca;Brighenti Alfredo;Schena Federico (2010). **Cardiorespiratory responses during incremental ramp test in mild acute hypoxia** ,4th International Symposium for Hypoxia in Medical Research, Training and Rehabilitation Location: Innsbruck, Austria (January 29th-30th, 2010)

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