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"Effects of small versus large muscle mass exercise training on maximal and sub-maximal exercise parameters in solid organ transplanted recipients"

PhD student

Dr. del Torto Alessio

Supervisor

Prof. Lazzer Stefano

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List of publications

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Effect of small *vs* large muscle mass endurance training on maximal oxygen uptake in organ transplanted recipients. Submitted for publication at the *Applied Physiology, Nutrition and Metabolism* (In press).

del Torto A, Skattebo Ø, Hallén J, Capelli C. Cardiac output with modified cardio-impedance against inert gas rebreathing during sub-maximal and maximal cycling exercise in healthy and fit subjects. *Eur J Appl Physiol.* 2019 Jan;119(1):163-170. doi: 10.1007/s00421-018-4011-z.

Zuccarelli L, do Nascimento Salvador PC, Del Torto A, Fiorentino R, Grassi B. Skeletal muscle $\dot{V}O_2$ kinetics by the NIRS repeated occlusions method during the recovery from cycle ergometer exercise. *J Appl Physiol* (1985). 2020 Mar 1;128(3):534-544. doi: 10.1152/japplphysiol.00580.2019.

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Abstract published at international congress

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Abstract

Achieving a high cardio-respiratory fitness is pivotal in healthy and clinical populations, in fact it is a strong predictor of mortality; in addition, since the most common daily activities are carried out at submaximal intensities and require continuous transitions from one exercise intensity to another one, also the rate of adjustment of pulmonary O2 uptake (i.e. $\dot{V}O_2$ kinetics) is a key feature that determine the exercise tolerance. Solid organ transplant recipients suffer from a reduced maximal O2 consumption ($\dot{V}O_{2max}$) and previous studies suggested a remarkable contribution of the peripheral, muscular factors, to this impairment. Likewise, pulmonary $\dot{V}O_2$ kinetics is slower in organ transplant recipients likely because of the oxidative metabolism defects of skeletal muscle that affect these patients. In fact, organ transplant patients suffer from defects of the skeletal muscles and these abnormalities are, likely, induced by the side effects of immunosuppressive therapies and the deconditioning/disuse. It is of note that endurance training (ET) protocols carried out with a small muscle mass (i.e. single leg cycling (SL)) result in greater improvements of the peripheral factors affecting O2 diffusion and utilization if compared to ET performed with large muscle masses (i.e. double leg cycling (DL)). Therefore, the effect of SL-ET *vs* DL-ET on $\dot{V}O_2$max and pulmonary $\dot{V}O_2$ kinetics (i.e. O2 deficit (O2Def)), mean response time (MRT) and the amplitude of the pulmonary $\dot{V}O_2$ slow component (SCamp)) were investigated in heart, kidney and liver transplanted recipients (HTx, KTx and LTx, respectively). Moreover, the role of the cardiovascular, central, and peripheral factors in affecting improvement after ET has never been quantified in these patients. Therefore, the application of the multifactorial model of $\dot{V}O_{2max}$ limitation is proposed in order to determine the contributions of cardiovascular and local factors to better quantify the origin of the main limitation of $\dot{V}O_2$max in these patients. 33 patients (HTx = 13, KTx = 11 and LTx = 9) were recruited and divided in SL-ET and DL-ET groups and completed 24 sessions of ET. After the exercise program, the SL-ET group increased the $\dot{V}O_2$max by 13.8% ± 8.7 ($p < 0.001$) likely because

of a larger maximal O2 systemic extraction. The DL-ET group increased the $\dot{V}O_{2max}$ by 18.6% ± 12.7 ($p < 0.001$) due to the concomitant central and peripheral adaptations. However, no difference was found for the $\dot{V}O_{2max}$ improvement ($p = 0.237$) between the two groups. Furthermore, following the exercise program, SL-ET group decreased O2Def, MRT and Scamp by 16.4% (13.7) ($p = 0.008$), by 15.6% (13.7) ($p = 0.004$) and by 35% (31) ($p = 0.002$), respectively. Likewise, the DL-ET group, O2Def, MRT and SCamp dropped by 24.9% (16.2) ($p < 0.0001$), by 25.9% (13.6) ($p < 0.0001$) and by 38% (52) ($p = 0.0003$), respectively. Additionally, the magnitude of improvement for the O2Def, MRT and SCamp were not significantly different between SL-ET and DL-ET groups ($p = 0.277$, $p = 0.083$ and $p = 0.601$, respectively).

In conclusion the results indicate that SL-ET is a valid ET typology, in fact was as effective as DL-ET to improve i) $\dot{V}O_{2max}$ and ii) pulmonary $\dot{V}O_2$ kinetics in HTx, KTx and LTx. Finally, it is suggested that the impaired peripheral O2 extraction and/or utilization play a remarkable and important role, but not superior to the central factors, in limiting $\dot{V}O_{2max}$ and exercise capacity in this type of patients.

Introduction

The current PhD thesis comprises three parts. Firstly, the following themes are introduced and illustrated: the general and descriptive physiology of maximal oxygen consumption in heathy subjects, the central and peripheral factors limiting the maximal oxygen intake, the decline of the cardio-respiratory fitness, the possible mechanism behind this impairment in solid organ transplanted patients (SOT) and the effect of small muscle mass exercise training in healthy and clinical populations on the maximal and sub-maximal aerobic exercise related parameters. In the second part, the main studies of the three years PhD experience are presented. This section addresses the topic of the effect of endurance training on maximal oxygen consumption in SOT comparing two different training modalities, namely dynamic exercise involving a small muscle mass opposed to the one recruiting extensive muscle masses. In addition, an attempt is made to clarify if the central or the peripheral factors are the main limiting components for the cardio-respiratory fitness in transplanted patients. Afterwards, a study comparing the effect of above mentioned training typologies on the pulmonary oxygen uptake kinetics in transplanted patients is presented.

Thirdly, the last part of the current thesis touches the other topics that the PhD student has investigated during the last 3 years. Thus, this final section regards i) a study evaluating the level of agreement and the repeatability of two non-invasive techniques for the cardiac output assessment during exercise, ii) an investigation evaluating the assessment of skeletal muscle oxygen uptake recovery kinetics, obtained by means of the near-infrared spectroscopy and the repeated occlusions method, during the recovery from cycle ergometer exercise and lastly, iii) a study investigating the level of agreement and the repeatability of cardiac output determination, by means of Physioflow device, in different types (constant load *vs* incremental) of maximal cycling exercises.

CHAPTER I

General introduction

1.1) O_2 cascade and maximal O_2 consumption

In mammals, adenosine-triphosphate (ATP) is the key molecule where energy, produced by the different metabolic pathways occurring in our cells, is stored in the form of chemical bonds, therefore it is a pivotal compound (McArdle et al., 2010). Our cells can rely on aerobic and anaerobic metabolic pathways in order to produce ATP; however, the contribution of those not requiring O_2 to the total energy homeostasis is marginal and of limited applicability. Therefore, the aerobic energy-generating process must be able to fully satisfy the cellular metabolic needs. Indeed, carbohydrates and lipids are processed to generate NADH and FADH2, molecules that contain electrons with high transfer potent and, therefore, energy-rich. Subsequently and within the mitochondria, those electrons are transferred to O_2 and, thanks to this step, energy is liberated and immediately stored in the form of chemical bond linking inorganic phosphate (P*i*) to adenosine diphosphate generating ATP, by the process known as oxidative phosphorylation. Conversely, when ATP is broken-down, the liberated energy became available in order to fuel the diverse cellular activities, for example, the contractile activity of skeletal muscles (McArdle et al., 2010). Therefore, O_2 is crucial for the human beings: thanks to it, our cells are able to synthesize aerobically ATP, through the oxidative phosphorylation, to supply the metabolic demands and guarantee the homeostatic condition, both at rest and during exercise. It is noteworthy that i) muscles do not benefit from a large content of ATP (~ 8 mmol · kg^{-1} wet weight of muscle), ii) these ATP storages are kept relatively stable and, to some extent, preserved even during intense exercise to exhaustion, thus iii) this imply that ATP must be regenerated at the very same rate with which it is utilised (Barker et al. 2010). O_2 must travel from the ambient air across the human organism to reach the mitochondria where it is used for energetic purposes; this pathway is well known as the O_2 cascade and represent all the, in series, steps that O2 must travel to reach the intracellular environment (di Prampero & Ferretti, 1990).

The respiratory system through the ventilation, allows the O_2 of the atmosphere to reach the alveolar-capillary units where, thanks to the pressure gradient occurring across the alveolar-capillary membrane, the O_2 diffuses into the blood where it binds to the haemoglobin (Hb) located within the red blood cells (McArdle et al., 2010). Subsequently, the cardiovascular system pumps arterialized blood to the whole body and, thanks to the complex network of arterial vessels, O_2 is delivered to the tissues. At peripheral level, blood flow converges in the capillary network that surround other tissues and the skeletal muscles, wherein peripheral gas exchanges occur. In fact, the $O2$ carried by the red blood cells, detach from Hb and diffuses into the myocytes; contrariwise, the carbon dioxide ($CO2$), deriving from the metabolic activity, diffuses into the blood loading the free binding sites of Hb (Bassett & Howley, 2000). Thereafter, the de-oxygenated blood continues its course in the circulatory three reaching again the alveolar-capillary in order to excrete the $CO2$, newly bind $O2$ and re-start the same cycle. Once $O2$ has diffused inside the myocytes, it is utilised through the electron transport chain, within the mitochondria, for the aerobic re-synthesis of ATP (Bassett & Howley, 2000).

The systemic oxygen consumption ($\dot{V}O_{2max}$) in resting conditions correspond, on average, to 3-4 mL $\cdot$ min^{-1} $\cdot$ kg^{-1} of body mass or 150-400 mL $\cdot$ min^{-1} for a young healthy human weighing 50-100 kg (Joyner & Casey, 2015). On the other hand, this scenario deeply changes during exercise, especially in the case of exercise performed with large muscle mass: the oxygen utilization of the skeletal muscle undergoes to a remarkable increase in order to support the elevated metabolic rate, reaching values between 30-90 mL$\cdot$ min$^{-1}\cdot$ kg^{-1} of body mass. This increment is linearly related to the metabolic demand imposed by the motor task and its maximal level depends on several factors, such as level of physical activity, age, gender and poorly understood genetics (Joyner & Casey, 2015). As mentioned before, during physical activity $\dot{V}O_2$ undergoes to a considerable augmentation in response to the external power requirement; however, is clear that it does not increases boundless.

The increment shows an upper limit that was highlighted for the first time by Hill and Lupton (1923). The two researcher, with their experimental works, reached the following conclusions: i) $\dot{V}O_2$ has an upper limit, the so called maximal O2 consumption ($\dot{V}O_2max$), ii) $\dot{V}O_2max$ may be principally due to the limitation of cardiovascular system to deliver O2 to the working muscles, iii) there is no universal value of $\dot{V}O_2max$, instead there are individual maximal rates of O2 intake for each person, iv) $\dot{V}O_2max$ is a prerequisite for success in middle and long-distance running (Bassett & Howley, 2000). Point (1) is especially noteworthy and implies additional explanations. Once the subject has reached his / her $\dot{V}O_{2max}$, further increments in external workload (f.i. speed of running or work rate) are not matched by a further increase of $\dot{V}O_2$. In other words, a so called plateau has been attained (Bassett & Howley, 2000; Hawkins et al., 2007; Levine, 2008). However, not all subjects show a clear plateau when they are stressed up to maximal effort: at least 50% of practitionersdo not attain it (Howley et, 1995; Duncan et al., 1997; Bassett & Howley, 2000). Hence, secondary criteria have to be applied to verify if the exercising subject reached his / her maximal limit in absence of a clear $\dot{V}O_2$ plateau: (1) a the attainment of at least 85% of the age-predicted maximal heart rate; (2) a respiratory exchange ratio value ≥ 1.1; (3) blood lactate concentration higher than 10 mM at maximal exercise; and (4) a rate of perceived exertion on the Borg scale, at least, of 19/20 (Åstrand et al., 2003). When at least two of these subsidiary criteria for $\dot{V}O_{2max}$ establishment are met at the end of the test, there is sufficiently high guarantee that is terminated when subject reached exertion and likely the $\dot{V}O_2$ (Howley et al., 1995). However, these criteria were recently criticized (Poole et al 2008), but they are still used to determine if subjects reached true exhaustion at the end of a maximal test (Zuccarelli et al., 2020). Recently, exercise protocols have been introduced to overcome this problem, which is particularly frequent when a cycling ramp test is used to evaluate $\dot{V}O_2max$. Briefly, after the end of an incremental steps and/or ramp maximal test, a verification phase is implemented after that the subject recovers for 5 minutes. For this latter

exercise, the subject resumes pedalling or running at mechanical power or velocity equal to 105% of that attained at the end of the first test (Poole et al., 2008). If a true $\dot{V}O_{2max}$ was reached during the incremental steps and/or ramp maximal exercise, a further test performed at a greater mechanical power or velocity should not elicit a greater $\dot{V}O_{2max}$ value. However, contrasting findings have indicated that the verification phase is not supported by the experimental data (Green & Askew, 2018; Murias et al., 2018; Iannetta et al., 2020; Possamai et al., 2020; Wagner et al., 2021); moreover, no studies have investigated the validity and feasibility of the verification phase in different patient populations. Despite this, when possible, the implementation of the verification phase during maximal exercise testing could be valuable. The difficulty met in obtaining, in some circumstances, a clear plateau of $\dot{V}O_2$ during a maximal test has convinced some authors to introduce the definition of peak O2 consumption ($\dot{V}$O2peak). This is the highest value of $\dot{V}O_2$ reached by the subject during a maximal test. It is also of remarkable importance to note that theterm refers to the highest value of $\dot{V}$O2 measured at exhaustion during a maximal test when we cannot show any clear plateau also in the presence of some of the validating criteria. $\dot{V}$O2max has been always one of the most popular measurement in the exercise physiology. However, some confusion still exists concerning the definitions and terminology used to define terms related to it. As a matter of fact, the same meaning is often attributed to the terms maximal aerobic power and capacity. It is essential to differentiate these denominations the former is basically $\dot{V}O_2max$ expressed in the appropriate units of power, i.e. is the maximal rate of the aerobic synthesis of ATP, in turn proportional to $\dot{V}O_2max$ (Basset & Howley, 2000). The latter, i.e. the aerobic capacity, denotes the total chemical energy available from the aerobic metabolism to perform work and, despite different parameters used to estimate it (e.g. time to exhaustion and endurance performance), no single index of aerobic capacity is accepted yet (Malina et al., 2004). After its first characterization, $\dot{V}O_2max$ has been widely examined by researchers and sport scientists who have provided a huge

body of knowledge regarding its predictive meaning in exercise physiology. They demonstrated that $\dot{V}O_2max$ is up to twice as much in endurance athletes compared with sedentary individuals. Moreover, they soon understood the importance to express $\dot{V}O_2max$ in relative terms - normalized to body mass ($mL \cdot min^{-1} \cdot kg^{-1}$) or in absolute terms ($L \cdot min^{-1}$) - in the case the athletes perform or do not perform activities that foresee antigravitational work, like in uphill cycling, or not, like in cycling on flat tracks. (Ferretti, 2014; Saltin & Åstrand, 1967). Men are typically characterized by $\dot{V}O_2max$ 15 - 30% larger than women and this discrepancy is likely due to difference in body mass composition and total haemoglobin mass; this difference between sexes is maintained even considering the decline of $\dot{V}O_2max$ with aging (Talbot et al., 2000). $\dot{V}O_2max$ decreases with age. In fact, after around 25 years old, it declines steadily at a rate of about 1% per year, with athletes maintaining higher values along the entire life span; this fall is largely consequence of the decrease of maximal heart rate and of the development of muscle hypotrophy and it is augmented in the oldest ages (Ferretti, 2014). Therefore, endurance training, with both continuous or intervals-training protocols, produces two main effects: from one side it leads to an increase of $\dot{V}O_2max$, from the other one it slows down its decline induced by the aging (Ferretti, 2014). By the other hand the opposite occurs with prolonged inactivity (Ferretti, 2014; Ferretti & Capelli, 2009). Training and detraining are not the only interventions that induce the modification of the $\dot{V}O_{2max}$; in fact, several other conditions can cause alterations of the maximal aerobic power. Indeed, it has been shown that $\dot{V}O_2max$ diminishes both in acute and chronic hypoxia: it is reduced progressively and linearly by 0.6% per 100m of altitude (Ferretti, 2014; Levine, 2008; Wehrlin & Hallén, 2006). However, breathing elevated oxygen pressure gases leads only to a slight, if any, raise in $\dot{V}O_{2max}$ and the effect of hyperoxia is particularly evident in athletes with very high maximal cardiac output ($\dot{Q}_{max}$) who undergo a progressive exercise-induced hypoxemia as the intensity increases. This phenomenon, also known as Dempsey effect, is due to the progressive shortening of the transit time of the blood

along the capillary of the lung. This prevents the attainment of the equilibrium between the partial pressure of O_2 in the alveolar air with that of the blood at the end of the capillary. The effects of acute manipulation of cardiovascular oxygen transport system on $\dot{V}O_{2max}$ were also investigated, showing that $\dot{V}O_{2max}$ is lower in subject affected by acute anemia than in healthy individuals and higher in acute polycythaemia compared with heathy subjects (Ferretti, 2014). As it will be explained below, the alterations of $\dot{V}O_{2max}$ are mainly associated with consensual changes of $\dot{Q}_{max}$, although also peripheral adaptations, such as variations in muscle capillarity, mitochondrial volume density and muscle oxidative enzyme activities are capable to induce long-term adaptations of $\dot{V}O_{2max}$. No wonder, therefore, it has been demonstrated a strong relationship between $\dot{V}O_{2max}$ and $\dot{Q}_{max}$; indeed, selective and non-selective beta-adrenergic blockade, causing a drop of maximal heart rate (HR), were shown to decrease $\dot{V}O_{2max}$ in healthy and well trained men (Kaiser et al., 1986). Furthermore, after 90 days of bed rest exposure, the fast component of $\dot{V}O_{2max}$ decline was reported to be strongly related with the decreasing of $\dot{Q}_{max}$; however, the slow component of $\dot{V}O_{2max}$ decrese was associate to the negative alterations of peripheral gas exchange, resulting probably the development of muscle hypotrophy. (Ferretti, 2014; Ferretti & Capelli, 2009). As a matter of fact, the relationship between cardiac $\dot{Q}_{max}$ and $\dot{V}O_{2max}$ is illustrated by the Fick's principle, where $\dot{V}O_2$ is given by $\dot{Q}$ times the arteriovenous oxygen difference (a-$\bar{v}$O2diff),

$$\dot{V}O_2 = \dot{Q} \cdot (\text{a-}\bar{v}\text{O2diff}) \quad (1.$$

where

$$\dot{Q} = \text{Stroke Volume (SV)} \cdot \text{Heart Rate (HR)} \quad (2.$$

Therefore

$$\dot{V}O_2 = (\text{SV} \cdot \text{HR}) \cdot (\text{a-}\bar{v}\text{O2diff}) \quad (3.$$

As such, this equation suggests that $\dot{V}O_{2max}$ is strictly reflected by the ability of the cardiovascular

system to deliver oxygen to exercising muscles and to the capability of metabolically active tissues to extract and utilizeO_2. Consequently, in the latter equation is shown that the interaction of central and peripheral physiological factors is a key element for the investigation concerning the $\dot{V}O_2$ (Tanner& Gore, 2013). If we apply Fick's equation to the maximal exercise condition, the $\dot{V}O_{2max}$ is determined by the product between $\dot{Q}max$ and the maximal arteriovenous oxygen difference (a-$\bar{v}$O2diff)max, as follow:

$$\dot{V}O_{2max} = \dot{Q}max \cdot (a\text{-}\bar{v}O2diff)max \quad (4$$

1.2) Limiting factors of $\dot{V}O_{2max}$

As mentioned above, the O2 uptake of a subject who is performing a maximal effort does not increase boundless, but it reaches a phase of plateau where further augmentation in power output are not followed by any raising in $\dot{V}O_2$, showing that maximal oxygen consumption must be limited at some level along the respiratory system (Hawkins et al. 2007; Ferretti, 2014). Taking into account the Fick's equation applied to maximal condition, $\dot{V}O_{2max} = \dot{Q}max \cdot (a\text{-}\bar{v}O2diff)max$, and recalling that $\dot{Q}max$ is given by SVmax times HRmax, we can understand that $\dot{V}O_{2max}$ is restrained by both systemic and peripheral components (Basset & Howley, 2000). The quest for the main limiting factors of $\dot{V}O_{2max}$ has stirred a heated scientific debate between who hypothesized only a main limiting factor, either peripheral or central, and who are in favor of a multifactorial limitation. The pathway for O2 from the atmosphere to the mitochondria follows a series of steps, each of which may produce a potential limiting effect to O2 flux. The physiological factors that could limit $\dot{V}O_{2max}$ namely are: the pulmonary diffusing capacity, the maximal systemic O2 delivery ($\dot{Q}_aO_{2max}$), the oxygen carrying capacity of the blood and the skeletal muscle characteristics (Basset & Howley, 2000). The first three are usually classified as "central" factors and the last one is defined as

"peripheral". A brief explanation of these components will follow. However, I will not explain this topic in details since it is beyond the main aim of the present investigation. Nevertheless, I consider important to outline it in order to understand the cause-effect relationship between $\dot{V}O_{2max}$ and $\dot{Q}_{max}$.

Considering the pulmonary system, in the average individual exercising at submaximal and maximal level, lungs perform their function saturating arterial blood with O_2 extremely well, as arterial oxygen saturation ($\%S_aO_2$) remains around 95% across a broad range of exercise intensities. However, it has been already mentioned that highly trained endurance athletes undergo substantial arterial O_2 desaturation when exercising at maximal intensity compared with less trained or un-fit individuals. High level, trained endurance athletes have a much higher $\dot{Q}_{max}$ than untrained (40 vs 25 $L \cdot min^{-1}$). This leads to the decrease of the transit time of the blood in the pulmonary capillary. Consequently, there may not be enough time to attain the equilibrium between the partial pressure of O_2 in the alveoli and that prevailing at the end of the capillary, therefore the erythrocytes leaving the lungs are not fully loaded with O_2 (Dempsey et al., 1984; Levine, 2008). This condition was defined *exercise-induced arterial hypoxemia* (EIAH) (Dempsey, 1986). Furthermore, this scenario is extremely evident in hypoxic condition, for instance exercising at moderately high altitudes (3,000 -5,000 m), and in patients with chronic obstructive pulmonary disease (COPD) that are characterized by an impairment of the diffusion capacity of lungs. When these conditions are met, the pulmonary system represents a factor limiting the $\dot{V}O_{2max}$ (Levine, 2008; Basset & Howley, 2000). $\dot{Q}_{max}$ represents the main factor affecting $\dot{Q}_aO_{2max}$ when blood O2 carrying capacity is fixed to the value of 200 mL O2 / L of blood (i.e. with [Hb] in the range of normal values):

$$\dot{Q}_aO_{2max} = \dot{Q}_{max} \cdot CaO2 \qquad (5.$$

The raised $\dot{Q}$ is the main responsible for the increased $\dot{Q}_aO_2$ to working muscles, that in turns

can benefit from a higher amount of O_2 due to the greater blood flow (Wilmore et al., 2011). By changing, acutely, the (Hb) content of the blood we can manipulate O_2 transport to the skeletal muscles. In this case, the $\dot{V}O_{2max}$ can be consensually modified; for instance, by using autologous blood transfusion or blood withdrawal, alteration in the $\dot{V}O_{2max}$ are achieved (Basset & Howley, 2000). In fact, it has been reported that the reinfusion of 900–1,350 mL of blood elevates the oxygen carrying capacity of the blood, leading to an increase of 4–9% of $\dot{V}O_{2max}$ (Gledhill, 1982). As a matter of fact, by using a multifactorial model of the limiting factors of $\dot{V}O_{2max}$ it can be estimated that the systemic $\dot{Q}_aO_{2max}$ is responsible for 75-80% overall limitation to the $\dot{V}O_{2max}$ when exercise with large muscles mass is performed at sea level by healthy subjects (di Prampero & Ferretti, 1990). Researchers have also deeply investigated the role of peripheral factors as potential constraints of $\dot{V}O_{2max}$. Some authors have reported that endurance athletes have not only higher $\dot{Q}_{max}$, but also a larger fraction of type I oxidative muscle fibers, a greater capillary density and a higher activity of oxidative enzymes when compared with untrained individuals (Hoppeler & Weibel, 2000). Then, it has been also found that aerobic training increases the muscle capillary supply, muscle mitochondrial volume and muscle oxidative enzyme activities (Andersen & Henriksson, 1977; Costill et al., 1976). Furthermore, it was shown that the $\dot{V}O_{2max}$ of altitude-acclimatized subjects in chronic hypoxia, when exposed to normoxic gas mixtures, did not return to the pre-acclimatization levels (Cerretelli, 1976), meaning that a possible decrease in muscle mass and oxidative capacity induced by altitude acclimatization might be responsible for this phenomena. On the base of these findings, some researchers concluded that muscle oxidative capacity, rather than cardiovascular oxygen transport, was the limiting element for the $\dot{V}O_{2max}$ and also a highly significant relationship between $\dot{V}O_{2max}$ and both mitochondrial mass and capillary density was established (Hoppeler, 1990). Moreover, Saltin et al. (1985) observed the behavior of a small muscle mass performing a maximal effort and two interesting findings have been reported: i) a great portion

of $\dot{Q}$ was flowing to the isolated area; ii) the $\dot{V}O_{2peak}$ determined in the working small muscle mass was 2-3 times higher than the one measured in the same muscle group during a whole-body maximal exercise. Therefore, they concluded that skeletal muscles have a huge capacity to increase blood supply and $\dot{V}O_2$, being somehow "flooded" by blood during exercise performed with small group of muscles. In this case, therefore, $\dot{V}O_{2peak}$ is not limited by O_2 delivery, but peripheral factors. Moreover, we can also hypothesize that vascular resistances in the muscular vascular bed during exercise performed by using large masses of muscle do not decrease to such an extent to induce hypotension as it would be the case during exercise performed with a limited mass of muscle.

In conclusion, nowadays in the exercise physiology field is accepted that if we consider the factors limiting $\dot{V}O_{2max}$ we have to specify the type and conditions of exercise. In normoxia and during whole-body exercise $\dot{V}O_{2max}$ is mainly limited by central factors; by contrast, when a maximal effort is performed by recruiting a small mass of muscle, $\dot{V}O_{2max}$ is primarily limited by the peripheral factors (Basset & Howley, 2000; Levine 2008).

1.3) The multifactorial model of $\dot{V}O_{2max}$ limitations

The role of central and peripheral factors to set the limitation to whole body $\dot{V}O_{2max}$ can be quantified in the light of the multifactorial model of di Prampero (di Prampero, 2003; di Prampero & Ferretti, 1990). In fact, other studies applied this line of reasoning in order to disclose the role of cardiovascular and/or muscular alteration (i.e. micro-gravity and supine vs upright exercise) on $\dot{V}O_{2max}$ (Bringard et al., 2010; Capelli et al., 2006).

The oxygen cascade, i.e. the progressive drop of the PO_2 along the pathway from air to mitochondria, is composed by several steps in series, each one could be considered as a resistance (R_i) that has to be overtaken by a pressure gradient (ΔP_i). The oxygen flow, through each resistance, whenever we are at steady-state and/or when we are at $\dot{V}O_{2max}$, can be described as

$$\dot{V}O_{2max} = \frac{\Delta P_T}{R_T} = \frac{\Delta P_i}{R_i} \quad \text{(A1)}$$

where R_T represents the total resistance to the oxygen flow that has to be overtaken by ΔP_T that corresponds to the total pressure gradient. Considering that the resistances are located in-series, the sum of the whole pool of individual R_i gives R_T; likewise, ΔP_T is given by the sum of all individual ΔP_i, and it corresponds to:

$$\Delta P_T = P_{IO2} - P_{mO2} \quad \text{(A2)},$$

Where P_{IO2} and P_{mO2} respectively represent the partial pressure of oxygen in inspired ambient air and in the mitochondria, usually set to 0 mmHg, and for *n* resistance in series, we have:

$$\dot{V}O_{2max} = \frac{P_{IO_2}}{R_T} = \frac{\Delta P_1}{R_1} = \frac{\Delta P_2}{R_2} = \ldots = \frac{\Delta P_{(n-1)}}{R_{n-1)}} = \frac{\Delta P_n}{R_n} \quad \text{(A3)};$$

where ΔP_n corresponds to the individual O_2 partial pressure difference needed to overcome the corresponding R_n.

In normoxia, ΔP_T remains constant also in presence of marked modifications of $\dot{V}O_{2max}$ induced, f.i. by training or immobilization. Therefore, the observed modification in $\dot{V}O_{2max}$ will be induced either by the reduction or increase of R_T, in turn affected by the consensual modifications of one or more of its elements in series, R_i. In fact, if we develop the algebra of the model after having imposed a given modification of $\dot{V}O_{2max}$, we obtain:

$$\dot{V}O_{2max} + \Delta\dot{V}O_{2max} = \frac{P_{IO_2}}{R_T + \Delta R_T} \quad \text{(A4);}$$

where $\dot{V}O_{2max} + \Delta\dot{V}O_{2max}$ is the change in $\Delta\dot{V}O_{2max}$ caused by a modification in R_T (ΔR_T). In Eq. (A4) $\dot{V}O_{2max}$ represents the value preceding the intervention and $\dot{V}O_{2max} + \Delta\dot{V}O_{2max}$ is the value obtained in response to the intervention: an increase of $\dot{V}O_{2max}$ will correspond to a decrease of R_T (ΔR_T will be negative) and vice versa. Dividing Eqs. (A3) by (A4), we will then obtain the following expression:

$$\frac{\dot{V}_{O_{2max}}}{\dot{V}_{O_{2max}} + \Delta\dot{V}_{O_{2max}}} = 1 + \frac{\Delta R_T}{R_T} \quad \text{(A5),}$$

which can be also written as follows:

$$\frac{\dot{V}_{O_{2max}}}{\dot{V}_{O_{2max}} + \Delta\dot{V}_{O_{2max}}} = 1 + \frac{\Delta R_1 + \Delta R_2 + \cdots + \Delta R_{(n-1)} + \Delta R_n}{R_T} \quad \text{(A6a)}$$

or:

$$\frac{\dot{V}_{O_{2max}}}{\dot{V}_{O_{2max}} + \Delta\dot{V}_{O_{2max}}} = 1 + \frac{\Delta R_1}{R_T} + \frac{\Delta R_2}{R_T} + \ldots + \frac{\Delta R_{(n-1)}}{R_T} + \frac{\Delta R_n}{R_T} \quad \text{(A6b)}$$

We now can also introduce the concept that the fraction F_i of the total limitation to $\dot{V}O_{2max}$ represented by any given individual R_i is simply the ratio between a given resistance R_i and R_T:

$$F_i = \frac{R_i}{R_T} \quad \text{(A7)}$$

Therefore, (A6b) can be expressed by substituting R_T with the corresponding F_i to obtain:

$$\frac{\dot{V}_{O_{2max}}}{\dot{V}_{O_{2max}} + \Delta\dot{V}_{O_{2max}}} = 1 + \frac{F_1 \cdot \Delta R_1}{R_1} + \frac{F_2 \cdot \Delta R_2}{R_2} + \ldots + \frac{F_{(n-1)} \cdot \Delta R_{(n-1)}}{R_{(n-1)}} + \frac{F_n \cdot \Delta R_n}{R_n} \quad \text{(A8)}$$

If one and only one of the in-series resistances underwent a modification leading to a change in $\dot{V}O_{2max}$, the approach summarised in Eq. (A8) can be substantially simplified, in fact all the other ratios will be zero, with exception of the one referred to modified resistance. For instance, should only the cardiovascular resistance R_Q, be acutely changed, Eq (A8) can be written as follows:

$$\frac{\dot{V}_{O_{2max}}}{\dot{V}_{O_{2max}} + \Delta\dot{V}_{O_{2max}}} = 1 + \frac{F_Q \cdot \Delta R_Q}{R_Q} \quad \text{(A9).}$$

Now F_Q represents the fractional limitation imposed by R_Q to $\dot{V}O_{2max}$. The theoretical line reported in Fig 1 (see below) is algebraically represented by Eq. (A9) and it was obtained in the particular case where only R_Q was acutely changed and the consequent and corresponding increases-decreases in $\dot{V}O_{2max}$ measured. In this case, if we plot on the y-axis the ratio of the values preceding and following the intervention (left arm of Eq. (A9) as a function of the corresponding ΔR_Q / R_Q, a linear relationship is drawn having the y-intercept and the slope equal to 1 and F_Q (slopes of lines in Fig 4 and 5), respectively. R_Q is the reciprocal of $\dot{Q}$ multiplied by the oxygen transfer coefficient in the blood phase (βb), that in turns corresponds to the average slope of the oxygen equilibrium curve:

$$R_Q = \frac{1}{\dot{Q} \cdot \beta_b} \approx \frac{1}{\dot{Q} \cdot c_{aO_2}} \approx \frac{1}{\dot{Q}_{aO_2}} \quad \text{(A10),}$$

where $\dot{Q}_aO_2$ represents the systemic, bulk O_2 delivery. In the case of acute modifications of $\dot{Q}_aO_{2max}$, and hence of R_Q, F_Q turned out to be equal to about 0.70-0.75. This indicates that maximal systemic O_2 delivery accounts for about 70 % of the total factors limiting $\dot{V}O_{2max}$ downstream the lungs, the remaining 30 % being represented by all the lumped peripheral factors. In order to better highlight this

concept, we can perform a hypothetical experiments wherein we are able to double $\dot{Q}_aO_{2max}$. This increase in maximal O_2 delivery will increase $\dot{V}O_{2max}$ by about 70-75 %.

Even if βb is not assessed, the SpO_2 does not substantially change in response to a training intervention, only modifications in arterial oxygen concentration (CaO_2) might lead to changes in βb, as we properly considered in the calculations. Furthermore, another example might help to better realize the practical application of this insightful model, in fact figure 1, taken from Ferretti and Capelli (2009), illustrates the graphical representation of the algebra describing di Prampero's multifactorial model. More precisely, the figure at stake represents the modifications in maximal oxygen consumption, given by the ratio between the values before ($\dot{V}O_{2max}$) and after ($\dot{V}O_{2max} + \Delta$) the modifications, as a function of $\Delta R_Q/RQ$, where ΔR_Q is the induced change in the cardiovascular resistance to $\dot{V}O_{2max}$ and R_Q is the very same resistance before the alteration taken in consideration. If we considered that one and only one resistance along the oxygen pathway is modified (R_Q in the current context), this would cause $\dot{V}O_{2max}$ changes such that the relation between the two parameters on y- and x-axes turns out linear, with y-intercept equal to 1 and slope equal to the fractional limitation to $\dot{V}O_{2max}$ imposed by the resistance at stake (F_Q in the current context). The line drawn in Fig 1 is theoretical and has a slope of 0.70, corresponding to the F_Q value reported by di Prampero and Ferretti (1990) for humans in normobaric condition. Moreover, the data showed in the legend, belong to to three bed rest investigations. It is noteworthy that the data-point for the 14- and 42 days of immobilisation are located close to the theoretical line at stake, whereas the 90-days immobilisation data-points lie well beyond. In other words, in the first case (i.e. 14- and 42 days of bed rest), the modification in R_Q completely explained the changes in $\dot{V}O_{2max}$ found following the period of immobilisation; on the other hand, in the second condition (i.e. 90-days of bed rest) the modification in $\dot{V}O_{2max}$ were greater than that can be estimated by supposing a change only in R_Q, implying that the alteration of another resistance must be put into play, namely the peripheral resistance R_p.

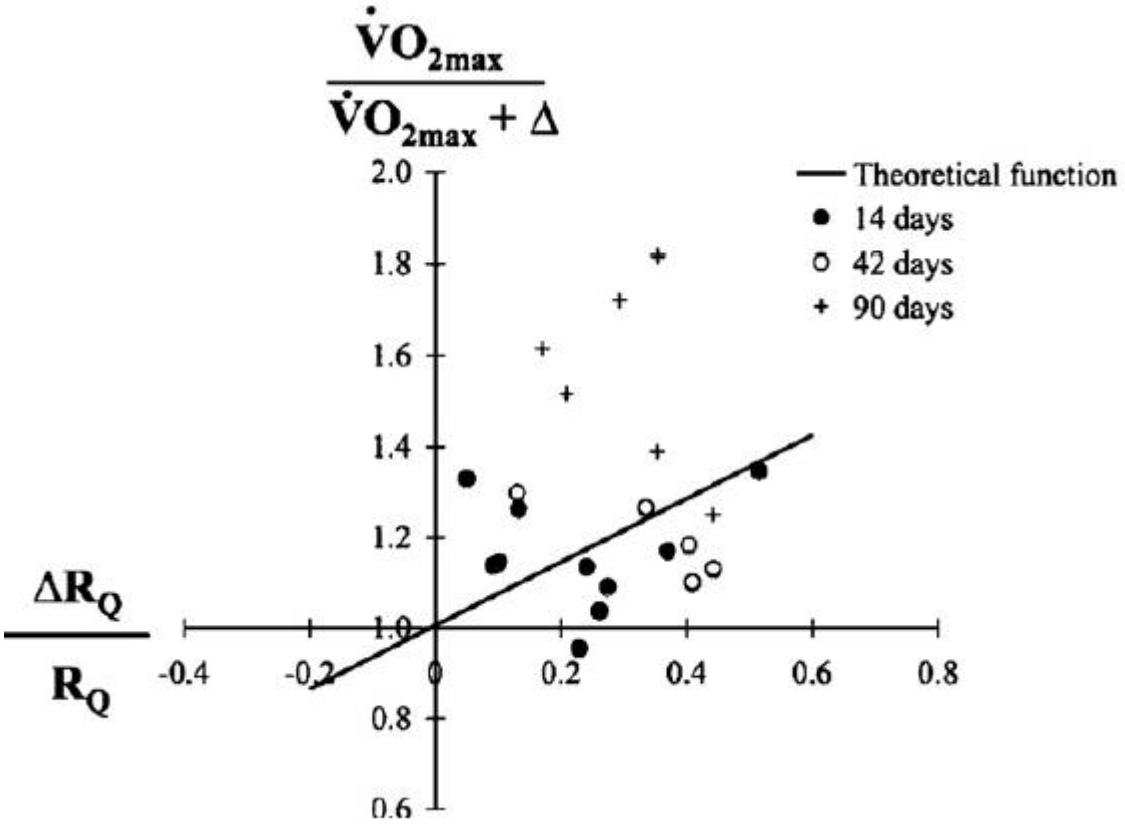

Figure 1. Graphical representation of the multifactorial model of $\dot{V}O_{2max}$ limitations, taken from (Ferretti & Capelli, 2009). The black strait line represents the theoretical line with slope of 0.70 and intercept 1 (di Prampero & Ferretti, 1990). The full black and withe dots are the data-point for the 14-day and 42-day of bedrest campaigns, respectively. The cross stand for the data-point for the 90-day of bed rest study. For further details see the text (paragraph *"The multifactorial model of $\dot{V}O_{2max}$ limitations")*.

1.4) The importance of exercise training as a lifestyle in health and diseases

Exercise training is essential in order to increase the $\dot{V}O_{2max}$ and to excel in endurance sporting events and is also a key behavior that strongly affects the health condition of human beings (Ferretti, 2014). As a matter of fact, the inadequate level of physical activity was identified as the fourth leading risk factor (https://www.ncbi.nlm.nih.gov/books/NBK305057/) for the incidence of non-communicable diseases (i.e. cardiovascular disease and diabetes). The prevalence of insufficient physical activity (i.e. 150 minutes of moderate intensity aerobic activity every week or 75 min of vigorous-inteisty activity) amounted to almost 50% and 40% for women and men, respectively, belonging to USA regions adhering to World Health Organization in 2008; furthermore, globally physical inactivity amount to 31% in male adult population, causing by 3.2 million of deaths each year (https://www.who.int/dietphysicalactivity/factsheet_inactivity/en/). In addition, peak exercise capacity, expressed in terms of metabolic equivalents (MET), has been found to be a powerful predictor of mortality, indeed the risk of death for any cause in subjects reaching less than 5 MET was estimated to be double than in subjects with 8 or more MET; furthermore, it was also observed that for every increase in1 MET, the survival rate is raised by 12% (Myers et al., 2002).

In spite of this, physical activity is not the only one modifiable risk factor for the development of chronic diseases (https://www.who.int/chp/chronic_disease_report/media/Factsheet1.pdf); hypertension (i.e. systemic blood pressure >140/90), abnormal blood glucose level, elevated blood lipids (i.e. dyslipidemia) and excess body fat (i.e. overweight and obesity) fall into this category (Fontana et al., 2007). As a matter of fact, hypertension is the most common preventable risk factor for the cardiovascular disease (Pescatello et al., 2015), dyslipidemia and, more precisely the elevated concentration of the low density lipoprotein cholesterol, is associated with higher risk of cardiovascular disease, coronary artery disease and stroke (Brunzell et al., 2008). Furthermore, the increased visceral adiposity (i.e. mesenteric and omental fat) induces insulin resistance (i.e. greater

plasma insulin during a glucose tolerance test) (Borst et al., 2005) by the production of resistin (Fontana et al., 2007) and increases the low-grade systemic inflammation caused by the release of pro- inflammatory cytokines (Shin et al., 2019). Both, insulin resistance and low-grade systemic inflammation, in turns, are established risk factors for chronic diseases (Fontana et al., 2007). In addition, the lack of chronic exposure to physical activity is one of the causes responsible for development of sarcopenia, that is a conditionclinically defined by low muscle mass, functional decline of mobility and muscle strength (Migliavacca et al.,2019). In fact, the loss of muscle mass of lower limbs was reported after just 2 day of leg immobilisationa (Kilroe et al., 2020) and after 5 (Mulder et al., 2015) to 10 days of bed rest (Suzukiet al., 1994) and this muscle hypotrophy, at least in part, can be responsible for the reduced cardio-respiratory fitness due to the deleterious effect of immobilisation on peripheral gas exchanges (Capelli et al., 2006; Ferretti & Capelli, 2009). The prevalence estimate of sarcopenia amount from 2 to 20% in community dwelling older people; however, it can reach up to 33% among patients in long term care (Cruz-Jentoft et al., 2014); furthermore, the sarcopenia turned out to be a predictor for disability and mortality (Cruz-Jentoft & Sayer, 2019) and this condition is also associate to an increased mortality in SOT (Englesbe et al., 2010).

It is noteworthy that SOT suffer from a remarkable development of cardiovascular diseases and its associated risk factors, which in turns represent one of the leading causes of morbidity and mortality in this clinical population (Warden & Duell, 2019). In fact, in kidney transplant recipients (KTx) dyslipidemia is observed in 50 to 60% of the patients, hypertension is detected in > 70% of KTx and the new onset-diabetes has a cumulative incidence that raises with the post-transplant time, being about 6 % and 29.8% after 6 monthand 15 years from the transplant, respectively (Gill, 2008). Liver transplanted recipients (LTx) have a higher risk of cardiovascular deaths and ischemic events if compared with the age- and sex-matched peers (Johnson et al. 2002); in fact, a previous study

investigating a group of 110 LTx, reported that 44% and 69% of them were found with dyslipidemia and hypertension, respectively (Johnson et al. 2002). In addition, data from retrospective studies indicated that the accumulated incidence for the onset of post-transplant diabetes mellitus amounted to ~40%, after 5 years, in LTx (Jenssen & Hartmann, 2019). Furthermore, sarcopenia is a condition that commonly affects LTx (Kallwitz, 2015) and it is associated with increased mortality in this patients. Indeed, Englesbe and colleagues (2010) previously showed strong association between psoas area and post- transplantation mortality in LTx. These researchers found that the risk of mortality was inversely related to the psoas area; in fact, they estimated that the hazard ratio increased by 3.7 per 1000 mm^2 decline in the total psoas area (Englesbe et al., 2010). Previous studies found that the 12-month cumulative incidence of new onset diabetes of 13% (Bodziak & Hricik, 2009) on heart transplanted recipients (HTx), furthermore a systematic review pointed out that the incidence of new onset diabetes in HTx ranged from 7% to 26% (Heisel et al. 2004). Dyslipidemia affect about 60% to 81% of HTx and the elevated total cholesterol has been indicated as a predictor of mortality for HTx (Arora et al., 2009); in addition, the incidence of hypertension has increased to 90% of HTx (Urs et al., 1990).

It is noteworthy that physical activity is a modifiable risk factor, in fact several pieces of evidences demonstrated that a regular endurance exercise training is extremely effective in order to increase the peak exercise capacity and, as highlighted previously, reducing the risk of mortality if compared to sedentary individuals in both healthy subjects and patients (Myers et al., 2002). Indeed, physical activity can be implied as a preventive therapy for non-communicable diseases given its efficacy in preventing and controlling the cardio-metabolic risk factors (Warburton et al., 2006). Previous studies showed the benefits of physical activity in reducing the risk of death from any cause and from cardiovascular disease; in fact, a higher than 50% reduction in the risk was correlated with being fit or active (Myers et al., 2004). Furthermore, for each 2000-kcal·wk^{-1}

increment in energy expenditure, obtained by physical activity, there is a 24% reduction in the risk of developing noninsulin-dependent diabetes mellitus (Helmrich et al. 1994). Physical activity positively influences also resting systemic blood pressure, in fact after a single bout of aerobic exercise an immediate decrease in resting systemic blood pressure of 5-7 mmHg occurs in subjects affected by hypertension, phenomenon known as post-exercise hypotension which last up to 24h (Kenney & Seals, 1993; Pescatello & Kulikowich, 2001). However, the beneficial effect of physical activity on blood pressure extends also to the chronic repetition of exercise sessions, that has been reported as an effective intervention in order to lower blood pressure. Indeed, several metanalysis pointed out that exercise training decreased systolic blood pressure by 5-6 mmHg in hypertensive adults (Costa et al., 2018; Leal et al.,2020) and this amelioration has been associated with a 7–14% lower incidence of all-cause mortality, stroke, and coronary heart disease (Costa et al., 2018). Therefore, participation in regular exercise is recognized as powerful modifiable determinant of hypertension.

Physical activity, if applied properly, turned out to be useful in order to reduce the dyslipidemia (i.e. increased concentration of low density lipoprotein cholesterol), body weight and the visceral adiposity in normal weight, over-weight and obese subjects (Fontana et al., 2007; Miller et al., 1997). By the other hand, itwas reported that lowering the level of daily physical activity, by means of daily steps reduction (i.e. from 10000 to 1500 steps per day), increases the visceral fat mass by 7% and resulted in the development of metabolic unfavorable modifications, suggestive of decreased insulin sensitivity, in only 3 weeks in healthy men (Olsen et al., 2008).

Therefore, it seems clear that regular exercise training is an optimal strategy to manage the active role of the modifiable factors, reducing the incidence of cardiovascular and other chronic diseases. Since the cardio-metabolic alterations, leading to the non-communicable diseases, are common in HTx, LTx and KTx, it is of pivotal importance to submit SOT patients to structured

physical activity programs. However, the optimal exercise prescription has not yet been found; indeed, several issues limit the cardio-respiratory fitness and the exercise capacity in this class of patients (Williams and McKenzy, 2012).

1.5) Solid organ transplanted recipient and their exercise limitations

Several pieces of evidence reported that SOT suffer from a reduced cardio-respiratory fitness and exercise capacity, indeed they do not reach the predicted level for their, age and sex matched healthy counterparts (Williams & McKenna, 2012). It is noteworthy that these patients are affected by skeletal muscle dysfunctions. As a matter of fact, the detrimental drawbacks induced by the immunosuppressive therapy (i.e. cyclosporine and/or corticosteroids) can be the main cause for these abnormalities (Hokanson et al., 1995; Mercier et al., 1995; Lampert et al., 1998; Williams & McKenna, 2012). Previous studies on mice skeletal muscle reported that cyclosporine negatively altered the respiratory function of mitochondria, more precisely affecting state 3 and uncoupled mitochondrial respiration (Hokanson et al., 1995); moreover, it was reported that the suppression of muscle mitochondrial electron chain capacity, induced by cyclosporine administration, impaired endurance exercise performance in mice (Mercier et al., 1995). Accordingly, abnormalities in the oxidative metabolism of skeletal muscle have been reported in SOT (Williams & McKenna, 2012). Kempeneers et al. 1990 showed the presence, in the m. vastus lateralis, of impaired skeletal muscle oxidative capacity and the skeletal muscle fiber distribution was only 33% of type I fibers in relatively young KTx (i.e. 33 years on average) under immunosuppressive therapy (Kempeneers et al., 1990). Previous studies showed that, in the m. vastus lateralis of healthy and young subjects the type I fibers distribution amount to 49% and 55% on average in female and men, respectively (Komi & Karlson, 1978). This suggests that the study of Kempeneers et al., highlighted an abnormal muscle fiber type shift in KTx. In addition, a reduced cross sectional area of muscle fibers, a drop in

mitochondrial volume density and an inferior myofibril volume density was also evident on a sedentary homozygotes twin, after receiving the kidney transplant from his sibling, suggesting that peripheral dysfunctions might exist also in the absence of immunosuppressant medications (Painter et al., 2003). Severalpieces of evidence reported the presence of skeletal muscle alterations in HTx induced by both the deleterious effect of chronic heart failure and immunosuppressive therapy (Braith et al., 2005; Bussières et al., 1997; Stratom et al., 1994). In addition, Lampert and colleagues showed that sedentary HTx, when comparted to theheathy matched control group, display a reduced capillary density and capillary-to-fiber ratio (Lampert et al., 1996, Lampert et al., 1998). This deficiency can be ascribed, at least in part, to the detrimental side effect induced by cyclosporine on angiogenesis and endothelial function (Petrakopoulou et al., 2006; Ramzy et al., 2005). Regarding LTx, sarcopenia is often reported in these patients (Kallwitz, 2015) and it was demonstratedthat is a pathological condition characterized by mitochondrial bioenergetics dysfunction, with negative alterations impairing the oxidative metabolism of skeletal muscle (Migliavacca et al., 2019). As previously explained, capillary density and mitochondrial enzyme levels are the components that determine the peripheralgas exchanges, which in turns provide the 20-30 % of $\dot{V}O_{2max}$ limitation in healthy subject exercising with large muscle masses in normoxia (Bassett & Howley, 2000). Therefore, if both dysfunction in oxidative metabolism and, likely, a reduced capillary density affects the skeletal muscles of HTx, KTx and LTx, the peripheral gas exchanges would be impaired in this class of patients. This body of evidence has prompted some authors to argue that the cardio-respiratory fitness and the exercise capacity might be remarkably limited by the muscular factors in SOT (Williams & McKenna, 2012).This hypothesis is also supported by previous studies; indeed, Kempeneers and colleagues, reported that, even after 6 months of whole body (i.e. running) endurance exercise training, the oxidative metabolism was still impaired and the negative morphological alterations were still present in the *vastus lateralis* muscle of the investigated

transplanted patients. In fact, it was found a higher proportion of type II fibers and a lower oxidative capacity, reduced approximately by 37%, when compared to healthy subjects (Kempeneers et al., 1990). It is noteworthy, that the exercise training was effective in order to ameliorate the cardio-respiratory fitness and skeletal muscle function in KTx; however, the presence of skeletal muscle defects even after the training period lead the authors to argue that the exercise capacity in KTx might be mainly limited by the peripheral rather than the central factors (Kempeneers et al., 1990). Moreover, also Lampert and colleagues showed the beneficial effect of a relatively short period (i.e. 6 weeks) of endurance training on the $\dot{V}O_{2peak}$ in HTx (Lampert et al., 1998). The volume density of total mitochondria was larger after the training period; in spite of this, the capillary-to-fiber ratio was not positively changed by the exercise training, showing that skeletal muscle abnormalities persisted also after the exercise training intervention in HTx (Lampert et al., 1998). Furthermore, a strong association was found between the poor $\dot{V}O_{2max}$ and quadriceps muscle strength in KTx ($r = 0.866$; $p < 0.001$), suggesting that degraded leg muscle strength might strongly contribute to the $\dot{V}O_{2max}$ impairment (Van Den Ham et al., 2005). In addition, a previous study evaluated the effect of organ transplantation on the rate of $\dot{V}O_2$ and HR adjustment (i.e. $\dot{V}O_{2max}$ and HR kinetics, respectively) from a resting condition to level imposed by the external workload. The speed of $\dot{V}O_2$ kinetics directly affects muscular metabolic stability; in fact, for a given work-load, a faster $\dot{V}O_2$ kinetics is translated into a more rapid attainment of the so called $\dot{V}O_2$ at steady-state exercise, leading to a lower intracellular perturbation (e.g., accumulation of H^+ and lactate, and depletion of phosphocreatine) and, therefore, to a greater exercise tolerance (Poole & Jones, 2012). A previous study compared the $\dot{V}O_2$ and HR kinetics in a group of LTx, KTx and HTx during the six-minute walking test. It was reported a slower HR kinetics for the cardiac transplanted group when compared to the non-cardiac transplanted group (i.e. KTx and LTx together). Since the HR kinetics can be considered a good proxy for the O_2 transport kinetics (MacPhee et al., 2005) it can be expected that

HTx might have slower $\dot{V}O_2$ kinetics if compared to non-cardiac transplanted counterpart. In spite of this, no significant difference was found for the speed of $\dot{V}O_2$ kinetics between the cardiac and non-cardiac transplanted groups (Tomczak et al., 2008). In addition, the slower $\dot{V}O_2$ kinetics assessed for the transplant groups were significantly slower when compared to the healthy control group (Tomczak et al., 2008). Furthermore, when the O_2 transport kinetics (i.e. cardiac output kinetics) wherespeeded, by means of prior exercise, no acceleration of $\dot{V}O_2$ kinetics were reported for HTx (Grassi et al., 1997). Considering these findings together, it is likely that the prolonged $\dot{V}O_2$ kinetics might origin from the oxidative metabolism derangements in SOT; this further highlight the role of the peripheral factors in impairing the exercise capacity in this population. Stephenson et al. evaluated eight moderately active LTX, $\dot{V}O_{2peak}$ and $\dot{V}O_2$ at the anaerobic threshold were decreased amounting to 66% and 47% of predicted value, respectively. Pulmonary function at rest was not impaired, haemoglobin concentration was found to be 13.9 $g \cdot dL^{-1}$, no desaturation was reported and heart rate at peak exercise corresponded to 88% of predicted values (Stephenson et al., 2001). Moreover, subjects reported a moderate rate of perceived exertion rating for dyspnea ($RPE_{dyspnea}$) (6.6 ± 2.2), but greater for leg pain ($RPE_{leg\ fatigue}$) (8.5 ± 22). Since there were no evidences for an impaired cardiac and/or ventilatory response during exercise and the precocious anaerobic threshold, the authors speculated that exercise limitation was likely of peripheral nature (Stephenson et al., 2001). Richard and colleagues reported a significantly higher $\dot{V}O_2$ needed to run at a given velocity in well-trained KTx and HTx when compared to heathy peers matched also for the physical characteristics and performance level (Richard et al., 2005). Since mechanical efficiency (energy cost) is given by the ratio between the work done and the energy expended to perform it, the increased $\dot{V}O_2$ for each speed for the transplanted group (both HTx and KTx) reflects a greater energy cost for the same running speed if compared with the control group. The authors suggested that the peripheral abnormalities were behind the increased energy cost and

these muscular defects still affect well-trained HTx and KTx limiting their exercise capacity (Richard et al., 2005).

Finally, several pieces of evidence demonstrated that endurance training is an effective interventionif the aim is to improve the cardio-respiratory fitness in HTx, LTx and KTx (Kempeneers et al., 1990; Hermanet al., 2011; Nytrøen et al., 2012; Riess et al., 2014; Moya-Nájera et al., 2017). Since several authors pointed out the substantial contribution of the peripheral factors to limit the exercise capacity, the implementation of exercise interventions that can induce a greater stress on the muscular rather than the cardiovascular factors, such as small muscle mass endurance training, might be beneficial in this class of patients.

1.6) Small muscle mass exercise training

When dynamic exercise is carried out with a small muscle group, maximal $\dot{Q}$ is not attained and the majority of the blood flow is directed to the working muscles and the relative $\dot{V}O_{2peak}$ normalized per kg of muscle mass involved increases 2-3 times more than the $\dot{V}O_{2peak}$ normalized per kg of body mass assessed during whole-body maximal exercise (Bassett & Howley, 2000). Thus, the recruited skeletal muscles show a remarkable peripheral reserve that make them able to increase O_2 utilization, indicating that, in this scenario, the muscles $\dot{V}O_{2peak}$ is not constrained by $\dot{Q}_aO_2$, but the main limitation is provided by the ability of the involved muscle to extract and utilize the delivered O_2. In this conditions, a greater mass-specific blood flow and a higher O_2 delivery and availability will fully sustain the oxidative metabolism of the active muscle mass, meaning that the oxidative capacity responsible for the aerobic ATP re-synthesis would be fully exploited.

As a matter of fact, several pieces of evidence demonstrated that small muscle group endurance trainingwas effective to elicit the beneficial adaptations on the factors responsible for the peripheral gas exchanges in both sedentary and trained subjects; more precisely, increasing both the level of oxidative enzymes and the capillary net that perfuse the skeletal muscle. Previous studies on

sedentary individuals reported that the peripheral adaptations induced by small muscle mass endurance training were accompanied by the increase of $\dot{V}O_{2max}$ when tested on exercise involving large muscle masses (Klausen et al. 1981; Munch et al., 2018). On the contrary, previous investigations on trained participants (i.e. from a moderate to a high level) reported improved muscle oxidative and O_2 diffusion capacities leading to a greater O_2 extraction of the conditioned limb, after a training period of small muscle mass endurance training. These ameliorations resulted in the increase of muscle $\dot{V}O_{2max}$ of the trained leg, but not of the whole body $\dot{V}O_{2max}$ (Rud et al., 2012; Skattebo et al., 2020). This difference can be ascribed to the notably lower level of oxidative enzymes and capillary density of untrained subjects; therefore, in these subjects the muscular factors provide a greater limitation to the whole body $\dot{V}O_{2max}$ if compared to their active counterparts (Bassett & Howley, 2000; Ferretti, 2014; Hellsten & Nyberg, 2016). Since the training stimulus, provided by the small muscle mass endurance training, elicits the adaptations responsible for improvement of the peripheral gas exchanges; when sedentary subjects undertake this type of training, the enhancement of the peripheral factors lead to improvement of the whole body $\dot{V}O_{2max}$. By the same token, small muscle group endurance training can be applied with beneficial results in the clinical population where the peripheral gas exchanges are impaired due to the deconditioning/disuse, the side effects of medications and by the pathologies that directly affect skeletal muscles (Esposito et al., 2011). In fact, heart failure patients whom performed knee extension endurance training for 8 weeks improved muscle oxidative and O_2 diffusion capacities, which were paralleled not only by a greater muscle $\dot{V}O_{2max}$, but also by an increase of whole body $\dot{V}O_{2max}$ (Esposito et al., 2011; Tyni-Lenné et al., 1997; Tyni-Lenné et al., 1999). Indeed, in the studies where the effect of small muscle group endurance training on whole body $\dot{V}O_{2max}$ was investigated in several heart-failure subjects, the cardio-respiratory fitness was increased by ~24% (from 1.63 to 2.01 $L \cdot min^{-1}$ on average), ~12% (from 2.1 to 2.4 $L \cdot min^{-1}$ on average) and ~19% (from 1.31 to 1.57 $L \cdot min^{-1}$ on average) (Esposito et al., 2011; Munch et al., 2018; Tyni-Lenné et al.,

1999). Other investigations, implementing the aerobic training of small muscle group (i.e. knee extension exercise or single leg cycling) in clinical population and healthy elderly subjects, reported the positive effect on whole body $\dot{V}O_{2max}$ when tested on exercise carried out with large muscle mass (i.e. double leg cycling) (Bjørgen et al., 2009; Esposito et al., 2010; Gordon et al., 2019). In chronic obstructive pulmonary disease patients, whom were trained with the single leg cycling for 7 and 8 weeks, with 3 sessions per week, the whole body $\dot{V}O_{2max}$ was increased by ~12 (from 1.48 to 1.68 L·min-1 on average) and 22% (0.87 to 1.06 L·min-1 on average) (Bjørgen et al., 2009; Dolmage & Goldstein, 2008).

The effectiveness of endurance exercise training for the amelioration of cardio-respiratory has been consistently demonstrated in organ transplanted subjects; however, the optimal exercise training modality has not yet been found. Moreover, the effect of small muscle mass endurance training has never been tested in HTx, KTx and LTx, in which the detrimental effect of immunosuppressive therapy and extreme deconditioning negatively impact the skeletal muscle inducing abnormalities altering the peripheral gas exchanges. As previously mentioned, $\dot{V}O_{2max}$ is mainly limited by $\dot{Q}_aO_{2max}$ (i.e. 70 to 80%) in healthy humans performing maximal whole body exercise at sea level, meanwhile the peripheral factors account to a minor, but still important, extent (di Prampero & Ferretti, 1990; Ferretti, 2014). However, in clinical population where the peripheral factors are impaired due to skeletal muscle abnormalities, such as HTx, LTx and KTx, this scenario can be different and the peripheral gas exchanges might substantially limit $\dot{V}O_{2max.}$

1.7) Aim of the thesis

Based on the premises presented in the introduction, clearly emerges that HTx, KTx and LTx commonly suffer from skeletal muscle defects. These deficiencies are likely to origin from the deleterious backwards elicited by the immunosuppressive treatments and the prolonged deconditioning/ disuse; in turn, these abnormalities alter the proper development of peripheral gas exchanges. Additionally, dynamic exercise training involving a small muscle mass produces remarkable adaptations in skeletal muscle characteristics responsible for the improvement of O_2 diffusion and utilization in healthy and clinical populations. In view of this, it can be hypothesized that i) the role of muscular factors in limiting the $\dot{V}O_{2max}$ might be remarkable in transplanted recipients and ii) endurance training performed with small muscle mass might be as beneficial as traditional whole-body endurance training if the aim is the enhancement of the $\dot{V}O_{2max}$ and the sub-maximal aerobic exercise related parameters in transplant recipients.

To the best of my knowledge, no investigations have been carried out comparing the effect of single leg cycling opposed to the traditional double-leg cycling training on the maximal and sub-maximal aerobic exercise parameters in HTx, KTx and LTx. Therefore, this thesis aimed to compare the responses on $\dot{V}O_{2max}$, maximal mechanical power, $\dot{V}O_2$ and power output at the first ventilator threshold, energy cost of cycling and

$\dot{V}O_2$ kinetics, tested on whole body cycling, after 8 weeks of single leg versus double leg endurance training in HTx, KTx, and LTx. Finally, the multifactorial model of $\dot{V}O_{2max}$ limitation is implemented to provide insight about the role of cardiovascular and local factors in limiting the cardio-respiratory fitness in this class of patients.

CHAPTER II

Experimental part

Aim of chapter II

1) The first study related to this section deals with the effect of small vs large muscle mass endurance training on the cardiorespiratory fitness in SOT and the possible role of central and peripheral factors in limiting $\dot{V}O_{2max}$ in these patients. The impaired cardiorespiratory fitness in SOT herald the presence of deficiencies in one or more steps of the oxygen pathway. Several pieces of evidence showed that metabolic detrimental alterations affect the skeletal muscle of these patients, namely defects of the mitochondrial oxidative capacity. The causesof this condition can be ascribed to the chronic immunosuppressive (i.e. cyclosporine) intake and the deconditioning/disuse. Regarding the pharmacological therapy, animal studies showed that the immunosuppressive (i.e. cyclosporine) use causes deleterious effects on the oxidative apparatus of skeletal muscle, resulting in a poor endurance exercise capacity. These muscular factors are essential for the proper peripheral O_2 diffusion and utilisation, which in turns are key components that determine a fraction of the whole-body $\dot{V}O_{2max}$. It is noteworthy that endurance training involving a small muscle mass (i.e. single leg cycling) elicits greater adaptations in skeletal muscle characteristics, enhancing the O_2 utilization, if compared to exercises carried out with large muscle masses (i.e. double leg cycling). In the light of this premise, it can be hypothesised that single leg cycling endurance training can be as effective as double leg cycling if the aim is to enhance the $\dot{V}O_{2max}$ in SOT. Therefore, a group of SOT (HTx, KTx and LTx) were recruited for the current investigation. Firstly, the enrolled subjects performed an incremental double leg cycling test to exhaustion in order to assess the whole-body $\dot{V}O_{2max}$, the maximal systemic O_2 delivery and the maximal systemic O_2 extraction. Then, the patients were divided into two training groups to participate in two different endurance training protocols. The single leg cycling group performed high intensity interval training cycling with only one leg the first half of the training session and with the other leg the other half. The double leg cycling group performed the entire high intensity interval training cycling with both leg

simultaneously. After24 training sessions, the enrolled patients were asked to repeat the very same battery test, carried out at the beginning of the study, in order to assess the amelioration in the $\dot{V}O_{2max}$ and in the factors that contributes toits improvement.

2) The second study related to this section deals with the effect of small vs large muscle mass endurance training on the pulmonary $\dot{V}O_2$ kinetics during moderate intensity exercise and indexes of pulmonary $\dot{V}O_2$ slow component ($\dot{V}O_{2SC}$) during heavy intensity exercise in SOT. As previously mentioned, the whole-body $\dot{V}O_{2max}$ is impaired in HTx, KTx and LTx if compared to healthy age- and sex- matched subjects and this is of note since the $\dot{V}O_{2max}$ is a strong predictor of cardiovascular mortality. However, the most common daily activities are carried out at submaximal intensities and require continuous transitions from rest to exercise and/or to oneexercise intensity to another one. In these conditions (i.e. transient exercises) the rate of pulmonary O_2 uptakeadjustment from rest to a level imposed by the external workload directly affects muscular metabolic stability. Indeed, in healthy subjects and in normoxia, the speed of pulmonary O_2 uptake kinetics, at the exercise onset, likely represents the rate of adjustment of the muscles' oxidative metabolism to the imposed work rate; that, in turn, is presumably set by the rate of muscle O_2 utilisation. In addition, when pathological conditions affect the oxidative metabolism of skeletal muscle, the pulmonary O_2 uptake kinetics became prolonged, heralding poor exercise tolerance and, likely, limiting physical performance. As previously showed, transplanted recipients suffer from skeletal muscle abnormalities that affect the peripheral O_2 utilization and these defects might be responsible for a slower rate of adjustment of the muscles' oxidative metabolism resulting in slower pulmonary O_2 uptake kinetics. As a matter of fact, slow pulmonary O_2 uptake kinetics have been reported for transplanted patients and these studies pointed out the role of peripheral O_2 utilization as the major

culprit for the impaired pulmonary O_2 uptake kinetics. Single leg cycling, when compared to double leg cycling, elicits greater adaptations for the factors affecting peripheral gas exchanges, enhancing the muscle O_2 utilization. Therefore, the effect of single vs double leg cycling on the pulmonary $\dot{V}O_2$ kinetics were evaluated in HTx, KTx and LTx. In the light of this premise, it can be hypothesised that single leg cycling endurance training can be as effective as double leg cycling if the aim is to improve the pulmonary $\dot{V}O_2$ uptake kinetics during moderate and heavy intensity exercise. As detailed before, the recruited HTx, KTx and LTx were divided in two training groups (i.e. single vs double leg endurance training) and performed a double-leg cycling incremental test, two repetitions of double-leg moderate constant load exercise (DL-MOD) and a double-leg heavy constant work rate cycling (DL-HVY) exercise. After the training intervention (see *Aim of chapter II, paragraph 1*) the very same constant work rate tests (i.e. at the same mechanical power used for the tests before the exercise program) were carried out to evaluate the changes in the pulmonary $\dot{V}O_2$ kinetics.

Main results of chapter II

1) The results regarding the first investigation showed that both groups significantly increased their whole- body $\dot{V}O_{2max}$; however, the data indicates that the mechanisms leading to the improved whole body-$\dot{V}O_{2max}$ might differ between the two training groups. In fact, the single leg cycling group increased the whole body-$\dot{V}O_{2max}$ by 13.8 % ± 8.7 ($p < 0.001$) thanks to a larger maximal O_2 systemic extraction, meanwhile, $\dot{V}O_{2max}$ in the double leg cycling group increased by 18.6 % ± 12.7 ($p < 0.001$) because of concomitant central and peripheral adaptations. Furthermore, by applying the multifactorial model of whole body-$\dot{V}O_{2max}$ limitation, an attempt was made to quantify the relative weights of the mechanisms behind the whole body-$\dot{V}O_{2max}$ improvement induced by the two different training typologies. The multifactorial model suggested (confirming the former results) that

the single leg cycling group ameliorated the whole body-$\dot{V}O_{2max}$ because of the larger maximal O_2 systemic extraction; meanwhile, double leg cycling group increased the $\dot{V}O_{2max}$ mainly thanks to the improvement of central factors.

2) After the training program, both groups significantly decreased the O_2 deficit (O_2Def) and the mean response time (MRT) during the DL-MOD; likewise, both the amplitude of the $\dot{V}O_{2}$sc (i.e. the difference of $\dot{V}O_2$, expressed in $mL \cdot min^{-1}$, between the 6^{th} and the 3^{rd} min of exercise) and the slope of the linear increase of $\dot{V}O_2$ as a function of time were significantly lower in the two groups. It is of note that the magnitude of the reduction of O_2Def, MRT and the indexes of $\dot{V}O_{2}$sc were not different between the two groups, indicating that single leg cycling is as effective as double leg cycling when the aim is to improve the pulmonary $\dot{V}O_2$ kinetics in HTx, KTx and LTx. HR kinetics were significantly speeded following the exercise program and no difference were found in the magnitude of the improvement between the two groups. It is not possible make inferences whether the ameliorations originated from an enhanced O_2 availability or an improved O_2 utilization at muscular level. In addition, the minute ventilation, the O_2 cost of respiratory muscles, the pulmonary $\dot{V}O_2$, the heart rate and the peak blood lactate concentration were all significantly reduced at the end of DL-HVY after the training program in both groups and without difference between them.

Effect of small vs large muscle mass endurance training on maximal oxygen uptake in organ transplanted recipients

ALESSIO DEL TORTO[1,2], CARLO CAPELLI[3], ROBERTO PERESSUTTI[4], ADRIANA DI SILVESTRE[4], UGOLINO LIVI[1,5], CHIARA NALLI[1,5], SANDRO SPONGA[1,5], GIANPAOLO AMICI[6], UMBERTO BACCARANI[1,7], STEFANO LAZZER[1,2]

[1]Department of Medicine, University of Udine, Udine, ITALY; [2]School of Sport Sciences, University of Udine, Udine, ITALY; [3]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, ITALY; [4]Regional Transplantation Centre, Friuli Venezia Giulia Region, Udine, ITALY; [5] Cardiac Surgery Unit, University Hospital of Udine, ITALY; [6]Nephrology and Dialysis Unit, San Daniele del Friuli Hospital, Udine, ITALY; [7]Liver-Kidney Transplant Unit, University Hospital of Udine, Udine, ITALY

Corresponding Author:

Dr Alessio del Torto

Department of Medicine, University of Udine

P.le Kolbe 4 – 33100. Udine (Italy)

e-mail: alessio.deltorto12@gmail.com

ABSTRACT

Maximal oxygen consumption ($\dot{V}O_{2max}$) is impaired in heart (HTx), kidney (KTx), and liver (LTx) transplanted recipients and the contribution of the cardiovascular, central, and muscular, peripheral, factors in affecting $\dot{V}O_{2max}$ improvement after endurance training (ET) has never been quantified in these patients. ET protocols involving single leg cycling (SL) elicit larger improvements of the peripheral factors affecting O_2 diffusion and utilization than the double leg (DL) cycling ET. Therefore, this study aimed to compare the effects on $\dot{V}O_{2max}$ of SL-ET vs DL-ET. We determined the DL-$\dot{V}O_{2max}$ and maximal cardiac output before and after 24 SL-ET vs DL-ET sessions on 33 patients (HTx = 13, KTx = 11 and LTx = 9). The DL-$\dot{V}O_{2max}$ increased by 13.8% ± 8.7 ($p<0.001$) following the SL-ET, due to a larger maximal O_2 systemic extraction, meanwhile, $\dot{V}O_{2max}$ in DL-ET increased by 18.6% ± 12.7 ($p<0.001$) because of concomitant central and peripheral adaptations. We speculate that in transplanted recipients, SL-ET is as effective as DL-ET to improve $\dot{V}O_{2max}$ and that the impaired peripheral O_2 extraction and/or utilization play an important role in limiting $\dot{V}O_{2max}$ in this type of patients.

Key Words: solid organ transplant, single leg cycling, endurance training, small muscle mass, limiting factors, systemic oxygen extraction

Novelty

- SL-ET increases $\dot{V}O_{2max}$ in transplanted recipients because of improved peripheral O_2 extraction and/or utilization.
- SL-ET is as successful as DL-ET to improve the cardiorespiratory fitness in transplanted recipients.
- The model of $\dot{V}O_{2max}$ limitation indicates the peripheral factors as a remarkable limitation to the $\dot{V}O_{2max}$ in these patient.

INTRODUCTION

Heart, kidney, and liver transplant recipients (HTx, KTx, and LTx, respectively) suffer from reduced cardiorespiratory fitness compared to healthy sedentary peers: their maximal oxygen uptakes ($\dot{V}O_{2max}$) amount to 50 - 80 % of the ones predicted for age and sex (Williams and McKenna, 2012) and they are also hardly improved by endurance training (ET) (Kempeneers *et al.*, 1990; Herman *et al.*, 2011; Williams and McKenna, 2012).

In healthy individuals, $\dot{V}O_{2max}$ during exercise performed at sea level with large muscle masses (i.e., cycling and running) is determined by the interplay of central (i.e., the maximal systemic oxygen delivery, $\dot{Q}_aO_{2max}$) and peripheral factors (i.e. oxygen diffusion and utilisation). By applying a mechanistic model of the limiting factors of $\dot{V}O_{2max}$, it has been estimated, f.i., that $\dot{Q}_aO_{2max}$ imposes approximately 70 % of the total fractional limitation of $\dot{V}O_{2max}$, whereas the peripheral factors account for the remaining one (di Prampero and Ferretti, 1990).

However, previous studies suggested a remarkable contribution of the peripheral, muscular factors to the impairment of $\dot{V}O_{2max}$ in transplant recipients and other clinical populations (Esposito et al., 2011; Williams and McKenna, 2012; Gea et al., 2013). Skeletal muscle abnormalities are commonly reported in these patients and metabolic dysfunction and muscle fibre type alterations persisted in KTx after six months of aerobic training (Kempeneers *et al.*, 1990). Similarly, muscle myopathies, a reduction in capillary density, length density and endothelial dysfunctional were found in HTx (Lampert *et al.*, 1998; Braith *et al.*, 2005; Herman *et al.*, 2011; Williams and McKenna, 2012). In addition, a strong association was found in KTx between the poor $\dot{V}O_{2peak}$ and quadriceps muscle strength, suggesting that diminished leg muscle mass might contribute to the $\dot{V}O_{2peak}$ impairment (Van Den Ham *et al.*, 2005). Considering LTx, sarcopenia is a common feature in these patients (Williams and McKenna, 2012; Kallwitz, 2015) and it was recently demonstrated that this condition is characterized by mitochondrial bioenergetics dysfunction impairing the oxidative metabolism of

skeletal muscles (Migliavacca et al., 2019). Indeed, LTx, reported a greater perceived exertion rating for leg pain ($RPE_{leg\ fatigue}$) than dyspnoea ($RPE_{dyspnoea}$), while no cardio-respiratory impaired response during exercise was evident (Stephenson et al., 2001). A limitation of peripheral origin was finally suggested in sedentary (Borrelli et al., 2003) and in well-trained KTx and HTx (Richard et al., 2005) because of their increased energy cost of running in comparison to healthy counterparts. Therefore, these findings strongly suggest that skeletal muscle defects can be present in these patients.

It is noteworthy that HTx, KTx and LTx also exhibit some common sequelae, namely, i) the usage of immunosuppressive drugs that negatively affect skeletal muscles (Hokanson et al., 1995; Mercier et al., 1995) and ii) disuse/deconditioning that can worsen fitness eliciting ominous detraining adaptations both at the central and peripheral level (Ferretti *et al.*, 1997; Capelli *et al.*, 2006).

An insightful approach applied to investigating the role of peripheral factors in limiting $\dot{V}O_{2max}$ consists in using dynamic exercises performed with a small muscle mass [i.e., knee extension exercises and single-leg (SL) cycling] (Ferretti, 2014). When exercise is performed with a reduced muscle mass , the majority of cardiac output ($\dot{Q}$) is directed to the involved tissue and the relative $\dot{V}O_{2max}$ increases 2–3 times more than the $\dot{V}O_{2max}$ normalized per kg of body mass assessed during whole-body maximal exercise (MacInnis et al., 2017; Iannetta et al., 2019). A greater mass-specific blood flow and a higher O_2 delivery and availability will fully sustain the oxidative metabolism of the active muscle mass, meaning that the oxidative capacity responsible for the aerobic ATP re-synthesis would be fully exploited.

Since we know that: a) muscular abnormalities and sarcopenia are commonly found in transplanted recipients, and; b) ET performed with a small muscle mass results in substantial adaptations in skeletal muscle characteristics, leading to a larger O_2 utilization (Esposito et al 2011; Klausen et al., 1981; Rud et al., 2012), we can hypothesize that this type of training might be as effective as traditional whole-body ET if we aim to increase their cardio-respiratory fitness.

To our knowledge, no study has evaluated the effect of SL versus traditional double-leg (DL) cycling on DL-$\dot{V}O_{2max}$ and DL maximal power output (PO_{max}) in HTx, KTx, and LTx. We aimed to compare the responses on DL-$\dot{V}O_{2max}$, $\dot{V}O_2$ at the gas exchanges threshold ($\dot{V}O_2$-GET) and DL-PO_{max} after 8 weeks of SL-ET versus DL-ET in HTx, KTx, and LTx under the hypothesis that training performed involving small muscular masses can be as effective as traditional double-leg exercise in patients where peripheral gas exchanges are impaired by multifactorial causes. Then, an attempt to determine the contributions of cardiovascular and local factors is proposed to better quantify the origin of the main limitation of $\dot{V}O_{2max}$ in transplanted recipients.

METHODS

Patients

Thirty-eight sedentary and clinically stable transplant recipients (i.e. not presenting conditions that that required hospitalization) (n: 14, HTx; n: 13, KTx; n: 11, LTx) were enrolled in the present study. The investigation was approved by the local Institutional Review Board (n: 8/IRB DAME) and was conducted in compliance with the principles of the Declaration of Helsinki. Before beginning the study, all patients underwent a full medical anamnesis and physical examination and were informed about the potential risks associated with the experiments; then, they gave their informed consent. Criteria for exclusion were pregnancy, cardiopulmonary diseases, cancer and orthopaedic conditions that might impede or contraindicate cycling exercise; moreover, patients were only included at least 1 year after transplantation. Before the beginning of the training period, 5 volunteers dropped out of the study: 2 because of orthopaedic injury, 1 for malignant diseases, and 2 for personal reasons; finally, 33 patients (male = 28; female = 5) participated in the study (HTx= 13, KTx= 11 and LTx= 9).

Study protocol

Incremental step exercise tests, including assessment of anthropometric and physical capacities, were performed two weeks before (PRE) and immediately after (POST) the completion of the ET. Before the study, participants were familiarized with the equipment and the procedures, and they were asked to avoid strenuous exercise the day before the experimental sessions and any caffeinated drinks (5 h). They also abstained from meal consumption in the 3 hours preceding the tests.

Patients came to the laboratory on two different days. During the first visit, after medical anamnesis and anthropometric measurements, all individuals performed the DL ramp cycling test (DL-INC); on the second day, all participants performed a SL ramp cycling test (SL-INC). The experimental sessions were separated by at least 2 days. Thereafter, the patients visited the laboratory twice to perform two cycling tests of moderate and heavy intensity in order to familiarize with the ranking of $RPE_{leg\ fatigue}$ and $RPE_{dyspnoea}$. After these supplementary familiarization trials, the 33 patients were randomly divided into SL and DL cycling endurance training groups ($SL\text{-}ET_{GRP}$ and $DL\text{-}ET_{GRP}$, respectively), 17 were randomly allocated to the $SL\text{-}ET_{GRP}$ (HTx= 6, KTx= 6 and LTx= 5) and 16 to the $DL\text{-}ET_{GRP}$ (HTx= 7, KTx= 5 and LTx= 4).

Anthropometric characteristics

Body mass was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany) with the patients dressed only in light underwear and no shoes. Stature was measured to the nearest 0.5 cm on a standardized wall-mounted height board. Body mass index was calculated as the ratio between body mass and the stature (m) squared.

Incremental exercise tests

The INC tests were conducted under medical supervision and monitoring the electrical activity of the heart with standard 12-lead ECG; the exercise test was interrupted if systolic blood pressure reached or exceeded 240 mmHg of and if abnormalities in the ECG trace were observed. After instrumentation, the patients sat on an electromechanically braked cycling ergometer (Ergomed 839E, Monark, Vansbro, Sweden); the handlebar and seat heights were properly adjusted and then kept constant for all exercise tests for each volunteer. Breath-by-breath $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$) and minute ventilation ($\dot{V}_E$) were continuously assessed using a metabolic unit (CPET, Cosmed, Italy); HR was obtained from the ECG signal. Before each experiment, O2 and CO2 analysers and the turbine flowmeter were calibrated by utilizing gas mixtures of a known composition (16.00 % O2, 4.00 % CO2, N2 as balance) and a 3 L syringe according to the indications of the producer. During DL-INC, after 3 min at rest, the patients pedalled for 6 min at 25 W or 40 W. Immediately after the baseline, a symptom-limited (leg fatigue or shortness of breath) DL-INC was performed, increasing the mechanical power by 15 $W \cdot min^{-1}$ until exhaustion. Participants were asked to keep a constant pedalling cadence, which was digitally displayed, at their preferred rate (60–75 RPM). They were asked to cycle until volitional exhaustion or until they were not capable to maintain the cadence above 60 rpm in spite of strong verbal encouragement. After exhaustion, the work rate was decreased to 25 W for 5 min to cool down. The rate of perceived exertion for leg fatigue ($RPE_{leg\ fatigue}$) and dyspnoea ($RPE_{dyspnoea}$) were registered ((Borg, 1982)). DL-$\dot{V}O_{2max}$, DL maximal $\dot{V}CO_2$ (DL-$\dot{V}CO_{2max}$), DL maximal $\dot{V}_E$ (DL-$\dot{V}_{Emax}$) and DL maximal HR (DL-HR_{max}) were determined as the highest average assessed in a 30 s epoch. Briefly, if during the last step of the DL- and SL-INC, a subject reached the exertion before the end of the stage, the corresponding PO_{max} was calculated as (Lepretre et al., 2004):

$$POmax = POf + [(t/60) \cdot 15]$$

where POf and t are the mechanical power (W) and the time (s) of the last, completed stage, respectively, and 15 is the PO increment in watt per minute. $\dot{V}O_2$-GET was assessed by two independent and trained researchers using the V-slope method; ventilatory equivalents ($\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$) and pressure end tidal (PET O_2 and PET CO_2) were used as ancillary criteria (Whipp et al., 1981). If agreement was not found between them, a third independent assessor applied the three methods and $\dot{V}O_2$-GET was determined only when the three investigators agreed on the results. Then, for each patient, the $\dot{V}O_2$ vs power relationship was left shifted by a fixed amount of time, 30sec, to calculate the mechanical power corresponding $\dot{V}O_2$-GET. Afterwards, for the supplementary familiarization trials, we calculated the 80% of the power associated to the $\dot{V}O_2$-GET and used for the 10 min constant load exercise; meanwhile for the 14 min heavy constant load exercise we calculated the power associated to the 40% of delta between gas exchange threshold and $\dot{V}O_{2max}$.

During SL-INC, participant's instrumentation and the testing protocol were identical to the one applied in the first visit with the exception of the work rate during the warm-up that was set to 20 W and the ramp increase of the workload was set to 7.5 $W \cdot min^{-1}$. As for DL-INC, maximal cardio-respiratory parameters were determined as the highest average measured in a 30 s epoch.

During SL the contralateral crank was fitted with a 10 kg counterweight and the participant's leg was placed on a wooden support on the floor so that the non-exercising limb was protected from the rotating counterweight (Abbiss et al., 2011).

Cardiovascular assessments during incremental exercises

HR, $\dot{Q}$, blood pressure and percent saturation of oxyhaemoglobin (S_aO_2) were assessed at rest and during the exercise trials. Beat-by-beat $\dot{Q}$ and HR were measured by using a modified cardio-impedance method by means of Physioflow™ (Manatec Biomed., France). This methodology was validated against an invasive, gold-standard method and is well described elsewhere (Charloux *et al.*, 2000). $\dot{Q}$ was calculated as stroke volume (SV) times HR derived for the ECG signal and maximal $\dot{Q}$ ($\dot{Q}_{max}$) was defined as the highest average measured in a 30 s epoch. Haemoglobin concentration ([Hb]) was determined by means of a photometric technique (**Hemo Control,** EKF Diagnostics, Germany) from an 8 µ capillary blood sample withdrawn from the fingertip at rest and S_aO_2 was measured via an infrared oximetry (Xpod, PFT/CPET, Cosmed, Italy). Maximal oxygen delivery, $\dot{Q}_aO_{2max}$ in $L \cdot min^{-1}$, was then computed as:

$$\dot{Q}_aO_{2max} = \dot{Q}_{max} \cdot S_aO_2 \cdot [Hb] \cdot \sigma \quad (1,$$

where σ indicates the physiological O_2 binding coefficient of haemoglobin, which is 1.34 $ml \cdot g^{-1}$, $\dot{Q}_{max}$ is expressed in $L \cdot min^{-1}$ and [Hb] in $mg \cdot dl^{-1}$". Moreover, maximal systemic artero-to-mixed venous blood differences in O_2 concentrations (a-$\bar{v}O_2diff_{max}$) and maximal systemic oxygen extraction ($O_{2extr\ max}$) were calculated as the ratio between $\dot{V}O_{2max}/\dot{Q}_{max}$ and $\dot{V}O_{2\ max}/\dot{Q}_aO_{2\ max}$, respectively.

Endurance training

Volunteers in the SL-ET and DL-ET groups started an 8-week cycling training programme with 3 supervised sessions per week. The training pattern consisted in high-intensity, interval training (HIIT), which has been shown to be a safe and feasible exercise mode for transplanted patients (Herman *et al.*, 2011). Two protocols of training were used, alternatively between each session, to increase participant

adherence to the training programs, as previously applied (Saltin et al., 1976; Skattebo et al., 2020). These two training protocols were reported to be effective in imporving the peak $\dot{V}O_2$ in SOT (Herman *et al.*, 2011; Nytrøen et al., 2012). Therefore, the patients underwent i) 12 ET sessions that included 4 min at high intensity followed by 3 min of active recovery; each bout was repeated 4 times and: ii) 12 ET consisting of 2 min at high intensity followed by 2 min of active recovery; in this case, each bout was repeated 6 times. Each HIIT training session was always preceded and followed by 5 min of warm-up and 5 min of cool down, respectively. The DL-ET group performed DL training for the whole session; conversely, the SL-ET group completed the first half of the training pedalling with one leg and the second half with the other leg. With this configuration, both groups trained for the same interval of time. Furthermore, patients in both DL-ET_{GRP} and SL-ET_{GRP} were asked to keep a fixed cadence, within the range of 60 to 70 RPM, during the ET sessions; hence the mechanical work performed by the skeletal muscle was similar for DL-ET and SL-ET.

For the SL exercise, the bikes were arranged as for SL-INC (see below). The workload imposed for the HIIT was set to elicit a value equal to or higher than 15 of $RPE_{dyspnoea}$ during the DL-ET (6-20 Borg's scale) and equivalent to or greater than 5 of $RPE_{leg\ fatigue}$ during the SL-ET (1-10 Borg's scale) (Buchheit and Laursen, 2013). The intensity of the active recovery was tailored to achieve a score equal to or lower than 12 on $RPE_{dyspnoea}$ and equivalent to or lower than 2 on $RPE_{leg\ fatigue}$ in the two exercise modalities, respectively. The training load was adjusted weekly; HR, $RPE_{dyspnoea}$ and $RPE_{leg\ fatigue}$ were continuously monitored during HIIT and during the active recovery phases. The utilization of the RPE (i.e. Borg's scale) was previously showed to be an effective criterion for high intensity exercise prescription (Ciolac et al., 2015; Gordon et al., 2019). For the $RPE_{leg\ fatigue}$ and for the $RPE_{dyspnoea}$ the 1-10 and 6-20 scales were used, respectively. HR was recorded throughout the training and HR values occurring in the last15 sec before the end of each interval of high and low intensity were averaged; $RPE_{leg\ fatigue}$ and $RPE_{dyspnoea}$ values were recorded at the end of each interval of high

and low intensity. Research assistants were responsible for verifying that each subject participated in each training session, performed the exercises correctly, and completed at least 90 % of the training sessions.

Statistics

Data were analysed with Prism, version 8.0 (GraphPad Software, La Jolla, CA, USA). Data in the text and tables are presented as the means ± standard deviation (SD), unless stated otherwise.

A Shapiro–Wilk test was used to verify the normal distribution of the data. The PRE and POST investigated cardiovascular and cardio-respiratory parameters were analysed using a two-way, within-subject ANOVA, with group (SL-ET and DL-ET) and time (PRE- and POST- training) as the two factors. If a significant main effect or interaction effect was found, a post hoc test (Bonferroni's multiple comparisons test) was performed. Alpha level was set to ≤ 0.05. An unpaired Student's t-test was utilized to assess differences between SL-ET and DL-ET prior to the training for the anthropometric parameters and to determine the feasibility of the comparison between HTx and KTx together with LTx. Variables measured during each training and the number of ET sessions performed by each group were analysed using unpaired Student's t-tests. Partial eta squared (ηp^2) was calculated as previously reported (Del Vecchio *et al.*, 2019); ηp^2 for the main effect of time, unless otherwise stated, and 95 % confidence intervals of the mean differences between the PRE and POST values were calculated and reported. The post-hoc analysis of the statistical power, calculated with the 33 participants and setting a size effect of 0.25 for the main outcome ($\dot{V}O_{2max}$), yielded a power value of 0.80 (G*Power).

RESULTS

Patient characteristics and the exercise training regimen

The main anthropometric characteristics, pharmacological therapies and the morbidities that led to organ transplants for the SL-ET_{GRP} and DL-ET_{GRP} are reported in Table 1 and Table 2, respectively. Volunteers participated in the 97.8 % (6.3) and 99.2 % (2.3) (P = 0.398) of the total exercise sessions for the SL-ET_{GRP} and DL-ET_{GRP}, respectively; no adverse events were reported. The DL-ET_{GRP} reported higher values for the $RPE_{dyspnoea}$ [16 (0.7)] than the SL-ET_{GRP} [14 (0.7), P < 0.001]; conversely, no differences were found for the $RPE_{leg\ fatigue}$ in the DL-ET_{GRP} and SL-ET_{GRP} [7 (0.7) and 7 (0.4), P = 0.826]. The two groups trained at the 91 % (7) and 87 % (11) (P = 0.269) of the HR_{max}, reached during the DL-INC, for the DL-ET_{GRP} and SL-ET_{GRP}, respectively. Data regarding the main respiratory and cardiovascular parameters for the DL-INC for the HTx vs. non-cardiac transplant recipients (i.e., KTx and LTx together; NCT), at PRE, are presented in the Supplementary Material, Table S1. HR was significantly lower in the HTx versus the NCT (P = 0.021) and SV was statistically greater in the NCT compared to HTx (P = 0.043) (Supplementary Material, Table S1). No differences were detected for the other variables between HTx and the NCT (Supplementary Material, Table S1).

Incremental exercise tests

The data related to DL-INC assessed in the PRE and POST sessions are presented in Table 3; the ones related to SL-INC in the two very same occasions are reported in Table 4 instead.

At PRE, only DL-HR_{max} was significantly lower in SL-ET (P = 0.041; Table 3), mainly because of the higher number of patients under β-blockade medications. DL-$\dot{V}O_{2max}$ was 74 % (16) and 77 % (21) of age and sex predicated values for DL-ET_{GRP} and SL-ET_{GRP}, respectively, highlighting an impaired cardio-respiratory fitness. $RPE_{dyspnoea}$ was 18 (1) and 19 (1) for DL-ET_{GRP} and SL-ET_{GRP}, respectively (P = 0.390), whereas $RPE_{leg\ fatigue}$ was 8 (1.0) and 9 (0.5), for DL-ET_{GRP} and SL-ET_{GRP},

respectively (P = 0.094). No arterial desaturation was evident at maximal exercise in SL-ET_{GRP} and DL-ET_{GRP} at PRE and POST.

At POST, absolute DL-$\dot{V}O_{2max}$ increased by 18.6 % (12.8) and 13.8 % (8.7) in DL-ET and SL-ET, respectively (P = < 0.001); DL-$\dot{V}O_{2max}$ relative to body mass improved by approximately 18.1 (12.7) % and 15.3 (9.0) % in DL-ET and SL-ET, respectively (P = < 0.001; Table 3). The ameliorations in both absolute and relative to body mass DL-$\dot{V}O_{2max}$ were not significantly different between the two groups (Table 3). Likewise, DL-PO_{max} increased significantly by 23.2 % (12.5) in DL-ET and by 19.7 % (11) in SL-ET (P ≤ 0.001), yet the changes were not significantly different between the two groups (Table 3). Similarly, $\dot{V}O_2$-GET was augmented by 15.3 % (11.8) and 15.2 % (7.8) in DL-ET and SL-ET, respectively, and was not significantly different between the two groups (Table 3). DL-HR_{max} and [Hb] did not significantly change in response to the exercise training programme in both groups (Table 3).

DL $\dot{Q}aO_{2max}$ augmented by 12.2 % (19.1) and diminished by 3.7 % (15.1) in DL-ET and SL-ET, respectively; however, it was not significantly different from PRE to POST. However, the interaction effect tended to be significantly different between the two groups (P = 0.053; Table 3) and this indicated that the effect of training on maximal oxygen delivery diverged in the two types of intervention.

DL $O_{2extr\text{-}max}$ was significantly improved in SL-ET, increasing by 19.4 (17.5) % (P = 0.009), while in DL-ET the amelioration of DL $O_{2extr\text{-}max}$ by 7.6 (20.2) % was not significant (P = 0.767); despite the greater amelioration was reported for the SL-ET, the interaction effect was not significantly different (P = 0.117; Table 3).

Likewise, (a-$\bar{v}O_2$diff)$_{max}$ turned out to be significantly augmented by 17.6 % (20.4) only in SL ET; it remained unchanged whereas it marginally increased by 9.1 % (17.7) in the DL-ET group.

These data support the view that the increase of $\dot{Q}aO_{2max}$ justified about 50% of the observed increase of $\dot{V}O_{2max}$ in DL-ET and the amelioration of the peripheral gas exchanges accounted for the remaining per cent increase. Conversely, the same data collected in the SL-ET seems to indicate that the better O_2 utilisation, revealed by the amplification of $(a\text{-}\bar{v}O_2diff)_{max}$, accounted for about the total percent increase of $\dot{V}O_{2max}$.

SL-$\dot{V}O_{2max}$ significantly increased ($P \leq 0.001$; Table 4) in DL-ET and SL-ET, without differences between the groups; likewise, SL-PO_{max} increased ($P \leq 0.001$; Table 4) in DL-ET and SL-ET, without differences between the groups.

DISCUSSION

In the present study, we investigated the effects of 8 weeks of SL-ET and DL-ET on DL-$\dot{V}O_{2max}$ in solid organ transplanted recipients. Furthermore, we aimed to understand the main mechanisms behind the different responses induced by SL-ET and DL-ET on DL-$\dot{V}O_{2max}$. Besides, by applying a multifactorial model of $\dot{V}O_{2max}$ limitation (di Prampero and Ferreti, 1990) we tried to quantify the relative roles of the improvements of the maximal, systemic O2 delivery and of peripheral gas exchanges in increasing DL-$\dot{V}O_{2max}$

The main results revealed that SL-ET was an effective exercise modality for increasing $\dot{V}O_{2max}$ during DL cycling, while both training modalities led to significant increases of DL-$\dot{V}O_{2max}$, DL-PO_{max}, SL-$\dot{V}O_{2max}$ and SL-PO_{max}.

The intensities for the training sessions were prescribed based on RPE, which is an effective criterion for high intensity exercise prescription (Ciolac *et al.*, 2015; Gordon *et al.*, 2019). For the DL-ET, the rated effort during exercise sessions based on $RPE_{dyspnoea}$ was 16 (0.7) and for $SL\text{-}ET_{GRP}$ it was 14 (0.7) ($P \leq 0.001$), suggesting that the central factors related to endurance performance were stressed more in DL-ET than in SL-ET, as expected (Bassett and Howley, 2000). On the other hand, no

difference was found for the $RPE_{leg\ fatigue}$ recorded during the training sessions, 7 (0.7) and 7 (0.4) (P= 0.826) for DL-ET_{GRP} and SL-ET_{GRP}, respectively.

Previous studies demonstrated the effectiveness of exercise training in increasing cardio-respiratory fitness and exercise capabilities in solid organ transplanted recipients (Herman *et al.*, 2011; Riess *et al.*, 2014; Moya-Nájera *et al.*, 2017). Thus, the achievement of a higher DL-$\dot{V}O_{2max}$ following the training period in SL-ET and DL-ET agrees with the findings reported by other studies. Other investigators who implemented aerobic training of small muscle mass in clinical populations (Bjørgen et al., 2009; Dolmage & Goldstein, 2008; Esposito et al., 2011; Munch et al., 2018; Tyni-Lenné et al., 1999) and in healthy elderly individuals (Gordon *et al.*, 2019) reported positive effects on DL-$\dot{V}O_{2max}$ similar to the ones obtained in the present study.

Previous investigations on trained participants reported improved muscle oxidative and O_2 diffusion capacities, leading to a greater O_{2extr} of the conditioned limb, after a training period of SL or knee extension exercise (Rud et al., 2012; Skattebo et al., 2020). These ameliorations resulted in an increase of muscle $\dot{V}O_{2max}$ of the trained leg, but not of DL-$\dot{V}O_{2max}$ (Rud et al., 2012; Skattebo et al., 2020). Conversely, heart failure patients who exercised with knee extension exercise for 8 weeks improved their muscle oxidative and O_2 diffusion capacities, which were paralleled by a greater muscle $\dot{V}O_{2max}$, and also by improved DL-$\dot{V}O_{2max}$ (Esposito et al., 2011; Tyni-Lenné et al., 1997; Tyni-Lenné et al., 1999). Considering that SL and knee extension exercise impacts mainly on the peripheral factors involved in O_2 transport and utilization, the higher DL $O_{2extr-max}$ estimated in our study in SL-ET_{GRP} may likely origin from the amelioration of these muscular factors. Our findings seems therefore to confirm that when peripheral gas exchanges are negatively affected by systemic diseases (Esposito *et al.*, 2011), medication (Hokanson et al., 1995; Mercier et al., 1995) and disuse (Capelli *et al.*, 2006; Bringard *et al.*, 2010), the maximal aerobic capacity is limited to a larger extent by peripheral factors than in healthy or/and trained individuals. The DL-ET improved DL-$\dot{V}O_{2max}$ with no significant

difference from SL-ET. However, the results indicate that the mechanisms behind the improvements might differ between the groups.

DL $\dot{Q}aO_{2max}$ was unchanged in SL-ET_{GRP} and, despite non significantly, it was 12.2 % higher in DL-ET_{GRP} and the interaction between groups and time clearly indicated that the effect of the intervention as for DL $\dot{Q}aO_{2max}$ was different in the two training modalities. On the other hand, the DL $O_{2extr-max}$ was significantly increased only in the SL-ET_{GRP} and it was non-significantly larger (7.6 %) than at PRE in the DL-ET_{GRP}; the interaction effect tended to be different, indicating a greater adaptive response at the muscular level in the SL training group. By applying the multifactorial model of $\dot{V}O_{2max}$ limitations (di Prampero and Ferretti, 1990) we are tentatively trying to quantify the relative weights of the mechanisms behind the DL-$\dot{V}O_{2max}$ improvement induced by the SL-ET and DL-ET (see paragraph *"The nature of double leg $\dot{V}O_{2max}$ limitations: the increasing influence of skeletal muscle"*).

Thereafter, the absence of sedentary age-matched control group a prevented us to identify if DL-ET or SL-ET might cause different adaptive responses between transplanted recipients and age-matched healthy individuals. However, the adaptations induced by ET have been well and extensively described elsewhere for healthy individuals (Bassett and Howley, 2000); moreover, the central and peripheral changes that are commonly caused by ET involving large muscle masses are similar to the ameliorations observed for our DL-ET_{GRP}. In addition, previous studies reported that a small muscle mass-ET was effective to increase DL-$\dot{V}O_{2max}$ and improve the factors affecting the peripheral gas exchanges of the trained limbs in sedentary, healthy, young and old individuals (Klausen, Andersen and Pelle, 1981; Bell *et al.*, 2001; Munch *et al.*, 2018). These findings are in line with the favourable changes observed for our SL-ET_{GRP}.

The nature of double leg $\dot{V}O_{2max}$ limitations: the increasing influence of skeletal muscle

The role of central and peripheral changes in determining the observed differences of DL-$\dot{V}O_{2max}$ in the two groups may be quantified in light of the multifactorial model of $\dot{V}O_{2max}$ limitations proposed by di Prampero and Ferretti (di Prampero and Ferretti, 1990). Other studies applied this line of reasoning in order to disclose the role of cardiovascular and/or muscular modifications on $\dot{V}O_{2max}$ after disuse (Capelli *et al.*, 2006; Bringard *et al.*, 2010). The reader is referred to the Appendix for the model's explanation.

The increase of $\dot{Q}_aO_{2max}$ induced by training translates into a reduced cardiovascular resistance to the O_2 flow (R_Q) at $\dot{V}O_{2max}$. Fig. 1, panel A, shows the model applied to the DL-ET. In the diagram, the ratio between DL-$\dot{V}O_{2max}$ at PRE and DL-$\dot{V}O_{2max}$ at POST is plotted as a function of $R_Q/\Delta R_Q$. In the same figure, the line with an intercept of 1 and a slope = to 0.70 (F_Q, i.e., the fractional limitation of $\dot{V}O_{2max}$ imposed by the cardiovascular system) resulting from the algebraic development of the model in the case of acute changes of only $\dot{Q}_aO_{2max}$, is also shown (di Prampero and Ferretti, 1990). With this representation, should only the cardiovascular resistance (R_Q) be changed in our patients, the resulting modification of $\dot{V}O_{2max}$ would be described by a linear relationship with slope equal to $F_Q = 0.70$.

Accordingly, the points describing the amelioration from the PRE to the POST conditions in DL-ET, although not symmetrically distributed around the theoretical line, are characterized by a residuals distribution (i.e., the differences between the experimental data and the ones calculated on the basis of a fixed value of $F_Q = 0.70$), with a mean not significantly different from 0 ($P = 0.689$). In the case of SL-ET, it appears that the residuals are not normally distributed and have a mean significantly different from zero ($P = 0.024$) (Fig. 1, panel B).

This suggests that, in the former case, the changes in R_Q were the main factor underpinning the observed improvements of DL-$\dot{V}O_{2max}$; in the latter case, in contrast, the observed amelioration of $\dot{V}O_{2max}$ after SL-ET were larger than the ones predicted by the sole modification of R_Q. Hence, we may

assume that additional changes in other resistances (e.g., peripheral - muscular resistance, R_m) may be responsible for the additional improvement of $\dot{V}O_{2max}$. Considering that no muscle biopsies were performed, it is not possible to disentangle which of the several steps affecting peripheral oxygen diffusion and utilization were the main ones responsible for the improvement of $\dot{V}O_{2max}$; we may only suggest that the SL-ET was able to elicit an improvement of the peripheral gas exchanges and O_2 utilisation.

Moreover, by following the lines of reasoning applied in the past for similar purposes, we tentatively calculated the relative change of R_m responsible of the amelioration in DL-$\dot{V}O_{2max}$ elicited by the two of training interventions under the assumption of a fractional cardiovascular limitation of 0,7 (see Appendix) (Capelli *et al.*, 2006; Bringard *et al.*, 2010). The results showed that in SL-ET $\Delta R_m/R_m$ decrease, on the average, by about 50 % in comparison with a drop of less than 30 % in DL-ET. This confirms the larger contribution of the beneficial peripheral adaptations to the observed increase of $\dot{V}O_{2max}$ in SL-ET and also suggests that muscular, peripheral factors account for a substantial limitation to the maximal aerobic power in HTx, KTx and LTx, which can be however effectively reversed with large muscle masses training.

Limitations of the study

A primary limitation is the lack of a verification phase to assess whether a true $\dot{V}O_{2max}$ was attained at exhaustion. A bout of supramaximal constant load exercise has been suggested in order to confirm the $\dot{V}O_{2max}$ (Poole and Jones, 2017) and should be implemented when possible. Even if contrasting findings have indicated that the verification phase is not supported by the experimental data (Green & Askew, 2018; Murias et al., 2018; Iannetta et al., 2020; Possamai et al., 2020; Wagner et al., 2021), and no studies have investigated the validity and feasibility of the verification phase in transplanted patients, a potential underestimation of $\dot{V}O_{2max}$ should not be excluded in our patients as

additional, unforeseen conditions may have prevented the participants from attaining $\dot{V}O_{2max}$. Other obvious limitations consist in the absence of muscle biopsies before and after training. This of course prevented us to evaluate the extents of any morphometric and/or biochemical defect that would have impaired muscular O_2 delivery, diffusion and utilisation and to describe in an invasive, quantitative manner the modifications undergone by these features. The absence of any gold-standard measurement of cardiac output is another important limitation. Besides, an additional limitation is the heterogeneity of the transplanted patients: individuals with different types of transplantation were grouped together. This allowed us to achieve a sufficient statistical power and improve the generalizability of the findings, but of course inserted some unpredictable bias and noise in the results. For instance, it is likely that the cardiovascular response to exercise was dissimilar between the heart and the solid organs transplanted patients.

Finally, also the absence of sedentary age-matched control group a prevented us from identifying if DL-ET or SL-ET might cause different adaptive responses between transplanted recipients and age-matched healthy participants. However, the adaptations induced by ET have been well and extensively described elsewhere for healthy volunteers (Bassett and Howley, 2000); and previous studies reported that a small muscle mass-ET was effective to increase DL-$\dot{V}O_{2max}$ and improve the factors affecting the peripheral gas exchanges of the trained limbs in sedentary, healthy, young and old individuals (Klausen et al., 1981; Bell et al., 2001; Munch et al., 2018).

CONCLUSION

8 weeks of DL-ET and SL-ET significantly improved DL-$\dot{V}O_{2max}$ and DL-PO_{max} without any differences between the training groups. Therefore, our results indicate that training by exercising with a small muscle mass may represents an effective and useful modality for improving exercise capacity in transplant recipients. Furthermore, the results suggest that the limitation of DL-$\dot{V}O_{2max}$ is equally

distributed between central and peripheral factors in HTx, KTx, and LTx, and that impaired peripheral gas exchanges might play a more relevant role in decreasing maximal exercise capacity in this type of patients. These findings may pave the path leading to develop effective and criterion-based training protocols for post transplantation patients.

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CONFLICT OF INTEREST The authors declare no conflict of interest, financial or otherwise.

Figure captions

Fig. 1, panel A Changes in double leg maximal oxygen intake, expressed as the ratio between the value pre- (DL-$\dot{V}O_{2max}$) and post- (DL-$\dot{V}O_{2max}$ +Δ) in the double leg cycling endurance training group (DL-ET_{GRP}) reported as a function of the relative change in the cardiovascular resistance to oxygen flow (ΔRQ_aO_2/ RQ_aO_2) on the x-axis. Represented in the figure, the theoretical dashed line, with a slope of 0.7 is derived from di Prampero and Ferretti (1990) for human exercising at sea level

Fig. 1, panel B Changes in double leg maximal oxygen intake, expressed as the ratio between the value pre- (DL-$\dot{V}O_{2max}$) and post- (DL-$\dot{V}O_{2max}$ +Δ) in the single leg endurance training group (SL-ET_{GRP}) reported as a function of the relative adaptation in the cardiovascular resistance to oxygen flow (ΔRQ_aO_2/ RQ_aO_2) on the x-axis. Represented in the figure, the theoretical dashed line, with a slope of 0.7 is derived from di Prampero and Ferretti (1990) for human exercising at sea level

Table 1: Main anthropometric parameters at PRE and pharmacological therapies

Anthropometrics	SL-ET_{GRP} (n: 17)	DL-ET_{GRP} (n: 16)	P
Age (years)	56 (10)	55 (10)	0.82
BM (Kg)	83 (15)	78(18)	0.35
BMI	26.7 (3.3)	26.3 (5.3)	0.76
Years post-transplant	6.2 (6.9)	8.9 (7.7)	0.30
Medications			
Immunosuppressant	17 (100%)	16 (100%)	
Corticosteroids	7 (41%)	4 (25%)	
NSAID	10 (59%)	9 (56%)	
ACE-inhibitors	2 (12%)	1 (6%)	
Angiotensin 2 receptor blockers	4 (23%)	1 (6%)	
α-blockers	6 (35%)	4 (25%)	
β-blockers	11 (65%)	4 (25%)	
Diuretics	3 (18%)	2 (13%)	
Calcium channel blockers	4 (23%)	3 (19%)	
Statins	6 (35%)	3 (19%)	
Lipid lowering agents	2 (12%)	0 (0%)	
Metformin	1 (6%)	1 (6%)	
Insulin	1 (6%)	0 (0%)	
Thyroid hormones	1 (6%)	4 (25%)	
Proton pump inhibitors	10 (59%)	6 (38%)	
Xanthine oxidase inhibitors	3 (18%)	4 (25%)	
Hypouricemic agents	7 (41%)	5 (31%)	
Kinase inhibitor agents	1 (6%)	2 (13%)	
Bisphosphonates	1 (6%)	1 (6%)	
Dopamine agonists	1 (6%)	0 (0%)	
Bronchodilators	1 (6%)	0 (0%)	
Antigout agents	1 (6%)	1 (6%)	
Antiarrhythmic agents	1 (6%)	0 (0%)	

Non-steroidal anti-inflammatory drugs (NSAID), dingle leg endurance training group (SL-ET_{GRP}), Double leg endurance training group (DL-ET_{GRP}), bod mass (BM), body mass index (BMI)

Table 2: List of diseases leading to the organ transplant

SL-ET_{GRP} (HTx, n= 6; KTx, n= 6; LTx, n= 5)				DL-ET_{GRP} (HTx, n= 7; KTx, n= 5; LTx, n= 4)			
Disease	HTx	KTx	LTx	**Disease**	HTx	KTx	LTx
Cardiac sarcoidosis	1 of 6	-	-	Primitive cardiomyopathy	4 of 7	-	-
Ischemic cardiomyopathy	4 of 6	-	-	Ischemic cardiomyopathy	2 of 7	-	-
Myocarditis	1 of 6	-	-	Infiltrative cardiomyopathy	1 of 7	-	-
Glomerulonephritis	-	1 of 6	-	Alport syndrome	-	1 of 5	-
Berger's disease	-	1 of 6	-	Polycystic kidney	-	3 of 5	-
Chronic kidney failure	-	1 of 6	-	Glomerulonephritis	-	1 of 5	-
Polycystic kidney	-	2 of 6	-	Primitive sclerosing cholangitis	-	-	1 of 4
Goodpasture syndrome	-	1 of 6	-	α-1 antitrypsin deficiency disease	-	-	1 of 4
Hepatitis C	-	-	3 of 5	Hepatitis B	-	-	1 of 4
Hepatic cirrhosis	-	-	2 of 5	Hepatic cirrhosis	-	-	1 of 4

Single leg endurance group (SL-ET_{GRP}), double leg endurance group (DL-ET_{GRP}), heart transplant recipients (HTx), kidney transplant recipients (KTx), liver transplant recipients (LTx).

Table 3: Main cardio-respiratory and cardiovascular variables assessed during double leg cycling incremental test (DL-INC) before (PRE) and after (POST) endurance training period. Single leg cycling endurance training group ($SL\text{-}ET_{GRP}$) and double leg cycling endurance training group ($DL\text{-}ET_{GRP}$) groups.

	$SL\text{-}ET_{GRP}$ (n= 17)			$DL\text{-}ET_{GRP}$ (n= 16)			Effect size	*P* values*		
Parameters DL-INC	**PRE**	**POST**	**Mean difference (95% CI)**	**PRE**	**POST**	**Mean difference (95% CI)**	**ηp^2**	**G**	**T**	**G x T**
$\dot{V}O_{2max}$ ($ml \cdot min^{-1}$)	1747 (420)	1966 (407)†	219 (133; 305)	1719 (483)	2001 (430)†	282 (193; 371)	0.75	0.981	<0.001	0.237
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	21.8 (7.7)	24.7 (7.9)†	2.9 (1.3; 3.8)	22.2 (4.7)	25.9 (4.7)†	3.7 (2.5; 4.9)	0.65	0.733	<0.001	0.342
$\dot{V}_{Emax}$ ($L \cdot min^{-1}$)	78.6 (16.9)	95.2 (22.6)†	16.6 (9.1; 24.2)	77.5 (21.3)	94.1 (18.1)†	16.6 (8.8; 24.4)	0.63	0.875	<0.001	0.995
$\dot{V}O_2$ GET ($ml \cdot min^{-1}$)	1311 (216)	1507 (255)†	196 (129; 261)	1275 (290)	1468 (318)†	186 (117; 255)	0.74	0.536	<0.001	0.803
Hgb ($mg \cdot dL^{-1}$)	14.5 (1.5)	14.3 (1.5)	0.2 (-0.6; 0.2)	14.2 (1.7)	14.4 (1.5)	0.2 (-0.2; 0.6)	0.00	0.849	0.969	0.055
HR_{max} (bpm)	124 (24)	129 (24) ‡	4 (-0.5; 9)	143 (22) §	147 (19)	4 (-0.8; 9)	0.21	0.022	0.079	0.945
SpO_2 (%)	96 (2)	96 (2)	0 (-1.5; 1.3)	96 (3)	96 (3)	0 (-1.8; 1.6)	0.00	0.954	0.821	0.833
¶$\dot{Q}aO_{2max}$ ($L \cdot min^{-1}$)	2.53 (0.57)	2.39 (0.56)	0.13 (-0.43; 0.16)	2.79 (1.00)	3.01 (0.75)	0.22 (-0.08; 0.52)	0.15‖	0.191	0.617	0.053
¶$\dot{Q}_{max}$ ($L \cdot min^{-1}$)	13.5 (2.5)	13.0 (2.0)	0.5 (-2.1; 1.1)	15.2 (4.1)	16.3 (3.6)	1.2 (-0.6; 2.8)	0.19‖	0.085	0.480	0.096
¶SV ($ml \cdot beat^{-1}$)	119 (13)	113 (19)	6.6 (-19.5; 6.2)	102 (30)	105 (24)	3.6 (-9.2; 16.5)	0.01	0.199	0.694	0.185
¶$(a\text{-}\bar{v}O_2diff)_{max}$ $mL \cdot L^{-1}$	118.7 (19.7)	137.4 (20.5)†	18.6 (3.2; 34)	121.1 (22.4)	129.7 (17.2)	8.7 (-6.7; 24.1)	0.34	0.799	0.005	0.609
¶$O_{2Extrc\ max(\%)}$	64 (9)	76 (12)†	12 (3; 21)	67 (12)	70 (8)	3 (-6; 12)	0.32	0.794	0.009	0.117
$Power_{max}$ ($\dot{W}$)	133 (32)	158 (34)†	25 (18; 32)	132 (33)	161 (34)†	28 (21; 36)	0.83	0.912	<0.001	0.372

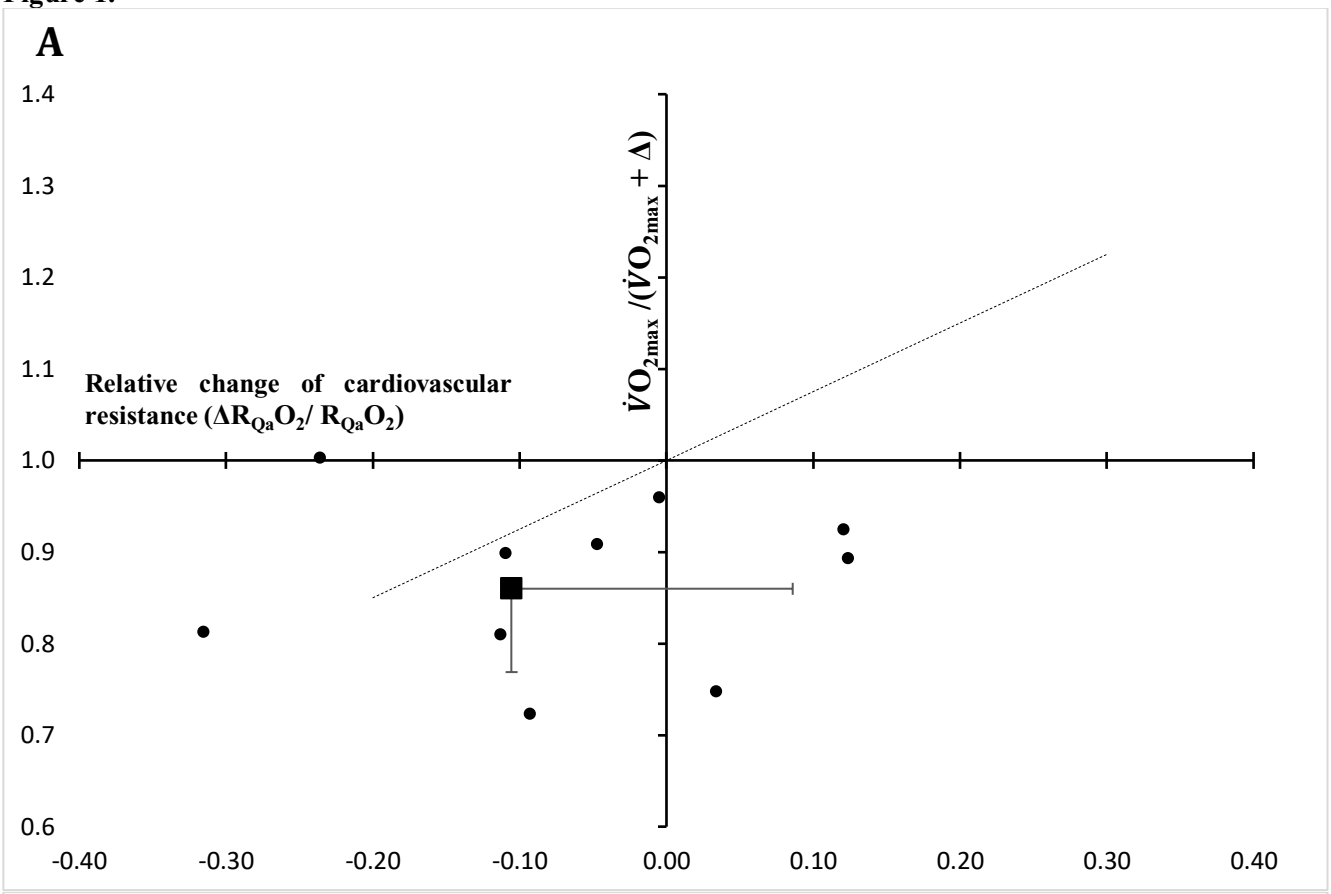
Values are expressed as mean ± standard deviation; *: *P* value from the two-way ANOVA are listed as group effect (G), time effect (T), groups x time effect (G x T); †: Post-hoc test identifies significance ($P \leq 0.05$) in differences between PRE and POST; ‡: Post-hoc test identifies significant trend ($0.05 < P \leq 0.1$) in differences between PRE and POST; ‖: partial eta squared calculated for interaction effect; §: Post-hoc test identifies significance ($P \leq 0.05$) in differences between the two groups PRE; ¶: SL-ET, n=10; DL-ET, n=10

Table 4: Main cardio-respiratory and cardiovascular variables assessed during single leg cycling incremental test (SL-INC) before (PRE) and after (POST) endurance training period. Single leg cycling endurance training (SL-ET) and double leg cycling endurance training group (DL-ET) groups.

	SL-ET$_{GRP}$ (n= 17)			DL-ET$_{GRP}$ (n= 16)			Effect size	*P* values*		
Parameters SL-INC	**PRE**	**POST**	**Mean difference (95% CI)**	**PRE**	**POST**	**Mean difference (95% CI)**	**ηp^2**	**G**	**T**	**G x T**
$\dot{V}O_{2max}$ (ml·min^{-1})	1383 (379)	1574 (306)†	191 (91; 290)	1416 (331)	1685 (334)†	269 (166; 371)	0.65	0.532	< 0.001	0.206
$\dot{V}O_{2max}$ (ml·kg^{-1}·min^{-1})	17.2 (6.3)	19.9 (6.3)†	2.7 (1.5; 3.9)	18.5 (4.1)	21.8 (4.1)†	3.3 (2.1; 4.6)	0.68	0.376	< 0.001	0.379
$\dot{V}_{Emax}$ (L·min^{-1})	62.9 (18.6)	81.2 (19.4)†	18.3 (12.3; 24.3)	61.5 (13.2)	76.3 (14.3)†	14.8 (8.6; 21)	0.73	0.566	< 0.001	0.344
HR max (bpm)	112 (22)	118 (24)†	6 (0.3; 11)	135 (22)‡	135 (22)	0.3 (-5; 6)	0.83§	0.014	0.079	0.106
SpO_2 (%)	96 (3)	97 (1)	1.4 (0; 3)	96 (1)	96 (2)	0 (-1.6; 1.7)	0.05	0.226	0.224	0.566
‖$\dot{Q}_{max}$ (L·min^{-1})	12.1 (4.3)	12.9 (2.7)	1.2 (-0.5; 3)	13.5 (4.1)	14.2 (3.5)	0.7 (-0.8; 2.2)	0.08	0.381	0.190	0.987
‖SV (ml·beat^{-1})	109 (20)	115 (14)	6.3 (-6.7; 19.3)	94 (21)	100 (25)	5.5 (-6.4; 17.3)	0.12	0.078	0.119	0.907
‖(a-$\bar{v}O_2$diff)$_{max}$ mL·L^{-1}	116.4 (12.7)	120.3 (12.6)	3.8 (-7; 14.7)	112.9 (24.1)	125.0 (26.7)†	12.2 (2.2; 22.1)	0.26	0.941	0.017	0.186
Power$_{max}$ ($\dot{W}$)	71 (20)	88 (21)†	17 (12; 22)	74 (20)	90 (20)†	16 (11; 20)	0.81	0.757	< 0.001	0.623

Values are expressed as mean ± standard deviation; *: *P* value from the two-way ANOVA are listed as group effect (G), time effect (T), groups x time effect (G x T); †: Post-hoc test identifies significance ($P \leq 0.05$) in differences between PRE and POST; ‡: Post-hoc test identifies significance ($P \leq 0.05$) in differences between the two groups PRE; §: partial eta squared calculated for interaction effect; ‖: SL-ET, n=10; DL-ET, n = 12.

Figure 1.


A
1.4
1.3
1.2
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1.0
0.9
0.8
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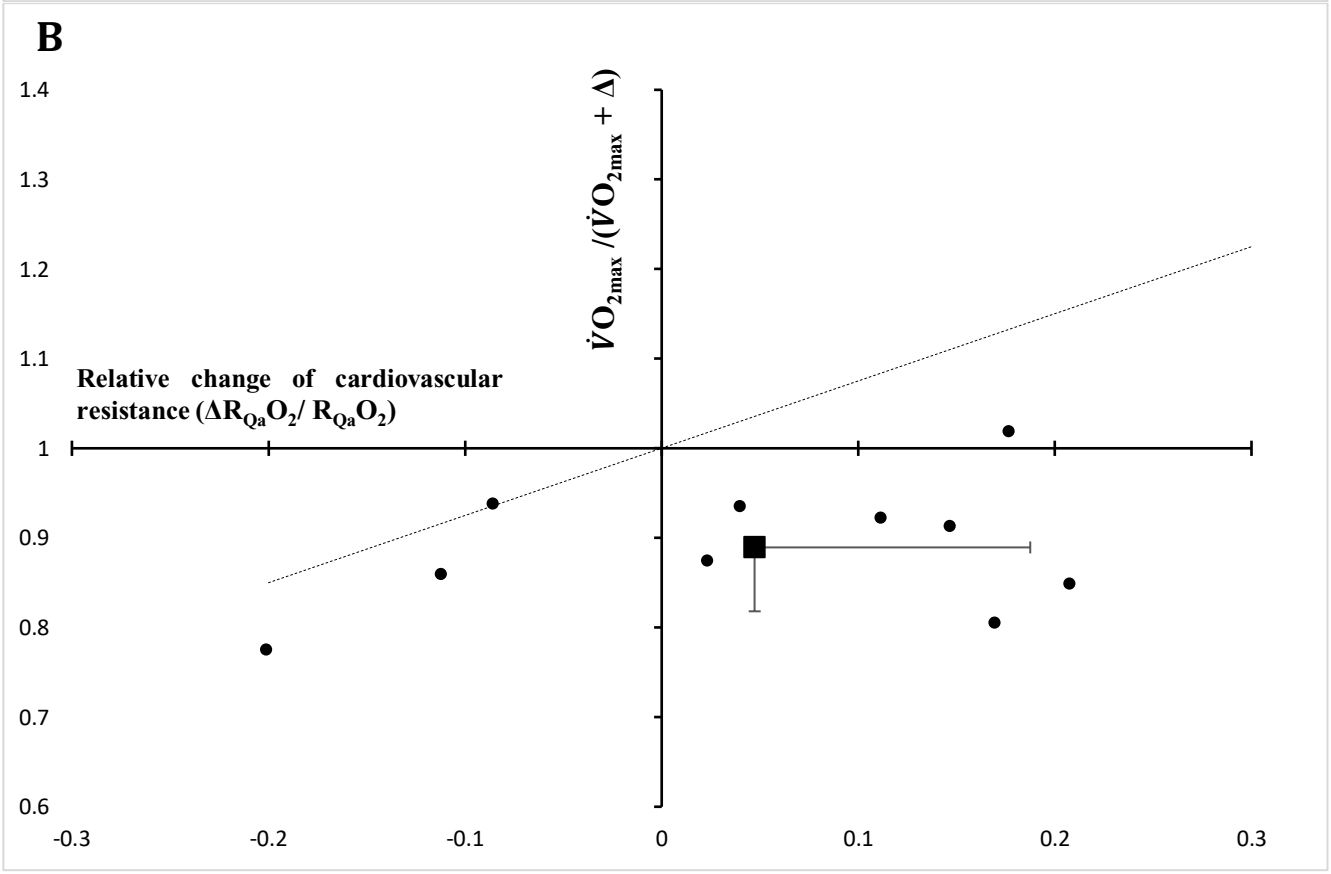
Relative change of cardiovascular resistance ($\Delta R_{Qa}O_2/ R_{Qa}O_2$)
$\dot{V}O_{2max}/(\dot{V}O_{2max} + \Delta)$
-0.40
-0.30
-0.20
-0.10
0.00
0.10
0.20
0.30
0.40


B
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
Relative change of cardiovascular resistance ($\Delta R_{Qa}O_2/ R_{Qa}O_2$)
$\dot{V}O_{2max}/(\dot{V}O_{2max} + \Delta)$
-0.3
-0.2
-0.1
0
0.1
0.2
0.3

Appendix

The following paragraphs illustrate the concepts behind the model of the conductance – resistance in-series to the oxygen flow that helped us quantifying the role of the physiological factors that limited $\dot{V}O_{2max}$ in our subjects. These concepts were clearly explained in insightful papers and they are only briefly summarized below (di Prampero and Ferretti, 1990; Capelli et al., 2006; Bringard et al., 2010).

The oxygen cascade, i.e. the progressive drop of the PO_2 along the pathway from air to mitochondria, is composed by several steps in series and each of them could be considered as a resistance (R_i) that has to be overcome by a pressure gradient (ΔP_i). The oxygen flow ($\dot{V}O_2$) along the pathway and through each resistance at $\dot{V}O_{2max}$ can be described as

$$\dot{V}O_{2max} = \frac{\Delta P_T}{R_T} = \frac{\Delta P_i}{R_i} \quad \text{(A1)},$$

where R_T represents the total resistance to the oxygen flow that has to be overcome by ΔP_T, the total pressure gradient. Considering that the resistances are located in-series, the sum of the individual R_i yields R_T; likewise, ΔP_T is given by the sum of all individual ΔP_i, and it corresponds to:

$$\Delta P_T = P_{IO2} - P_{mO2} \quad \text{(A2)},$$

where P_{IO2} and P_{mO2} represent the partial pressure of oxygen in inspired ambient air and in the mitochondria, usually set to 0 mmHg. For n resistance in series at $\dot{V}O_{2max}$, therefore, we have:

$$\dot{V}O_{2max} = \frac{P_{IO_2}}{R_T} = \frac{\Delta P_1}{R_1} = \frac{\Delta P_2}{R_2} = \dots = \frac{\Delta P_{(n-1)}}{R_{n-1)}} = \frac{\Delta P_n}{R_n} \quad \text{(A3)},$$

where ΔP_n corresponds to the individual O_2 partial pressure difference needed to overcome the corresponding R_n.

In normoxia, ΔP_T remains constant also in presence of marked modifications of $\dot{V}O_{2max}$ induced, f.i., by training or immobilization. Therefore, the observed modification in $\dot{V}O_{2max}$ will be induced either by the reduction or by the increase of R_T, in turn affected by the consensual modifications of one or more of its elements in series, R_i. If we develop the algebra of the model after having imposed a given modification of $\dot{V}O_{2max}$, we obtain:

$$\dot{V}O_{2max} + \Delta\dot{V}O_{2max} = \frac{P_{IO_2}}{R_T + \Delta R_T} \qquad \text{(A4);}$$

where $\dot{V}O_{2max} + \Delta\dot{V}O_{2max}$ is the change in $\Delta\dot{V}O_{2max}$ caused by a modification in R_T (ΔR_T). In Eq. (A4) $\dot{V}O_{2max}$ represents the value preceding the intervention and $\dot{V}O_{2max} + \Delta\dot{V}O_{2max}$ is the value obtained in response to the intervention: an increase of $\dot{V}O_{2max}$ will correspond to a decrease of R_T (ΔR_T will be negative) and vice versa. Dividing Eqs. (A3) by (A4), we will then obtain the following expression:

$$\frac{\dot{V}_{O2max}}{\dot{V}_{O2max} + \Delta\dot{V}_{O2max}} = 1 + \frac{\Delta R_T}{R_T} \qquad \text{(A5),}$$

which can be also written as follows:

$$\frac{\dot{V}_{O_{2max}}}{\dot{V}_{O_{2max}} + \Delta\dot{V}_{O_{2max}}} = 1 + \frac{\Delta R_1 + \Delta R_2 + \cdots + \Delta R_{(n-1)} + \Delta R_n}{R_T} \quad \text{(A6a)}$$

or:

$$\frac{\dot{V}_{O_{2max}}}{\dot{V}_{O_{2max}} + \Delta\dot{V}_{O_{2max}}} = 1 + \frac{\Delta R_1}{R_T} + \frac{\Delta R_2}{R_T} + \ldots + \frac{\Delta R_{(n-1)}}{R_T} + \frac{\Delta R_n}{R_T} \quad \text{(A6b)}$$

We now can also introduce the concept that the fraction F_i of the total limitation to $\dot{V}O_{2max}$ represented by any given individual R_i is simply the ratio between a given resistance R_i and R_T:

$$F_i = \frac{R_i}{R_T} \quad \text{(A7)}$$

Therefore, (A6b) can be expressed by substituting R_T with the corresponding F_i to obtain:

$$\frac{\dot{V}_{O_{2max}}}{\dot{V}_{O_{2max}} + \Delta\dot{V}_{O_{2max}}} = 1 + \frac{F_1 \cdot \Delta R_1}{R_1} + \frac{F_2 \cdot \Delta R_2}{R_2} + \ldots + \frac{F_{(n-1)} \cdot \Delta R_{(n-1)}}{R_{(n-1)}} + \frac{F_n \cdot \Delta R_n}{R_n} \quad \text{(A8)}$$

If one and only one of the in-series resistances undergoes a modification leading to a change in $\dot{V}O_{2max}$, the approach summarized in Eq. (A8) can be substantially simplified, in fact all the other ratios will be zero, with exception of the one referred to modified resistance. For instance, should only the cardiovascular resistance $R_{Q,}$ be acutely changed, Eq (A8) can be written as follows:

$$\frac{\dot{V}_{O_{2max}}}{\dot{V}_{O_{2max}} + \Delta\dot{V}_{O_{2max}}} = 1 + \frac{F_Q \cdot \Delta R_Q}{R_Q} \quad \text{(A9).}$$

Now F_Q represents the fractional limitation imposed by R_Q to $\dot{V}O_{2max}$. The theoretical line reported in Fig 1 (panel A and B) is the graphical representation of Eq. (A9) and it was obtained in the particular case where only R_Q was acutely changed and the consequent and corresponding increases-decreases in $\dot{V}O_{2max}$ measured. In this case, if we plot on the y-axis the ratio of the values preceding and following the intervention (left arm of Eq. (A9) as a function of the corresponding $\Delta R_Q/R_Q$, a linear relationship is drawn having the y-intercept and the slope equal to 1 and to F_Q (slopes of the straight lines in Fig 1, panel A and B), respectively.

R_Q can be quantified as the reciprocal of $\dot{Q}$ multiplied by the oxygen transfer coefficient in the blood phase (βb), which in turn corresponds to the average slope of the oxygen equilibrium curve:

$$R_Q = \frac{1}{\dot{Q} \cdot \beta_b} \approx \frac{1}{\dot{Q} \cdot C_{aO_2}} \approx \frac{1}{\dot{Q}_{aO_2}} \quad \text{(A10),}$$

where $\dot{Q}_aO_2$ represents systemic O_2 delivery. In the case of acute modifications of $\dot{Q}_aO_{2max}$, and hence of R_Q, F_Q turned out to be equal to about 0.70-0.75. This indicates that maximal systemic O_2 delivery accounts for about 70 % of the total factors limiting $\dot{V}O_{2max}$ downstream the lungs, the remaining 30 % being represented by all the lumped peripheral factors. In order to better highlight this concept, we can perform a hypothetical experiment wherein we are able to double $\dot{Q}_aO_{2max}$. This increase in maximal O_2 delivery will increase $\dot{V}O_{2max}$ by about 70-75 %.

In the present investigation, βb was not assessed; however, as S_aO_2 did not substantially change in response to the training intervention, only modifications in arterial oxygen concentration (CaO_2) might lead to changes in βb, as we properly considered in the calculations.

The intervention represented in panel A of Fig 1 refers to the DL-ET, where we can likely suggest that only the modification in $\dot{Q}_aO_2$ were responsible for the alteration in $\dot{V}O_{2max}$. Conversely, the experimental points, reported in panel B Fig 1, do not lie around the theoretical line $F_Q = 0.75$. This is because $\dot{V}O_{2max}$ underwent a larger improvement than the one predicted from the change of $R_{Q.}$ only. In other words, another resistance was reduced by the intervention and this could be only the peripheral (muscular) resistance R_m as $\dot{V}O_{2max}$ is not limited by the respiratory system in healthy human at sea level (Ferretti, 2014) and the model at stake considered only the limiting factors downstream the lungs (di Prampero and Ferretti, 1990; Capelli et al., 2006; Bringard et al., 2010).

In addition, the model would also allow to quantify the relative change of the peripheral, muscular resistance ($\Delta R_m/R_m$) under the proviso of assuming a fixed F_Q.

Since the experimental data of SL-ET do not seem to lie on the line for $F_Q = 0.75$, an additional peripheral resistance was decreased, and this could only be the peripheral (muscular) one (R_m). So, equation A1o can be now expanded as follows:

$$\frac{\dot{V}_{O_{2max}}}{\dot{V}_{O_{2max}} + \Delta\dot{V}_{O_{2max}}} = 1 + \frac{F_Q \cdot \Delta R_Q}{R_Q} + \frac{F_m \cdot \Delta R_m}{R_m} \qquad \text{(A11).}$$

By using the data appearing in Table 3 in Equation A11, the ratio $\Delta R_m/R_m$, showed that Rm decrease by about 50 % after single leg training suggesting a substantial improvement of the peripheral gas exchanges.

Supplementary Material

Table S1: Main cardio-respiratory and cardiovascular variables assessed during double leg cycling incremental test (DL-INC) at baseline (PRE) of the heart transplant (HTx) and non-cardiac transplant recipients (kidney and liver transplanted patients).

DL-INC	HTx (n= 13)	Non-cardiac transplant (n= 20)	P
$\dot{V}O_{2p\text{-}max}$ ($ml \cdot min^{-1}$)	1770 (486)	1710 (427)	0.710
$\dot{V}O_{2\ p\text{-}max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	20.6 (6)	23 (6.5)	0.294
$\dot{V}_{E\text{-}max}$ ($L \cdot min^{-1}$)	81.1 (17.6)	76 (19.9)	0.457
$\dot{V}O_{2p}$ at VT1	1322 (253)	1262 (253)	0.516
Hgb ($mg \cdot dL^{-1}$)	14.4 (2.1)	14.2 (1.7)	0.749
HR_{max} (bpm)	121 (27)	141 (20)	0.021
$*\dot{Q}aO_{2max}$ ($L \cdot min^{-1}$)	2.85 (1.06)	2.50 (0.51)	0.351
$*\dot{Q}_{max}$ ($L \cdot min^{-1}$)	15.1 (4.2)	13.8 (2.6)	0.412
*SV ($ml \cdot beat^{-1}$)	122 (21)	101 (23)	0.043
$*(a\text{-}\bar{v}O_2diff)_{max}$ $mL \cdot L^{-1}$	121 (16)	119 (24)	0.803
$*O_{2Extrc\text{-}max\ (\%)}$	66 (13)	65 (10)	0.828
$Power_{max}$ ($\dot{W}$)	135 (36)	131 (30)	0.689
Power at VT1 ($\dot{W}$)	80 (18)	74 (16)	0.332

Values are expressed as mean ± standard deviation.

*: HTx, n = 9; non-cardiac transplant, n = 11.

REFERENCE

1. Abbiss, C. R., Karagounis, L. G., Laursen, P. B., Peiffer, J. J., Martin, D. T., Hawley, J. A., Fatehee, N. N. and Martin, J. C. (2011) Single-leg cycle training is superior to double-leg cycling in improving the oxidative potential and metabolic profile of trained skeletal muscle, *J. Appl. Physiol.*, 110(5), 1248–1255. doi: 10.1152/japplphysiol.01247.2010.
2. Bassett, D. and Howley, E. T. (2000) Limiting factors for maximum oxygen uptake and determinants of endurance performance, *Med. Sci. Sport. Exerc.*, 32(1), 70–84. doi: 10.1097/00005768-200001000-00012.
3. Bell, C., Paterson, D. H., Kowalchuk, J. M., Moy, A. P., Thorp, D. B., Noble, E. G., Taylor, A. W. and Cunningham, D. A. (2001) Determinants of oxygen uptake kinetics in older humans following single-limb endurance exercise training, *Exp. Physiol.*, 86(5), 659–665. doi: 10.1113/eph8602209.
4. Bjørgen, S., Helgerud, J., Husby, V., Steinshamn, S., Richadson, R. R. and Hoff, J. (2009) Aerobic high intensity one-legged interval cycling improves peak oxygen uptake in chronic obstructive pulmonary disease patients; no additional effect from hyperoxia., *Int. J. Sports Med.*, 30(12), 872–878. doi: 10.1055/s-0029-1238292.
5. Borrelli E, Pogliaghi S, Molinello A, Diciolla F, Maccherini M, and Grassi B. (2003) Serial assessment of peak VO2 and VO2 kinetics early after heart transplantation. Med Sci Sports Exerc. 35(11), 1798-804. doi: 10.1249/01.MSS.0000093610.71730.02.
6. Borg, G. A. V. (1982) Psychophysical bases of perceived exertion. Med Sci Sports Exerc. 1982;14(5):377-81., *Med. Sci. Sports Exerc.*, 377–381.
7. Braith, R. W., Magyari, P. M., Pierce, G. L., Edwards, D. G., Hill, J. A., White, L. J. and Aranda, J. M. (2005) Effect of resistance exercise on skeletal muscle myopathy in heart transplant recipients, *Am. J. Cardiol.*, 95(10), 1192–1198. doi: 10.1016/j.amjcard.2005.01.048.

8. Bringard, A., Pogliaghi, S., Adami, A., De Roia, G., Lador, F., Lucini, D., Pizzinelli, P., Capelli, C. and Ferretti, G. (2010) Cardiovascular determinants of maximal oxygen consumption in upright and supine posture at the end of prolonged bed rest in humans, *Respir. Physiol. Neurobiol.*, 172(1–2), 53–62. doi: 10.1016/j.resp.2010.03.018.
9. Buchheit, M. and Laursen, P. B. (2013) High-intensity interval training, solutions to the programming puzzle: Part I: Cardiopulmonary emphasis, *Sport. Med.*, 43(5), 313–338. doi: 10.1007/s40279-013-0029-x.
10. Capelli, C., Antonutto, G., Kenfack, M. A., Cautero, M., Lador, F., Moia, C., Tam, E. and Ferretti, G. (2006) Factors determining the time course of V̇O2max decay during bedrest: Implications for V̇O2max limitation, *Eur. J. Appl. Physiol.*, 98(2), 152–160. doi: 10.1007/s00421-006-0252-3.
11. Charloux, A., Lonsdorfer-Wolf, E., Richard, R., Lampert, E., Oswald-Mammosser, M., Mettauer, B., Geny, B. and Lonsdorfer, J. (2000) A new impedance cardiograph device for the non-invasive evaluation of cardiac output at rest and during exercise: Comparison with the 'direct' Fick method, *Eur. J. Appl. Physiol.*, 82(4), 313–320. doi: 10.1007/s004210000226.
12. Ciolac, E. G., Mantuani, S. S., Neiva, C. M., Verardi, C. E. L., Pessôa-Filho, D. M. and Pimenta, L. (2015) Rating of perceived exertion as a tool for prescribing and self regulating interval training: A pilot study, *Biol. Sport*, 32(2), 103–108. doi: 10.5604/20831862.1134312.
13. Del Vecchio, A., Casolo, A., Negro, F., Scorcelletti, M., Bazzucchi, I., Enoka, R., Felici, F. and Farina, D. (2019) The increase in muscle force after 4 weeks of strength training is mediated by adaptations in motor unit recruitment and rate coding, *J. Physiol.*, 597(7), 1873–1887. doi: 10.1113/JP277250.
14. di Prampero, P. E. and Ferretti, G. (1990) Factors limiting maximal oxygen consumption in humans, *Respir. Physiol.*, 80(2–3), 113–128. doi: 10.1016/0034-5687(90)90075-A.

15. Dolmage, T. E. and Goldstein, R. S. (2008) Effects of one-legged exercise training of patients with COPD, *Chest*, 133(2), 370–376. doi: 10.1378/chest.07-1423.
16. Esposito, F., Reese, V., Shabetai, R., Wagner, P. and Richardson, R. (2011) Isolated quadriceps training increases maximal exercise capacity in chronic heart failure: the role of skeletal muscle convective and diffusive oxygen transport, *J Am Coll Cardiol*, 58(13), 1353–1362. doi: 10.1038/jid.2014.371.
17. Ferretti, G., Antonutto, G., Denis, C., Hoppeler, H., Minetti, A. E., Narici, M. V. and Desplanches, D. (1997) The interplay of central and peripheral factors in limiting maximal O2 consumption in man after prolonged bed rest, *J. Physiol.*, 501(3), 677–686. doi: 10.1111/j.1469-7793.1997.677bm.x.
18. Ferretti, G. (2014) Maximal oxygen consumption in healthy humans: theories and facts, *Eur. J. Appl. Physiol.*, 114(10), 2007–2036. doi: 10.1007/s00421-014-2911-0.
19. Gea J, Agustí A, and Roca J. (2013) Pathophysiology of muscle dysfunction in COPD. J Appl Physiol (1985)., 114(9):1222-34. doi: 10.1152/japplphysiol.00981.2012. Epub 2013 Mar 21. PMID: 23519228.
20. Gordon, N., Abbiss, C. R., Maiorana, A. J., James, A. P., Clark, K., Marston, K. J. and Peiffer, J. J. (2019) High-Intensity Single-Leg Cycling Improves Cardiovascular Disease Risk Factor Profile, *Med. Sci. Sports Exerc.*, 51(11), 2234–2242. doi: 10.1249/MSS.0000000000002053.
21. Green, S., & Askew, C. (2018). VO2peak is an acceptable estimate of cardiorespiratory fitness but not VO2max. *J Appl Physiol*, 125(1), 229–232. https://doi.org/10.1152/japplphysiol.00850.2017
22. Herman, T., Dall, C., Christensen, S., Goetze, J., Prescott, E. and Gustafsson, F. (2011) Effect of High Intensity Exercise on Peak Oxygen Uptake and Endothelial Function in Long-Term Heart, *Am. J. Transplant.*, 11(3), 536–541. doi: 10.1111/j.1600-6143.2010.03403.x.

23. Hokanson, J. F., Mercier, J. G. and Brooks, G. A. (1995) Cyclosporine A decreases rat skeletal muscle mitochondrial respiration in vitro, *Am. J. Respir. Crit. Care Med.*, 151(6), 1848–1851. doi: 10.1164/ajrccm.151.6.7767529.

24. Iannetta D, Passfield L, Qahtani A, MacInnis MJ, Murias JM. (2019) Interlimb differences in parameters of aerobic function and local profiles of deoxygenation during double-leg and counterweighted single-leg cycling. Am J Physiol Regul Integr Comp Physiol. 317(6),R840-R851. doi: 10.1152/ajpregu.00164.2019.

25. Iannetta, D., Azevedo, R. D. A., Ingram, C. P., Keir, D. A., & Murias, J. M. (2020). Evaluating the suitability of supra-POpeak verification trials after ramp-incremental exercise to confirm the attainment of maximum O2 uptake. *Am J Physiol Regul Integr Comp Physiol*, 319(3), 315–322. https://doi.org/10.1152/ajpregu.00126.2020

26. Kallwitz, E. R. (2015) Sarcopenia and liver transplant: The relevance of too little muscle mass, *World J. Gastroenterol.*, 21(39), 10982–10993. doi: 10.3748/wjg.v21.i39.10982.

27. Kempeneers, G., Noakes, T. D., van Zyl-Smit, R., Myburgh, K. H., Lambert, M., Adams, B. and Wiggins, T. (1990) Skeletal Muscle Limits the Exercise Tolerance of Renal Transplant Recipients: Effects of a Graded Exercise Training Program, *Am. J. Kidney Dis.*, 16(1), 57–65. doi: 10.1016/S0272-6386(12)80786-4.

28. Klausen, K., Andersen, L. B. and Pelle, I. (1981) Adaptive changes in work capacity, skeletal muscle capillarization and enzyme levels during training and detraining, *Acta Physiol. Scand.*, 113(1), 9–16. doi: 10.1111/j.1748-1716.1981.tb06854.x.

29. Lampert, E., Mettauer, B., Hoppeler, H., Charloux, A., Charpentier, A. and Lonsdorfer, J. (1998) Skeletal muscle response to short endurance training in heart transplant recipients, *J. Am. Coll. Cardiol.*, 32(2), 420–426. doi: 10.1016/S0735-1097(98)00227-7.

30. Lepretre, P. M., Koralsztein, J. P. and Billat, V. L. (2004) Effect of exercise intensity on

relationship between V̇O2max and cardiac output, *Med. Sci. Sports Exerc.*, 36(8), 1357–1363. doi: 10.1249/01.MSS.0000135977.12456.8F.

31. MacInnis M.J., Morris N, Sonne M. W., Zuniga A.F., Keir P. J., Potvin J. R., Gibala M. J. (2017) Physiological responses to incremental, interval, and continuous counterweighted single-leg and double-leg cycling at the same relative intensities. Eur J Appl Physiol. 117(7), 1423-1435. doi: 10.1007/s00421-017-3635-8.
32. Mercier, J. G., Hokanson, J. F. and Brooks, G. A. (1995) Effects of cyclosporine A on skeletal muscle mitochondrial respiration and endurance time in rats, *Am. J. Respir. Crit. Care Med.*, 151(5), 1532–1536. doi: 10.1164/ajrccm.151.5.7735611.
33. Moya-Nájera, D., Moya-Herraiz, Á., Compte-Torrero, L., Hervás, D., Borreani, S., Calatayud, J., Berenguer, M. and Colado, J. C. (2017) Combined resistance and endurance training at a moderate-to-high intensity improves physical condition and quality of life in liver transplant patients, *Liver Transplant.*, 23(10), 1273–1281. doi: 10.1002/lt.24827.
34. Munch, G. W., Iepsen, U. W., Ryrsø, C. K., Rosenmeier, J. B., Pedersen, B. K. and Mortensen, S. P. (2018) Effect of 6 wk of high-intensity one-legged cycling on functional sympatholysis and ATP signaling in patients with heart failure, *Am. J. Physiol. - Hear. Circ. Physiol.*, 314(3), H616–H626. doi: 10.1152/ajpheart.00379.2017.
35. Murias, J. M., Pogliaghi, S. and Paterson, D. H. (2018) Measurement of a true V·O2max during a ramp incremental test is not confirmed by a verification phase, *Front. Physiol.*, 9(143), 1–8. doi: 10.3389/fphys.2018.00143.
36. Nytrøen K, Rustad LA, Aukrust P, Ueland T, Hallén J, Holm I, Rolid K, Lekva T, Fiane AE, Amlie JP, Aakhus S, Gullestad L. High-intensity interval training improves peak oxygen uptake and muscular exercise capacity in heart transplant recipients. Am J Transplant. 2012 Nov;12(11):3134-42. doi: 10.1111/j.1600-6143.2012.04221.x. Epub 2012 Aug 17. PMID:

22900793.

37. Poole, D. C. and Jones, A. M. (2017) Measurement of the maximum oxygen uptake Vo2max: Vo2peak is no longer acceptable, *J. Appl. Physiol.*, 122(4), 997–1002. doi: 10.1152/japplphysiol.01063.2016.
38. Possamai, L. T., Campos, F. D. S., Cesar, P., Aguiar, R. A. De, Guilherme, L., Guglielmo, A., & de Lucas, R. D. (2020). Similar maximal oxygen uptake assessment from a step cycling incremental test and verification tests on the same or different day. *Appl Physiol Nutr Metab*, 45(4), 357–361.
39. Richard, R., Verdier, J., Doutreleau, S. and Piquard, F. (2005) Exercise Limitation in Trained Heart and Kidney Transplant Recipients : Central and Peripheral Limitations, *J Hear. Lung Transplant.*, 1774–1780. doi: 10.1016/j.healun.2005.03.017.
40. Riess, K. J., Haykowsky, M., Lawrance, R., Tomczak, C. R., Welsh, R., Lewanczuk, R., Tymchak, W., Haennel, R. G. and Gourishankar, S. (2014) Exercise training improves aerobic capacity , muscle strength , and quality of life in renal transplant recipients, 39(5), 566–571.
41. Rud, B., Foss, Krustrup, P., Secher, N. H. and Hallén, J. (2012) One-legged endurance training: Leg blood flow and oxygen extraction during cycling exercise, *Acta Physiol.*, 205(1), 177–185. doi: 10.1111/j.1748-1716.2011.02383.x.
42. Saltin, B., Nazar, K., Costill, D. L., Stein, E., Jansson, E., Essén, B. and Gollnick, P. D. (1976) The Nature of the Training Response; Peripheral and Central Adaptations to One-Legged Exercise, *Acta Physiol. Scand.*, 96(3), 289–305. doi: 10.1111/j.1748-1716.1976.tb10200.x.
43. Skattebo, Ø., Capelli, C., B.Rud, Auensen, M., Calbet, J. A. L. and Hallén, J. (2020) Increased oxygen extraction and mitochondrial protein expression after small muscle mass endurance training, *Scand. J. Med. Sci. Sports*, 30(9), pp. 1615–1631. doi: 10.1111/sms.13707.
44. Stephenson, A. L., Yoshida, E. M., Abboud, R. T., Fradet, G. and Levy, R. D. (2001) Impaired

exercise performance after successfull liver transplantation, *Transplantation*, 72(6), 1161–1164. doi: 10.5860/rusq.50n1.102.

45. Tyni-Lenné, R., Gordon, A., Jansson, E., Bermann, G. and Sylvén, C. (1997) Skeletal muscle endurance training improves peripheral oxidative capacity, exercise tolerance, and health-related quality of life in women with chronic congestive heart failure secondary to either ischemic cardiomyopathy or idiopathic dilated cardiomyopat, *Am. J. Cardiol.*, 80(8), 1025–1029. doi: 10.1016/S0002-9149(97)00597-3.
46. Tyni-Lenné, R., Gordon, A., Jensen-Urstad, M., Dencker, K., Jansson, E. and Sylvén, C. (1999) Aerobic training involving a minor muscle mass shows greater efficiency than training involving a major muscle mass in chronic heart failure patients, *J. Card. Fail.*, 5(4), 300–307. doi: 10.1016/S1071-9164(99)91334-9.
47. Van Den Ham, E. C. H., Kooman, J. P., Schols, A. M. W. J., Nieman, F. H. M., Does, J. D., Franssen, F. M. E., Akkermans, M. A., Janssen, P. P. and Van Hooff, J. P. (2005) Similarities in skeletal muscle strength and exercise capacity between renal transplant and hemodialysis patients, *Am. J. Transplant.*, 5(8), 1957–1965. doi: 10.1111/j.1600-6143.2005.00944.x.
48. Wagner, J., Niemeyer, M., Infanger, D., Hinrichs, T., Guerra, C., Klenk, C., Karsten, K., Cajochen, C., Schmidt-Trucksass, A., & Knaier, R. (2021). Verification-phase tests show low reliability and add little value in determining VO2 max in young trained adults. *PLoS ONE*, 16(1), 1–14. https://doi.org/10.1371/journal.pone.0245306
49. Whipp, B. J., Davis, J. A., Torres, F. and Wasserman, K. (1981) A test to determine parameters of aerobic function during exercise, *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.*, 50(1), 217–221. doi: 10.1152/jappl.1981.50.1.217.
50. Williams, T. J. and McKenna, M. J. (2012) Exercise limitation following transplantation, *Compr. Physiol.*, 2(3), 1937–1979. doi: 10.1002/cphy.c110021.

The effect of endurance training pulmonary on $\dot{V}O_2$ kinetics in solid organs transplanted recipients

ALESSIO DEL TORTO[1,2], CARLO CAPELLI[3], ROBERTO PERESSUTTI[4], ADRIANA DI SILVESTRE[4], UGOLINO LIVI[1,5], CHIARA NALLI[1,5], SANDRO SPONGA[1,5], GIAMPAOLO AMICI[6], UMBERTO BACCARANI[1,7], STEFANO LAZZER[1,2]

1) Department of Medicine, University of Udine, Udine, Italy.
2) School of Sport Sciences, University of Udine, Udine, Italy.
3) Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Italy.
4) Regional Transplantation Centre, Friuli Venezia Giulia Region, Udine, Italy.
5) Cardiac Surgery Unit, University Hospital of Udine, Italy
6) Nephrology and Dialysis Unit, San Daniele del Friuli Hospital, Udine, Italy
7) Liver-Kidney Transplant Unit, University Hospital of Udine, Udine, Italy.

Running title: Oxygen uptake kinetics in transplanted patients

Corresponding Author: Dr Alessio Del Torto

Department of Medicine, University of Udine

P.le Kolbe 4 – 33100. Udine (Italy)

e-mail: alessio.deltorto12@gmail.com

ABSTRACT

We investigated the effects of 24 sessions of single leg (SL-ET) and double leg (DL-ET) cycling endurance training on O_2 deficit (O_2Def) and mean response time (MRT), during square-wave moderate intensity exercise, and on the amplitude of $\dot{V}O_{2p}$ slow component (SC_{amp}), during heavy intensity exercise, on 33 patients (heart transplant = 13, kidney transplanted = 11 and liver transplanted = 9). After SL-ET, O_2Def, MRT and SC_{amp} decreased by 16.4% (13.7) (P = 0.008), by 15.6% (13.7) (P=0.004) and by 35% (31) (P = 0.002), respectively. After DL-ET, they dropped by 24.9% (16.2) (P < 0.0001), by 25.9% (13.6) (P < 0.0001) and by 38% (52) (P = 0.0003), respectively. The magnitude of improvement for the O_2Def, MRT and SC_{amp} were not significantly different between SL-ET and DL-ET (P = 0.277, P = 0.083 and P = 0.601, respectively) after training. We conclude that SL-ET is as effective as DL-ET if we aim to improve $\dot{V}O_{2p}$ kinetics in transplanted patients and suggest that the impaired $\dot{V}O_{2p}$ kinetics is mainly decelerated by the impairment of peripherals exchanges likely due the immunosuppressive medications and disuse.

Key Words: pulmonary oxygen uptake kinetics, solid organ transplant, single-leg cycling, endurance training, slow component

Novelty

- SL-ET is effective to accelerate $\dot{V}O_2$ kinetics and reduces the SC_{amp} in transplanted recipients.
- SL-ET is as successful as DL-ET to improve $\dot{V}O_2$ kinetics and indexes of exercise tolerance in transplanted recipients.

INTRODUCTION

Heart, kidney, and liver transplant recipients (HTx, KTx, and LTx, respectively) are characterized by lower values of peak pulmonary O_2 uptake ($\dot{V}O_{2p\text{-}peak}$) in comparison with mates of similar age (Williams & McKenna, 2012). A high $\dot{V}O_{2peak}$ is a strong predictor of cardiovascular mortality (Duscha et al., 2005); however, the most common daily activities are carried out at submaximal intensities and require continuous transitions from one exercise intensity to the other. The rate of adjustment of the muscles' oxidative metabolism may likely limit physical performance in these circumstances.

The kinetics of pulmonary O_2 uptake ($\dot{V}O_2$) from the rest to a constant external workload directly affects muscular metabolic stability. When a constant load exercise is performed at moderate intensity [below the 1st ventilatory threshold (1VT)], $\dot{V}O_2$, after a first and sudden increase (phase I) of cardiovascular origin, raises following mono-exponential kinetics (phase II) to attain $\dot{V}O_2$ steady-state ($\dot{V}O_{2ss}$). For a given work rate, the faster is the $\dot{V}O_{2p}$ kinetics, the more rapidly$\dot{V}O_{2ss}$ will be reached, leading to a lower intracellular perturbation (e.g., accumulation of H^+ and lactate, and depletion of PCr) and, therefore, to greater exercise tolerance (Poole and Jones, 2012).

For constant load exercise performed at heavy intensity (between the 1VT and the critical power), $\dot{V}O_2$ requires a longer time to reach $\dot{V}O_{2ss}$ because the so-called $\dot{V}O_2$ slow component ($\dot{V}O_2$sc) appears (Poole et al., 1991). A slower increase of, $\dot{V}O_2$ characterizes this phenomenon until the delayed $\dot{V}O_{2ss}$ is reached (Poole et al., 1991). During this more prolonged phase characterized by a delayed adjustment of the oxidative metabolism, a substantial amount of ATP comes from a more significant contribution of the anaerobic glycolysis to the overall energy turnover (Colosio, Caen, Bourgois, Boone, & Pogliaghi, 2020). As we approach $\dot{V}O_{2ss}$, there seems to be a shift in metabolic sources, as the contribution of oxidative phosphorylation increases, and one of the substrate phosphorylation decreases (O'Connell et al., 2017). Of course, the more extensive and transient accumulation of lactate

and proton may negatively affect the intramuscular milieu and again impair exercise capacity and herald a quicker development of exhaustion.

In healthy indivuduals and in normoxia the speed of $\dot{V}O_{2p}$ kinetics at the exercise onset is likely set by the rate of muscle O_2 utilization ($m\dot{V}O_2$), rather than by muscle O_2 delivery ($\dot{Q}_mO_2$); on the contrary, when pathological conditions reduce muscle $\dot{Q}_mO_2$, $\dot{V}O_{2p}$ kinetics becomes slower (Grassi, 2001). Besides, $\dot{V}O_2$ kinetics is decelerated when dysfunctions in the oxidative metabolism of skeletal muscle are present. Indeed, slower $\dot{V}O_2$ kinetics have been reported in patients with muscle abnormalities (i.e., mitochondrial myopathies and McArdle's disease) (Grassi et al., 2009). Notably, transplanted recipients (Tx) show several muscle defects that may impair peripheral gas exchanges. These sequelae are induced by several concomitant causes, such as immunosuppressive therapy, which negatively affect muscle tissue, and disuse/deconditioning (William and McKenna., 2012; Capelli et al., 2006; Mercier et al., 1995; Hokanson et al., 1995). Indeed, slower $\dot{V}O_{2p}$ kinetics were found in Tx. Still, most of the studies investigated HTx, and only one investigation focused on LTx and KTx, where peripheral muscular factors are more likely negatively affected, (Jendzjowsky et al., 2007; Tomczak et al., 2008; Tomczak et al., 2013). Accordingly, Tomczak et al. reported impaired $\dot{V}O_{2p}$ kinetics in thoracic Tx (i.e., HTx) and abdominal Tx (i.e., KTx and LTx). The authors concluded that the impaired ability to accelerate $m\dot{V}O_2$, rather than the slower rate of adjustment of $\dot{Q}_mO_2$, might be the leading cause for the degraded $\dot{V}O_2$ kinetics in HTx, LTx, and KTx (Tomczak et al., 2008; Grassi et al., 1997).

Is it well known that ET elicits favorable adaptations at cardiovascular and muscular levels, speeding $\dot{V}O_2$ kinetics, and reducing the $\dot{V}O_{2p}$sc amplitude (SC_{amp}) (Jones & Carter, 2000; Klausen et al., 1981; Rud et al., 2012). Small muscle mass [i.e., single-leg cycling (SL) or knee extension exercise] ET, f.i., seems to be more effective than double-leg cycling (DL) if the aim is to improve the oxidative metabolism of skeletal muscle (Abbiss et al., 2011). Moreover, knee extension training in elderly

subjects resulted in the speeding of $\dot{V}O_{2p}$ kinetics accompanied by a higher mitochondrial oxidative capacity, but without larger muscle blood flow velocity and a larger muscle capillarization (Bell et al., 2001). Furthermore, the only one training study on HTx disclosed that a combination of ET and resistance exercise was an effective intervention to accelerate $\dot{V}O_2$ kinetics. Unluckily, this finding has not been extended to KTx and LTx yet (Tomczak et al., 2013). Besides, no researches determined the effect of ET on SC_{amp} in HTx, LTx and KTx.

Considering that: i) disuse/deconditioning and immunosuppressant side-effects are common causes of the frequent muscular abnormalities found in HTx, KTx and LTx and; ii) small muscle mass training leads to favorable adaptations to peripheral gas exchanges, we can hypothesize that peripheral factors affecting the muscle's capacity of extracting and utilizing O_2 might be the main responsible for the slower $\dot{V}O_{2p}$ kinetics in transplant patients. Moreover, we may hypothesize that ET involving a small muscle mass (SL) might be as effective as traditional whole-body ET (DL), if one aims to improve $\dot{V}O_{2p}$ kinetics in this class of patients.

METHODS

Participants and anthropometric characteristics

Thirty-eight sedentary and clinically stable Tx (i.e. not presenting conditions that that required hospitalization) (n: 14, HTx; n: 13, KTx; n: 11, LTx) were recruited in the current investigation. This study was approved by the local Institutional Review Board (n: 8/IRB DAME) and was carried out in compliance with the principles of the Declaration of Helsinki. Before beginning the study, all patients underwent a full medical anamnesis and physical examination and were informed about the potential risks associated with the experiments; then, they gave their informed consent. Pregnancy was considered as an exclusion criterion, moreover, patients with cardiopulmonary diseases, cancer and orthopaedic issues compromising cycling exercise were excluded from the study. Patients were only

included at least 1 year after transplantation. Before the beginning of the training period, 5 volunteers dropped out of the study: 2 because of orthopaedic injury, 1 for malignant diseases, and 2 for personal reasons; finally, 33 subjects (male = 28; female = 5) participated in the study (HTx= 13, KTx= 11 and LTx= 9).

Body mass was measured to the nearest 0.1 kg with a mechanical scale (Seca 709, Hamburg, Germany) with the subject dressed only in light underwear and no shoes. Stature was measured to the nearest 0.5 cm on a standardized wall-mounted height board. Body mass index was calculated as body mass (body mass, kg) multiplied by the squared stature (m).

Study design

After the first visit, subjects were assigned randomly to SL ($SL\text{-}ET_{GRP}$; n = 17) and DL ($DL\text{-}ET_{GRP}$; n = 16) endurance training group. Preliminary tests, including assessment of anthropometric and physical capacities, were performed during two-weeks before (PRE) the beginning and immediately after (POST) completion of the ET period. Before the start of the study, participants were familiarized with the equipment and procedures. They were asked to avoid strenuous exercises the day before the experimental sessions and avoid any caffeinated drinks (5h) and meal consumption (3h) before every test. Subjects were referred to the laboratory in three subsequent days. On the first visit, after the medical screening and anthropometric measurement, subjects performed the double-leg cycling step exercise (DL-INC). On the second day, participants completed a double-leg moderate constant load exercise (DL-MOD), and on the third visit, they carried out two tests. Firstly, DL-MOD repetition and, secondly, a double-leg heavy constant load exercise (DL-HVY). The two exercise bouts were separated by at least 15-30 min of rest. All the individual experimental sessions were interspersed by at least two days of recovery.

Double leg incremental step test

During DL-INC, after 3 min at rest, the patients pedalled for 6 min at 25 W or 40 W. Immediately after the baseline cycling, a symptom-limited (leg fatigue or shortness of breath) DL-INC was performed, increasing the mechanical power by 15 W each minute until exhaustion. Participants were asked to keep a constant pedalling cadence, which was digitally displayed, at their preferred rate (60–75 RPM). They were asked to cycle until volitional exhaustion or until they were not capable to maintain the cadence above 60 rpm in spite of strong verbal encouragement. After exhaustion, the work rate was decreased to 25 W for five minutes to cool down. The rates of perceived exertion for leg fatigue ($RPE_{leg\ fatigue}$) and dyspnoea ($RPE_{dyspnea}$) were registered at the end of DL-INC (Borg, 1982). $\dot{V}O_{2p\text{-}peak}$, peak, CO_2 production ($\dot{V}CO_{2p\text{-}peak}$), minute ventilation ($\dot{V}_{Epeak}$), and heart rate ($HR_{peak}$) were determined as the highest averages measured in subsequent 30-s epochs. If, during the last step, a subject achieved exertion before the end of the stage, peak power output was calculated as previously reported (Lepretre et al., 2004). Briefly, if during the last step of the DL-INC, a subject reached the exertion before the end of the stage, the corresponding PO_{max} was calculated as:

$$PO_{max} = POf + [(t/60) \cdot 15]$$

where POf was the mechanical power (W) of the last terminated step, t represented the time maintained (s) for the last workload and 15W was the increment in the mechanical power for each stage. $\dot{V}O_2$-VT1 was assessed by two independent and trained researchers using the V-slope method; ventilatory equivalents ($\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$) and pressure end tidal (PET O_2 and PET CO_2) were used as ancillary criteria (Whipp et al., 1981). If agreement was not found between them, a third independent assessor applied the three methods and $\dot{V}O_2$-VT1 was determined only when the three investigators agreed on the results. Then, for each patient, the $\dot{V}O_2$ vs power relationship was left

shifted by a fixed amount of time, 30sec, to calculate the mechanical power corresponding $\dot{V}O_2$-VT1. $\dot{V}O_{2p}$, VCO_{2p}, and $\dot{V}_E$ were continuously assessed breath-by-breath by using a metabolic unit (CPET, Cosmed, Italy); HR was obtained from the ECG signal. Before each experiment, O_2 and CO_2 analysers and the turbine flowmeter, for the expiratory flow measurements, were calibrated by utilizing gas mixtures of a known composition (16.00 % O_2, 4.00 % CO_2, nitrogen as the balance) and with a 3 L syringe imposing three different flow rates.

Constant load exercises

After positioning the ECG electrodes in the second visit, a DL-MOD exercise test at a work rate corresponding to 80% of the power output at VT1 was carried out. After 3 minutes at rest sitting on the bike, participants immediately started cycling at constant work rate for 10 min and were asked to keep a constant pedaling cadence at their preferred rate (60-75 RPM), which was digitally displayed, and recorded for the subsequent experimental sessions. Once the cycling bouts were concluded, the $RPE_{leg\ fatigue}$ and $RPE_{dyspnea}$ were registered. Gas exchanges and $\dot{V}_E$ and HR were continuously measured throughout the test.

In the third experimental session, the DL-MOD was repeated. Once the first exercise bout was completed, volunteers rested sitting on the bike for ~15 to 30min. Afterward, a DL-HVY test was performed at a work rate corresponding to the 40% of the difference between $\dot{V}O_2$-VT1 and $\dot{V}O_{2p\text{-}peak}$. Subjects were asked to pedal until exhaustion. In any case, the exercise was always interrupted after 14 minutes of cycling. $RPE_{leg\ fatigue}$ and $RPE_{dyspnea}$ were registered at the end of the test. Blood lactate concentration ($[La]^b$) was assessed by an enzymatic method (Biosen C-line; EKF). Measurements were taken at rest, at the 1st min after the DL-MOD cessation, and at the 1st, 3rd, and 5th min after the end of DL-HVY; the highest value registered during the recovery phase was retained as peak blood lactate concentration ($[La]^b_{peak}$).

Endurance training

Volunteers in the SL-ET and DL-ET groups started an 8-week cycling training programme with 3 supervised sessions per week. The training pattern consisted in high-intensity, interval training (HIIT), which has been shown to be a safe and feasible exercise mode for transplanted patients (Herman *et al.*, 2011). Two protocols of training were used, alternatively, to increase participant adherence to the training programs, as previously applied (Saltin et al., 1976; Skattebo et al., 2020). These two training protocols were reported to be effective in imporving the $\dot{V}O_{2p\text{-}peak}$ in SOT (Herman et al., 2011; Nytrøen et al., 2012). Therefore, the patients underwent i) 12 ET sessions that included 4 min at high intensity followed by 3 min of active recovery; each bout was repeated 4 times and: ii) 12 ET consisting of 2 min at high intensity followed by 2 min of active recovery; in this case, each bout was repeated 6 times. Each HIIT training session was always preceded and followed by 5 min of warm-up and 5 min of cool down, respectively. The DL-ET group performed DL training for the whole session; conversely, the SL-ET group completed the first half of the training pedalling with one leg and the second half with the other leg. With this configuration, both groups trained for the same interval of time. Furthermore, patients in both $DL\text{-}ET_{GRP}$ and $SL\text{-}ET_{GRP}$ were asked to keep a fixed cadence, within the range of 60 to 70 RPM, during the ET sessions; hence the mechanical work performed by the skeletal muscle was similar for DL-ET and SL-ET.

For the SL exercise, the bikes were arranged as for SL-INC (see below). The workload imposed for the HIIT was set to elicit a value equal to or higher than 15 of $RPE_{dyspnoea}$ during the DL-ET (6-20 Borg's scale) and equivalent to or greater than 5 of $RPE_{leg\ fatigue}$ during the SL-ET (1-10 Borg's scale) (Buchheit and Laursen, 2013). The intensity of the active recovery was tailored to achieve a score equal to or lower than 12 on $RPE_{dyspnoea}$ and equivalent to or lower than 2 on $RPE_{leg\ fatigue}$ in the two exercise modalities, respectively. The training load was adjusted weekly; HR, $RPE_{dyspnoea}$ and RPE_{leg}

$_{fatigue}$ were continuously monitored during HIIT and during the active recovery phases. The utilization of the RPE (i.e. Borg's scale) was previously showed to be an effective criterion for high intensity exercise prescription (Ciolac et al., 2015; Gordon et al., 2019). HR was recorded throughout the training and HR values occurring in the last15 sec before the end of each interval of high and low intensity were averaged; $RPE_{leg\ fatigue}$ and $RPE_{dyspnoea}$ values were recorded at the end of each interval of high and low intensity. Research assistants were responsible for verifying that each subject participated in each training session, performed the exercises correctly, and completed at least 90 % of the training sessions.

Data treatment

$\dot{V}O_{2p\text{-}ss}$, $\dot{V}CO_{2p\text{-}ss}$, $\dot{V}_{Ess}$ and HR_{ss} were computed by averaging breath-by-breath values assessed in the last 2 min of the two DL-MOD. As for DL-HVY, the values of $\dot{V}O_{2p}$, $\dot{V}_E$ and HR recorded in the last minute of exercise were averaged. $[La]^b$ at the end of DL-MOD was calculated as the average of the two values obtained at the end of the DL-MOD tests. Oxygen deficit (O_2Def) was obtained as previously reported (Capelli et al., 2009). Briefly, B-by-B $\dot{V}O_2$ values of each DL-MOD repetition were interpolated to 1-s intervals (Lamarra et al., 1987), time aligned with the onset of the exercise test, and treated by subtracting the $\dot{V}O_2$ at rest. The two repetitions' data were then combined to obtain a single data file for each subject and condition. O_2Def was calculated as the difference between the O_2 that would have been consumed if $\dot{V}O_{2ss}$ had been attained immediately at the beginning of the exercise and the volume of O_2 taken up during exercise. The first quantity was calculated by multiplying $\dot{V}O_{2ss}$ in ml O_2 s^{-1} by the exercise duration (600 s). The O_2 volume consumed during exercise was calculated by summing progressively the $\dot{V}O_2$ values expressed in ml $O_2 \cdot s^{-1}$ from the trial's onset to 600 s. Mean response time (MRT) was then computed as the ratio between O_2Def and the corresponding $\dot{V}O_{2ss}$ (Capelli et al., 2009). Gross pedaling efficiency was calculated according to

Péronnet and Massicotte (1991), the oxygen cost of cycling (O_2cost) was computed dividing the difference between $\dot{V}O_{2ss}$ and resting $\dot{V}O_2$ for the corresponding W (Péronnet and Massicotte (1991).

To detect $\dot{V}O_2$sc during DL-HVY, we averaged and linearly fitted the $\dot{V}O_2$ values calculated every 30sec from the 3rd to the 6th of exercise. A positive slope significantly different from 0 would indicate the development of the $\dot{V}O_2$sc (Salvadego et al., 2017). Moreover, the SC_{amp} during the DL-HVY was determined as the difference between the $\dot{V}O_{2p}$ at the 3rd min and that at the last minute of exercise (Billat et al.,1998). $\dot{V}_E$ values were also averaged every 30sec and were used to estimate the work of breathing (WB) and the oxygen cost of respiratory muscles ($\dot{V}O_{2RM}$) according to (O'Connell et al., 2017):

$$WB = -0.430 + 0.050 \cdot (\dot{V}_E) + 0.00161 \cdot (\dot{V}_E)^2 \quad (1)$$

$$\dot{V}O_{2\text{-}RM} = (34.9 + 7.45 \cdot WB) \quad (2)$$

HR values coinciding with each single DL-MOD repetition were time aligned with the exercise onset and superimposed, then the HR values were averaged each 5-sec epoch. Afterward, the data were fitted by the function:

$$y(t) = y_{BAS} + A_f[1\text{-}e^{(t-TD_f)/\tau_f)}] \quad (3)$$

where y(t) represents HR as a function of time t; y_{BAS} is the baseline value of HR; A_f is the amplitude between baseline HR and that at steady-state of the fundamental component; TD_f is the time delay, and; τ_f is the is the time constant of the function for the fundamental component.

Statistics

Data were analyzed with Prism, version 8.0 (GraphPad Software, La Jolla, CA, USA). Data in text and tables are presented as means ± standard deviation (SD), unless otherwise stated. Besides, the mean difference is expressed as PRE minus POST values. Shapiro–Wilk test was used to verify the normal distribution of the data. PRE and POST investigated cardiovascular, cardio-respiratory and $\dot{V}O_2$ kinetics parameters were analyzed using a two-way, within-subject ANOVA, with groups (SL-ET_{GRP} and DL-ET_{GRP}) and time (PRE- and POST- training) as the two factors. If a significant main effect or interaction effect were found, post hoc Bonferroni's multiple comparisons test was performed. Paired Student's t-test was used to compare the 30sec averaged $\dot{V}O_{2p\text{-}ss}$ within the two-training groups during DL-MOD. The differences between SL-ET_{GRP} and DL-ET_{GRP} in the anthropometrics before training, the variables measured during training and the number of ET sessions, performed by each group, were analysed with unpaired Student's t-tests. Linear regressions were calculated by the least-squared residuals method (Motulsky and Christopoulos, 2004) and the difference between slopes was evaluated as indicated by Zar (Zar, 1974). Partial eta squared (ηp^2) was calculated as previously reported (Del Vecchio et al., 2019) and ηp^2 for main effect of time, unless otherwise stated, and 95% confidence interval of mean difference between PRE and POST values are reported in Tab 4, 5 and 6. Alpha level was set to ≤ 0.05, and values between > 0.05 and ≤ 0.10 were considered to indicate trends.

The HR model parameters were estimated using an iterative, weighted nonlinear least-squares procedure (Marquardt, 1963) implemented by the commercial software for data analysis Prism, version 8.0 (GraphPad Software, La Jolla, CA, USA). We entered initial guesses of the parameters of the model after visual inspection of the data.

RESULTS

Patient characteristics and the exercise training regimen

The current investigation mainly presents and discusses only the results related to the $\dot{V}O_{2p}$ and HR kinetics assessed during DL-MOD and DL-HVY. Three participants in the SL-ET_{GRP} and one in the DL-ET_{GRP} completed only one DL-MOD. Therefore we decided to exclude their data from the analysis of $\dot{V}O_{2p}$ and HR kinetics. The main anthropometric characteristics and maximal cardio-respiratory parameters, at PRE, are reported in Table 1. The pharmacological therapies and the morbidities that led to organ transplants for the SL-ET_{GRP} and DL-ET_{GRP} are reported in Table 2 and Table 3, respectively.

Volunteers participated to the 97.8 % (6.3) and to the 99.2 % (2.3) (P = 0.398) of total exercise sessions for the SL-ET_{GRP} and DL-ET_{GRP}, respectively; no adverse events were reported. The DL-ET_{GRP} reported higher values for the $RPE_{dyspnoea}$ [16 (0.7)] than the SL-ET_{GRP} [14 (0.7), P < 0.001]; conversely, no differences were found for the $RPE_{leg\ fatigue}$ in the DL-ET_{GRP} and SL-ET_{GRP} [7 (0.7) and 7 (0.4), P = 0.826]. The two groups trained at the 91 % (7) and 87 % (11) (P = 0.269) of the $HR_{max,}$ reached during the DL-INC, for the DL-ET_{GRP} and SL-ET_{GRP}, respectively

Double leg moderate constant load test

The data related to $\dot{V}O_2$ and HR kinetics are reported in Table 4. Those assessed during steady-state exercise are presented in Table 5. $\dot{V}O_2$ and HR kinetics parameters for HTx and non-cardiac Tx are shown in table 1 of Supplemental Material. The data of Tx treated with β-blocker medications and of the patients not treated with β-blockers are shown in table 2 of Supplemental Material.

At PRE HR_{ss} (P = 0.016) was slightly, but significantly, higher in DL-ET_{GRP} (Table 5). HR at baseline and the response amplitude of HR kinetics tended to be lower in SL-ET (P = 0.064 and P= 0.098, respectively) (P = 0.061) (Table 4).

The $\dot{V}O_{2ss}$ assessed during DL-MOD corresponded to 92 (6) % and 92 (6) % of $\dot{V}O_2$-VT1 for SL-ET_{GRP} and DL-ET_{GRP}, respectively. Moreover, the 30-sec averaged $\dot{V}O_2$ calculated during the 3rd minute (from 180th sec to the 210th sec) of the exercise was not significantly different from the one calculated during the 6th minute in SL-ET_{GRP} and DL-ET_{GRP} (P = 0.306 and P = 0.517, respectively). This finding confirmed that $\dot{V}O_{2p}$ attained a stable steady-state.

At POST, O_2Def significantly decreased by 16.4 % (13.7) (P = 0.008) and 24.9 % (16.2) (P < 0.0001) in SL-ET_{GRP} and DL-ET_{GRP}, respectively. Likewise, MRT dropped by 15.6 % (13.7) (P = 0.004) (P = 0.004) in SL-ET_{GRP} and by 25.9 % (13.6) (P < 0.0001) in the DL-ET_{GRP} (Table 4). The changes in O_2Def and MRT were not significantly different between the two groups (P = 0.277 and P = 0.083, respectively). HR_{Tau} decreased by 32 % (22), in SL-ET_{GRP} (P = 0.001), and by 23 % (41) DL-ET_{GRP} (P = 0.006); no differences were found between the two groups (Table 4). r^2 of the fitting ranged from 0.94 to 0.99 and from 0.95 to 0.99 for both groups at PRE and POST, respectively.

$\dot{V}_{Ess}$ tended to decrease by 4.7 % (6.5) in SL-ET_{GRP} (P = 0.065) and was significantly lower by 5.4 % (7.6) in DL-ET_{GRP} (P = 0.017). Moreover, also HR_{ss} was significantly reduced by 5 % (7.5) and 8.5 % (5.2) in SL-ET_{GRP} (P = 0.011) and DL-ET_{GRP} (P < 0.0001), respectively (Table 5). The change in $\dot{V}_{Ess}$ was not statistically different between the two groups (P = 0.154). HR_{ss}, tended to assume a lower value in DL-ET_{GRP} compared to SL-ET_{GRP} (interaction effect (G x T), P = 0.094). $[La]^b$ was significantly reduced by 25 % (18) in SL-ET_{GRP} (P = 0.0003) and by 37 % (17) in DL-ET_{GRP} (P = < 0.0001); the changes tended to be different between the two groups (G x T, P = 0.057) (Tab 5).

Double leg heavy constant load test

The results regarding cardio-respiratory parameters assessed during DL-HVY are reported in Table 6. The slopes of the linear relationship between the $\dot{V}O_2$ vs. time were significantly different from zero in SL-ET_{GRP} (P < 0.0001) and in DL-ET_{GRP} (P < 0.0001), indicating the presence of $\dot{V}O_{2SC}$ (Fig 1).

Moreover, the two slopes were not significantly different (P = 0.676). Only one subject was not able to reach the 6th min of DL-HVY. Therefore, he was excluded from the slope analysis. As for SL-ET_{GRP}, 15 volunteers reached the 8th min, 14 the 9th and 12 the 14th min of exercise; as for DL-ET_{GRP}, 15 subjects reached the 8th, 14 the 9th and 12 the 14th min of exercise.

At POST, all subjects in DL-ET_{GRP} terminated the test, whereas in SL-ET_{GRP} only one volunteer terminated the trial at the 7th min. In SL-ET_{GRP}, SC_{amp} decreased by 35 % (31) (P = 0.002); likewise, in DL-ET_{GRP} it dropped by 38 % (52) (P = 0.0003). The changes were not significantly different between the training groups (P = 0.654) (Table 6). The angular coefficients of the linear relationship between $\dot{V}O_{2p}$ and time were statistically different from zero in SL-ET_{GRP} and DL-ET_{GRP} also at POST (P = 0.0004 and P = 0.0002, respectively) (Fig 1); they were also significantly lower than those prevailing at PRE in both SL-ET_{GRP} and DL-ET_{GRP} (P < 0.0001 and P = 0.0012, respectively) (Fig 1).

$\dot{V}O_{2\text{-}RM}$ statistically decreased by 13 % (19) and by 23 % (15) in SL-ET_{GRP} and DL-ET_{GRP}, respectively (P = 0.023 and P = 0.0003, respectively) (Table 6). To assess if $\dot{V}O_{2\text{-}RM}$ substantially contributed to $\dot{V}O_{2pSC}$, "gross"$\dot{V}O_{2p}$ was correct by subtracting the estimated $\dot{V}O_{2\text{-}RM}$ and we calculated again the slope of the regression lines from the 3rd to the 6th min. The angular coefficients of the corrected $\dot{V}O_{2p}$ vs. time were not significantly different from the ones obtained from the "gross"$\dot{V}O_{2p}$ vs. time in SL-ET_{GRP} (P = 0.297) and DL-ET_{GRP} (P = 0.496). Besides, the slope of the corrected $\dot{V}O_{2p}$ vs. time was not significantly different between DL-ET_{GRP} and SL-ET_{GRP} (P = 0.370) (Fig. 2).

$\dot{V}O_2$ at the last min of DL-HVY decreased by 4.1 % (5) (P = 0.012) and 6.3 % (7.5) (P = 0.001) in SL-ET_{GRP} and DL-ET_{GRP}, respectively; there was no difference between the changes in the two groups (P= 0.469; Table 6). $\dot{V}_E$ was 10.6 % (10.4) (P = 0.001) and 17.1 % (9.3) lower (P < 0.0001) in SL-ET_{GRP} and DL-ET_{GRP}, respectively (Tab 6); O_2cost decreased, although not significantly so, by 4.1% (5.8) (P = 0.018) in SL-ET_{GRP}; it significantly dropped by 6.7 % (8.3) (P = 0.002) in DL-ET (Table 6).

Neither $\dot{V}_E$ nor O_2cost were significantly different between the two groups (P = 0.158 and P =0.160, respectively) (Table 6).

HR at the end of DL-HVY significantly decreased by 6.2 % (5.3) (P = 0.002) and 9.1 % (6.7) (P = 0.002) in SL-ET_{GRP} and DL-ET_{GRP}, respectively; a tendency towards a difference was found between the two groups (P = 0.098, Table 6). $[La]^b_{peak}$ statistically dropped in SL-ET_{GRP} and DL-ET_{GRP} by 26 % (35) (P = 0.026) and 63 % (70) (P < 0.0001), respectively; moreover, the difference between the two groups tended to be significant (P = 0.088, Table 6).

4) DISCUSSION

In the current study, we compared the effect 8 weeks of SL-ET vs. those of DL-ET on $\dot{V}O_{2p}$ and HR kinetics in transplanted patients for the first time in Tx.

The main results disclosed that: i) SL-ET was an effective strategy to improve $\dot{V}O_{2p}$ and HR kinetics and exercise tolerance during moderate and/or heavy intensity exercise, ii) no difference between SL-ET_{GRP} and DL-ET_{GRP} were found as for the improved $\dot{V}O_2$ and HR kinetics during DL-MOD after training, iii) no difference between SL-ET_{GRP} and DL-ET_{GRP} were reported for the attenuated SC_{amp} and for the reduction of the slopes of the linear increase of $\dot{V}O_2$ during heavy intensity exercise. The intensities for the training sessions were prescribed based on RPE, which is an effective criterion for high intensity exercise prescription (Ciolac et al., 2015; Gordon et al., 2019). For the DL-ET, the rated effort during exercise sessions based on $RPE_{dyspnoea}$ was 16 (0.7) and for SL-ET_{GRP} it was 14 (0.7) ($P \leq 0.001$), suggesting that the cardio-respiratory system was stressed more in DL-ET than in SL-ET, as expected. No difference was found for the $RPE_{leg\ fatigue}$ recorded during the training sessions, 7 (0.7) and 7 (0.4) (P= 0.826) for DL-ET_{GRP} and SL-ET_{GRP}, respectively, suggesting that the skeletal muscles were stressed similarly between the two groups.

SL-ET_{GRP} and the DL-ET_{GRP} included a combination of HTx, LTx and KTx, thus one can conjecture

that the three different Tx might have different physiological responses to exercise. HTx, because of the cardiac denervation, are characterized by the so-called chronotropic incompetence (Williams and McKenna., 2012). This condition is often responsible for the higher HR at rest, a lower HR_{peak} and sluggish HR adjustment during constant-load exercise that might prevent the attainment of large values of cardiac output and systemic O_2 delivery during submaximal exercise, possibly compromising the $\dot{Q}_mO_2$. In turns, the reduction of $\dot{Q}_mO_2$ can impair the $\dot{V}O_{2p}$ kinetics response at the onset of DL-MOD; indeed, HTx are commonly found with sluggish $\dot{V}O_{2p}$ kinetics (Jendzjowsky et al., 2007; Tomczak et al., 2008; Tomczak et al., 2013). Therefore, the prolonged HR kinetics, suggesting a lower rate of $\dot{Q}_mO_2$ (MacPhee et al., 2005), should result in slower $\dot{V}O_{2p}$ kinetics in HTx if compared with non-cardiac Tx. Despite the decelerated HR kinetics, a previous study reported that the $\dot{V}O_{2p}$ kinetics were not different between HTx and non-cardiac Tx (Tomczak et al. 2008). Additionally, the increase of $\dot{Q}_mO_2$ turned out to be ineffective in speeding $\dot{V}O_{2p}$ kinetics in HTx (Grassi et al., 1997). Our data confirmed this condition; in fact, HTx were found with significantly slower HR kinetics (P = 0.001); however, O_2Def and MRT were not different between HTx and non-cardiac Tx (P = 0.406 and P = 0.531, respectively) (see Table 1 in Supplemental Material).

Furthermore, when Tx treated with β-blockers were compared with those not treated, no differences were detected for HR_{Tau}, O_2Def, and MRT between the two groups (P = 0.995, P = 0.672 and P = 0.556, respectively) (see Table 2 in Supplemental Material). These findings strongly indicate that neither the cardiac denervation nor the β-blockade treatment has negatively influenced, *per se*, the rate of adjustment of the oxidative metabolism at the exercise onset in our Tx. Considering that the cardiac denervation affects only HTx, we can hypothesize that different mechanisms elicited the $\dot{V}O_{2p}$ kinetics speeding and the SC_{amp} attenuation in HTx if compared to non-cardiac Tx. The identification of the possible different physiological mechanisms, between HTx and non-cardiac Tx, behind the improvement of $\dot{V}O_{2p}$ kinetics at moderate and heavy exercise intensity is beyond the purpose of the

current investigation. In fact, the absence of skeletal muscle biopsies and the determination of $\dot{Q}_mO_2$ impedes to clarify if different adaptations occurred between HTx and non-cardiac Tx. Moreover, the magnitude of change in the SC_{amp}, O_2Def, and MRT was compared in cardiac Tx vs. non-cardiac Tx. SC_{amp} decreased by 22 % (38) in cardiac and by 45 % (43) in non-cardiac Tx, with no difference between them (P = 0.13). The O_2Def was lowered by 23.6 % (15.3) in cardiac and by 16.2 % (15.2) in non-cardiac Tx, with no difference between the groups (P = 0.212); similarly, MRT improved by 16.4 % (14.1) in cardiac and by 16.2 % (15.2) in non-cardiac Tx, with no difference between the type of transplant (P = 0.188).

The results showed that ET effectively induced beneficial adaptations of $\dot{Q}_mO_2$ or $m\dot{V}O_2$ and, regardless of the type of transplant, the $\dot{V}O_{2p}$ kinetics were accelerated and SC_{amp} smaller. This permitted the comparison between $DL\text{-}ET_{GRP}$ and $SL\text{-}ET_{GRP}$ formed by heterogeneous sets of Tx. The following paragraphs are devoted to discussing the possible mechanisms behind the observed improvements.

4.1) *$\dot{V}O_{2p}$ kinetics parameters at sub-threshold cycling*

The initial differences in HR_{ss}, HR at baseline and the amplitude response may be caused by more patients under β-blockade medications; however, the HR_{Tau} was not statistically different between the two groups.

Previous studies showed that ET speeded $\dot{V}O_{2p}$ kinetics during moderate exercise in older and young healthy subjects (Bell et al., 2001; Murias et al., 2011). We confirmed and extended these findings to HTx, LTx, and KTx. Indeed, MRT significantly reduced in $SL\text{-}ET_{GRP}$ and $DL\text{-}ET_{GRP}$ and, as a consequence, O_2Def significantly dropped after ET in both groups.

Our results agree with those reported by Tomczak et al. (2013) who showed a faster phase II $\dot{V}O_{2p}$ kinetics in a group of HTx following 12 weeks of combined endurance and strength training . Besides,

the faster $\dot{V}O_2$ kinetics assessed for the SL-ET_{GRP} agrees with the results provided by Bell and colleagues who reported accelerated $\dot{V}O_{2p}$ kinetics in the trained leg of elderly subjects after knee extension training (Bell et al., 2001). If, for a given work rate, a faster $\dot{V}O_{2p}$ kinetics leads to a reduced O_2Def, a lower intracellular perturbation (e.g., Δ[H+], Δ[lactate], and Δ[PCr]) is elicited, thus increasing the exercise tolerance (Poole & Jones, 2012). Therefore, the ameliorations of MRT and O_2Def conferred by SL-ET and DL-ET led to improved exercise tolerance in the studied patients. Also the lower $[La]^b$, HR_{ss} and $\dot{V}_{Ess}$ seem to indicate reduced cardio-respiratory stress and then an improved exercise tolerance after training.

The speeding of $\dot{V}O_{2p}$ kinetics was accompanied by a faster HR_{Tau}, suggesting an improved $\dot{Q}_mO_2$ adjustment at the DL-MOD onset (MacPhee et al.,2005). However, neither endothelial function nor skeletal muscle capillary density seem to improve following training in transplanted recipients. Indeed, a recent systematic review and meta-analysis concluded that the positive effect of exercise on endothelial function is not corroborated by scientific evidence (de Souza et al., 2019). Despite the fact that the genesis of new capillaries within the skeletal muscle is a well-known adaptation to training in healthy subjects, Lampert et al. did not find any improvement in capillary density after six weeks of ET in HTx (Hellsten & Nyberg, 2016; Lampert et al., 1998). This finding can be ascribed, in part, to the cyclosporine toxicity effect on endothelial function (Petrakopoulou et al., 2006; Ramzy et al., 2005). Cytokine transforming growth factor-beta (TGF-β) has been found to influence vascular endothelial growth factor (VEGF), which in turn represents a direct-acting angiogenic signal protein (Klagsbrun & D'Amore, 1991; Pepper et al., 1993). A high concentration of TGF-β exerts an inhibiting effect on VEGF; on the other hand, low concentrations of TGF-β reinforce the effect of VEGF (Pepper et al., 1993). Considering that TGF-β is triggered by cyclosporine, one might surmise that the increased content of TGF-β would have negatively affected VEGF, prevent the angiogenic process and cause the lack of capillarization in the trained muscle of transplanted recipients (Lampert

et al., 1998). Suppose this is so, an improved $m\dot{V}O_2$ cannot be ruled out as a possible mechanism behind the speeding of $\dot{V}O_2$ kinetics and the simultaneous acceleration of $\dot{V}O_{2p}$ and HR kinetics make it challenging to distinguish whether the ameliorations were due to a faster $\dot{Q}_mO_2$ or to an improved muscular capability to extract and utilize O_2.

Neither O_2cost nor gross pedaling efficiency were statistically changed ET, suggesting that some skeletal muscle defects might still be present even after the training period (Kempeneers et al., 1990; Richard et al., 2005). This further points towards the hypothesis that skeletal muscle, when negatively affected (i.e., by medications, disuse, and systemic organ failure) might provide a more significant limitation to the exercise capacity (del Torto et al., 2020; Capelli et al., 2006; Esposito et al., 2011)

4.2) Exercise responses to supra-threshold cycling

To our knowledge, this is the first study evaluating the effect of ET, and more specifically SL-ET vs. DL-ET, on SC_{amp} in HTx, KTx, and LTx. The reduction of SC_{amp} is a well described adaptation to ET; therefore, the reported drop of SC_{amp} (i.e. expressed in mL of O_2) and the lower slope of the linear regression of $\dot{V}O_2$ vs. time at POST, when compared to the one assessed at PRE, agree with other investigations (Jones & Carter, 2000). Moreover, no differences were found between $SL\text{-}ET_{GRP}$ and $DL\text{-}ET_{GRP}$ for the reduction of the SC_{amp} and the slopes, indicates that the SL-ET was as effective as the DL-ET to cause the favorable adaptations responsible for the $\dot{V}O_{2pSC}$ drop. Recent findings have suggested that the progressive recruitment of the less economic Type II fibers may be not strictly necessary to induce $\dot{V}O_{2SC}$. In contrast, it may derive from mechanisms inherent to the recruited fibers (Zoladz et al., 2008) that are responsible for the increase of O_2 cost of oxidative production of ATP. It is of note that a recent study (Zoladz et al., 2016) showed that endurance training in rats induced a temperature-dependent enhancement of mitochondrial oxidative phosphorylation and a significant drop of mitochondrial uncoupling. Therefore, the decrease of O_2 cost for oxidative ATP production in

each recruited muscle fiber may have substantially potentiated the effect of endurance training on $\dot{V}O_{2SC}$. Accordingly, the decreased $[La]^{b}_{peak}$ and the improved exercise economy support the view of improved oxidative metabolism after endurance training (Holloszy, 1967). However, they cannot disentangle whether this intrinsic muscular adaptation was more responsible than the modification of the pattern of motor units recruitment for eliciting $\dot{V}O_{2SC}$.

Direct muscular causes account for more of the 80% of SC_{amp} (Poole et al., 1991); meanwhile, factors such as the O_2cost of respiratory muscles (Carra et al., 2003), cardiac work and auxiliary muscles' contractions account for the remaining part (Jones et al., 2011). Indeed, Carra et al. (2003) provided direct experimental evidence pointing towards the role of ventilatory work regarding the development SC_{amp}, Salvadego et al. (2017) reported that respiratory muscle training resulted in a disappearance of SC_{amp} in obese patients during heavy intensity exercise. These factors are consistent with improved exercise tolerance. When DL-HVY was performed at POST, the same power output provoked a statistically lower $\dot{V}E$ and a reduced $\dot{V}O_{2\text{-}RM}$; in other words the respiratory muscle of our subjects became more efficient during heavy exercise, allowing a reduced O_2cost of breathing. Despite this, the reduced $\dot{V}O_{2\text{-}RM}$, *per se*, did not explain the reduction of SC_{amp}. Indeed, the slopes of the corrected $\dot{V}O_{2p}$ vs. time were not significantly different from the ones assessed from the "gross" $\dot{V}O_{2p}$ vs. time in $SL\text{-}ET_{GRP}$ and $DL\text{-}ET_{GRP}$. However, the lower $\dot{V}E$ and $\dot{V}O_{2\text{-}RM}$, together with the reduced $\dot{V}O_{2p}$ end-exercise, HR and $[La]^{b}_{peak}$ indicate an enhanced tolerance of intense exercise.

$\dot{Q}_mO_2$ and $m\dot{V}O_2$ alterations can profoundly influence the $\dot{V}O_2$ kinetics response (Koga et al., 1999; Murias et al., 2010), unfortunately in our study we were unable to investigate if the beneficial adaptations leading to the narrowing of SC_{amp} were induced by an enhanced of $\dot{Q}_mO_2$ and/or $m\dot{V}O_2$. To our knowledge, no studies have investigated the SC_{amp} and its related mechanisms after ET in HTx, KTx and LTx, making unfortunately impossible a comparison with other studies dealing with Tx.

CONCLUSIONS

In conclusion, 8 weeks of DL-ET and SL-ET significantly speeded $\dot{V}O_{2p}$ and HR kinetics and reduced O_2Def at exercise carried out with large muscle mass at moderate intensity; furthermore, the two training modalities resulted in the attenuation of the SC_{amp}, meaning that SL-ET is as effective as DL-ET when training aims to improve exercise capacity. More studies are required to evaluate the relative contribution of $\dot{Q}_mO_2$ vs. $m\dot{V}O_2$, to explain the mechanism behind the $\dot{V}O_{2p}$ kinetics improvement, and the long-term effect of small muscle mass training compared to more traditional endurance protocol in Tx.

STUDY LIMITATIONS

In the present study, the lack of muscle biopsies to assess the possible increase in capillary and mitochondrial densities, endothelial function, and the absence of leg blood flow measurment prevented us from discerning the effect of $\dot{Q}_mO_2$ vs $m\dot{V}O_2$ on $\dot{V}O_{2p}$ kinetics. Another limitation is that cardiac output kinetics were not assessed. However HR kinetics were suggested as a good proxy for the O_2 delivery adjustment to the imposed work rate (MacPhee et al., 2005). To identify the power output associated to the $\dot{V}O_2$-VT1, the $\dot{V}O_2$ vs time relationship was left shifted by 30 sec, instead of using an amount of time corresponding to the individual mean response time of the $\dot{V}O_{2p}$ kinetics. This approach may have caused the overestimation of the work rate used for the DL-MOD and DL-HVY. However, our data confirm that our patients were within the moderate and heavy intensity domains. Finally, the absence of an age-matched control group precluded the determination of potential differences, with respect to the healthy condition, in the adaptive mechanisms elicited by the two diverse exercise training modalities.

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AUTHOR CONTRIBUTIONS

ADT- patients enrolment, conception and design of the experiment, data collection, analysis of the data, interpretation of the results and draft of the paper; CC- concept and design of the investigation, interpretation of the results, critical revision of the paper; RP, DSA, UL, CN, SS, GA, BU- *patient* enrolment, interpretation of the results, critical revision of the manuscript; LS- conception and design of the experiment, interpretation of the results, critical revision of the paper, providing financial support for the project.

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CONFLICT OF INTEREST The authors declare no conflict of interest, financial or otherwise.

Figure captions:

Figure 1. $\dot{V}O_{2p}$ vs. time from the 3rd to 6th min of exercise during DL-HVY. Data show the linear increase of $\dot{V}O_{2p}$ vs. time before (PRE, empty circles) and after training (POST, black circles) in SL-

ET_{GRP} (diagram A) and in DL-ET_{GRP} (diagram B). Data in diagram C show the linear increase of $\dot{V}O_{2p}$ vs. time for SL-ET_{GRP} (black circles) and for DL-ET_{GRP} (black diamonds) at POST. Data are expressed as mean ± standard deviation. #: angular coefficient of the regression line is significantly different from zero. *: angular coefficient of the regression line is significantly different between groups.

Figure 2. $\dot{V}O_2$ vs. time from the 3rd to the 6th min of exercise during DL-HVY. Data in diagram A show the relationship between the $\dot{V}O_{2p}$ diminished by the estimated O_2 cost of breathing (white squares) and the gross $\dot{V}O_2$ (black circle) as a function of time in SL-ET_{GRP} after training. Data in diagram B show the same relationship as in diagram A, but in DL-ET_{GRP}. Data in diagram C show relationship between corrected $\dot{V}O_2$ as a function of time in SL-ET_{GRP} (black circles) and in DL-ET_{GRP} (black triangle). Data are expressed as mean ± standard deviation. #: angular coefficient of the regression line is significantly different from zero

Figure 1

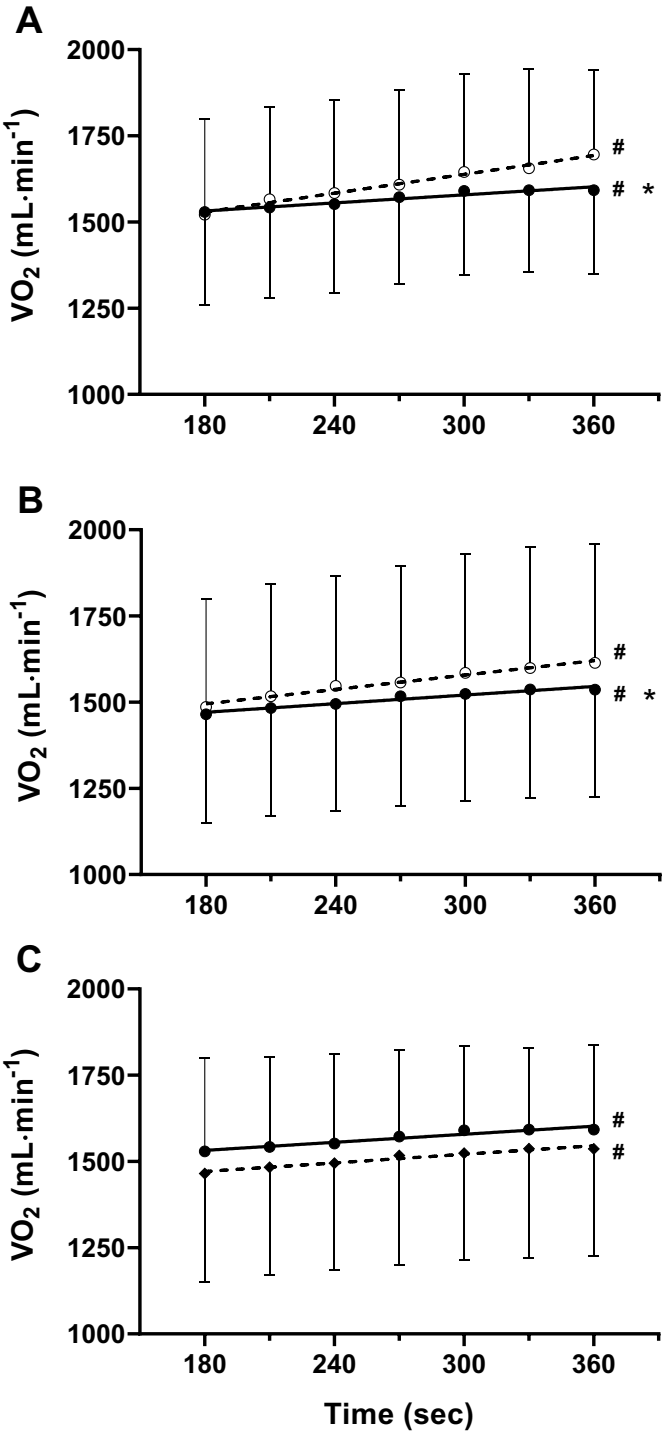
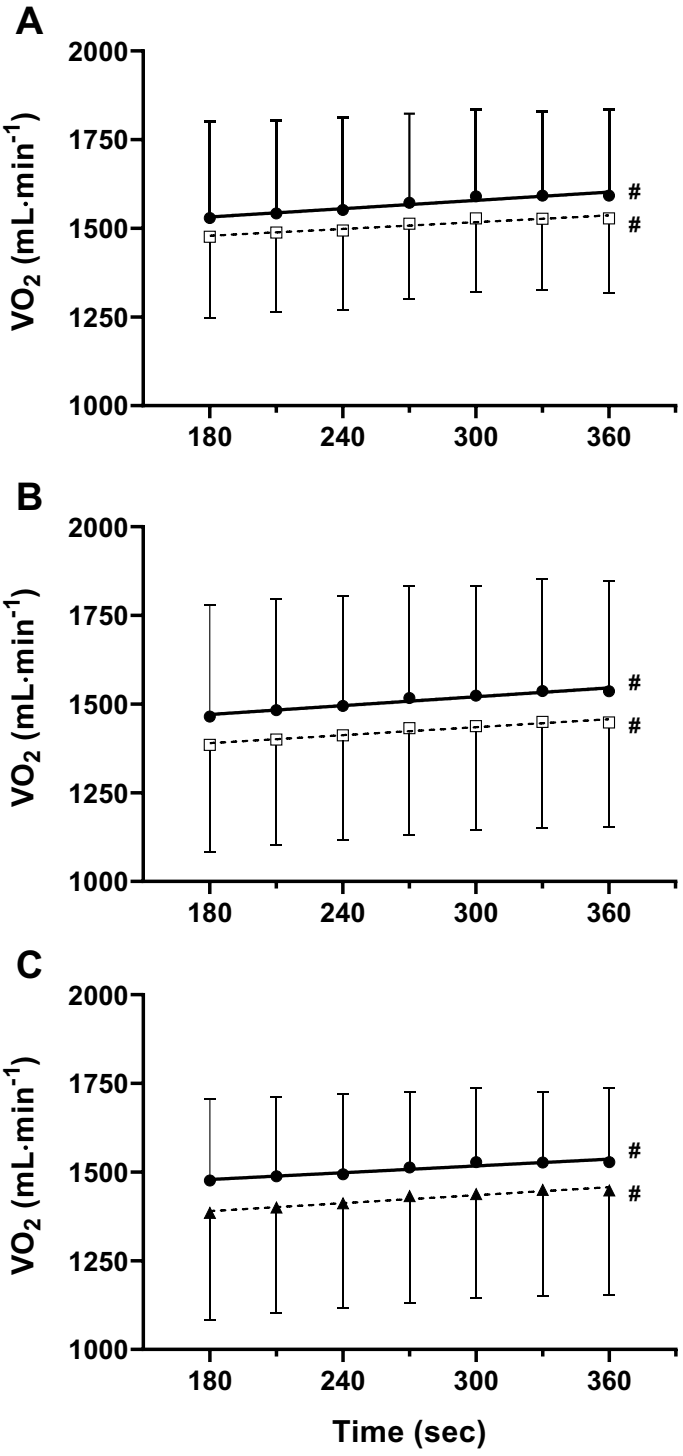

A
B
C
2000
1750
1500
1250
1000
180
240
300
360
VO2 (mL·min-1)
Time (sec)
#
*

Figure 2


A
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2000
1750
1500
1250
1000
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240
300
360
VO_2 (mL·min^{-1})
Time (sec)
#
#

Table 1. Main anthropometrics, cardio-respiratory and cardiovascular parameters assessed during double leg incremental test measured before the training period. Single leg endurance training group ($SL\text{-}ET_{GRP}$) and double leg endurance training group ($DL\text{-}ET_{GRP}$) groups.

Anthropometrics	$SL\text{-}ET_{GRP}$ (n= 17)	$DL\text{-}ET_{GRP}$ (n= 16)	P
Age (years)	56 (10)	55 (10)	0.82
BM (Kg)	83 (15)	78(18)	0.35
BMI	26.7 (3.3)	26.3 (5.3)	0.76
Years post-transplant	6.2 (6.9)	8.9 (7.7)	0.30
Cardio-respiratory parameters			
$\dot{V}O_{2peak}$ ($ml \cdot min^{-1}$)	1747 (420)	1719 (483)	0.86
$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	21.8 (7.7)	22.2 (4.7)	0.85
$\dot{V}_{Epeak}$ ($L \cdot min^{-1}$)	78.6 (16.9)	77.5 (21.3)	0.88
$\dot{V}O_2$ at VT1 ($ml \cdot min^{-1}$)	1311 (216)	1275 (290)	0.55
HR_{peak} (bpm)	124 (24)	143 (22)	0.03
SpO_2 (%)	96 (2)	96 (3)	0.93
Peak power output ($\dot{W}$)	133 (32)	132 (33)	0.95
Power output ($\dot{W}$) at VT1	76 (15)	76 (20)	0.87

Values are expressed as mean ± standard deviation.

Table 2: Pharmacological therapies

Medications	SL-ET_{GRP} (n: 17)	DL-ET_{GRP} (n: 16)
Medications		
Immunosuppressant	17 (100%)	16 (100%)
Corticosteroids	7 (41%)	4 (25%)
NSAID	10 (59%)	9 (56%)
ACE-inhibitors	2 (12%)	1 (6%)
Angiotensin 2 receptor blockers	4 (23%)	1 (6%)
α-blockers	6 (35%)	4 (25%)
β-blockers	11 (65%)	4 (25%)
Diuretics	3 (18%)	2 (13%)
Calcium channel blockers	4 (23%)	3 (19%)
Statins	6 (35%)	3 (19%)
Lipid lowering agents	2 (12%)	0 (0%)
Metformin	1 (6%)	1 (6%)
Insulin	1 (6%)	0 (0%)
Thyroid hormones	1 (6%)	4 (25%)
Proton pump inhibitors	10 (59%)	6 (38%)
Xanthine oxidase inhibitors	3 (18%)	4 (25%)
Hypouricemic agents	7 (41%)	5 (31%)
Kinase inhibitor agents	1 (6%)	2 (13%)
Bisphosphonates	1 (6%)	1 (6%)
Dopamine agonists	1 (6%)	0 (0%)
Bronchodilators	1 (6%)	0 (0%)
Antigout agents	1 (6%)	1 (6%)
Antiarrhythmic agents	1 (6%)	0 (0%)

Non-steroidal anti-inflammatory drugs (NSAID), dingle leg endurance training group (SL-ET_{GRP}), Double leg endurance training group (DL-ET_{GRP}).

Table 3: List of diseases leading to the organ transplant

SL-ET$_{GRP}$ (HTx, n= 6; KTx, n= 6; LTx, n= 5)				DL-ET$_{GRP}$ (HTx, n= 7; KTx, n= 5; LTx, n= 4)			
Disease	HTx	KTx	LTx	**Disease**	HTx	KTx	LTx
Cardiac sarcoidosis	1 of 6	-	-	Primitive cardiomyopathy	4 of 7	-	-
Ischemic cardiomyopathy	4 of 6	-	-	Ischemic cardiomyopathy	2 of 7	-	-
Myocarditis	1 of 6	-	-	Infiltrative cardiomyopathy	1 of 7	-	-
Glomerulonephritis	-	1 of 6	-	Alport syndrome	-	1 of 5	-
Berger's disease	-	1 of 6	-	Polycystic kidney	-	3 of 5	-
Chronic kidney failure	-	1 of 6	-	Glomerulonephritis	-	1 of 5	-
Polycystic kidney	-	2 of 6	-	Primitive sclerosing cholangitis	-	-	1 of 4
Goodpasture syndrome	-	1 of 6	-	α-1 antitrypsin deficiency disease	-	-	1 of 4
Hepatitis C	-	-	3 of 5	Hepatitis B	-	-	1 of 4
Hepatic cirrhosis	-	-	2 of 5	Hepatic cirrhosis	-	-	1 of 4

Single leg endurance group (SL-ET$_{GRP}$), double leg endurance group (DL-ET$_{GRP}$), heart transplant recipients (HTx), kidney transplant recipients (KTx), liver transplant recipients (LTx).

Table 4. Pulmonary O_2 uptake ($\dot{V}O_{2p}$) and heart rate (HR) kinetics parameters assessed during double leg moderate constant load exercise (DL-MOD) before (PRE) and after (POST) endurance training period. Single leg endurance training group ($SL\text{-}ET_{GRP}$) and double leg endurance training group ($DL\text{-}ET_{GRP}$) groups.

	$SL\text{-}ET_{GRP}$ (n= 14)			$DL\text{-}ET_{GRP}$ (n= 13)			Effect size	*** *P* values		
$\dot{V}O_{2p}$ kinetics	PRE	POST	Mean difference (95% CI)	PRE	POST	Mean difference (95% CI)	ηp^2	G	T	G x T
O_2 Def (mL O_2)	728 (168)	596 (131) †	132 (31; 233)	734 (278)	537 (204) †	197 (101; 293)	0.54	0.707	<0.0001	0.276
MRT (sec)	52.1 (15.9)	43.5 (15.2)†	8.5 (3 to 14)	53.3 (14.4)	38.6 (9.5) †	14.6 (9; 20)	0.63	0.707	<0.0001	0.083
SC_{amp} (mL O_2)	207 (57)	131 (68)†	75.5 (27; 124)	207 (84)	118 (90)†	90 (40; 138)	0.51	0.767	<0.0001	0.645
HR kinetics										
Baseline	71.6 (11.6)▲	70.3 (10.3)	1.3 (-4.2; 6.8)	81.2 (10.7)	78.3 (12.3)	3 (-2.3; 8.3)	0.07	0.040	0.193	0.615
Amplitude	21.6 (6.9)▲	19.6 (6.6)	2 (-1; 5)	26.4 (6)	22.9 (5.5) †	3.5 (0.7; 6.4)	0.29	0.082	0.038	0.375
Time delay (sec)	13.4 (10.7)	17.8 (19.5)	-4.4 (-12.3; 3.5)	11.9 (13.7)	12.3 (13.8)	-0.4 (-8.2; 7.3)	0.04	0.510	0.312	0.403
Time constant (sec)	78.4 (79.9)	50.6 (59)†	27.7 (11.8; 43.7)	62.9 (36.4)	41.9 (28.7) †	21 (5.7; 36.2)	0.53	0.557	<0.0001	0.750
MRT (sec)	91.7 (87)	68.4 (65.3)†	23.3 (8.2; 38.4)	74.8 (48.1)	54.2 (39.2) †	20.5 (6.2; 34.9)	0.51	0.513	<0.0001	0.655
95% CI for time constant	58 - 81	39 - 55	-	52 - 65	37 - 48		-	-	-	-

Values are expressed as mean ± standard deviation, note that $\dot{V}O_{2p}$ slow component amplitude (SC_{amp}) refers to double leg heavy constant load exercise.
***: *P* value from the two-way ANOVA are listed as group effect (G), time effect (T), groups x time effect (G x T).
†: Post-hoc test identifies significance ($P \leq 0.05$) in differences between PRE and POST.
▲: Post-hoc test identifies significant trend ($0.05 < P \leq 0.1$) between groups at PRE.

Table 5. Main cardio-respiratory parameters assessed during double leg moderate constant load exercise (DL-MOD) before (PRE) and after (POST) endurance training period. Single leg endurance training group ($SL\text{-}ET_{GRP}$) and double leg endurance training group ($DL\text{-}ET_{GRP}$) groups.

DL-MOD	$SL\text{-}ET_{GRP}$ (n= 14)			$DL\text{-}ET_{GRP}$ (n= 15)			Effect size	*** *P* values		
Steady-state parameters	PRE	POST	Mean difference (95% CI)	PRE	POST	Mean difference (95% CI)	ηp^2	G	T	G x T
$\dot{V}O_{2p\text{-}ss}$ ($mL \cdot min^{-1}$)	1206 (173)	1180 (169)	25 (-11; 61)	1135 (226)	1131 (229)	4 (-30; 40)	0.07	0.424	0.169	0.328
$\dot{V}CO_{2p\text{-}ss}$ ($mL \cdot min^{-1}$)	1153 (154)	1090 (141) †	62 (20 to 104)	1060 (197)	1019 (201) †	41 (1 to 81)	0.40	0.215	0.0002	0.398
$\dot{V}_{E\text{-}ss}$ ($L \cdot min^{-1}$)	41.8 (4.4)	39.8 (4.9) #	2 (-0.1 to 4.1)	38.5 (8.7)	36.1 (6.9) †	2.4 (0.4 ; 4.4)	0.32	0.154	0.001	0.734
RER	0.96 (0.04)	0.93 (0.04) †	0.03 (0.01; 0.06)	0.94 (0.04)	0.90 (0.03) †	0.03 (0.01; 0.06)	0.40	0.080	0.0002	0.873
Gross Efficiency (%)	14.7 (1.2)	15 (1.4)	-0.3 (-0.8 to 0.1)	14.4 (2.3)	14.5 (2.1)	0.1 (-0.5 to 0.3)	0.11	0.595	0.100	0.404
O_2 cost ($mL \cdot Watt^{-1}$)	14.0 (1.1)	13.9 (1)	0.1 (-0. to 0.7)	14.4 (3.2)	14.4 (2.4)	0 (-0.6 to 0.6)	0.00	0.524	0.727	0.807
HR_{ss} (bpm)	97 (17) ⁑	91 (15) †	5 (1 to 9)	111 (12)	102 (12) †	9 (5; 13)	0.58	0.022	<0.0001	0.094
$[La]^b$ ($mmol \cdot L^{-1}$)	3.15 (1.11)	2.45 (1.15) †	0.7 (0.3 to 1.1)	3.18 (0.84)	1.98 (0.75) †	1.15 (0.8; 1.5)	0.76	0.689	<0.0001	0.057
Power ($\dot{W}$)	62 (13)	62 (13)	-	58 (14)	58 (14)	-	-	-	-	-

Values are expressed as mean ± standard deviation.
***: *P* value from the two-way ANOVA are listed as group effect (G), time effect (T), groups x time effect (G x T).
#: Post-hoc test identifies significant trend ($0.05 < P \leq 0.1$) in differences between PRE and POST.
†: Post-hoc test identifies significance ($P \leq 0.05$) in differences between PRE and POST.
⁑: Post-hoc test identifies significance in differences between groups at PRE.

Table 6. Main cardio-respiratory parameters assessed during double leg heavy constant load exercise (DL-HVY) before (PRE) and after (POST) endurance training period. Single leg endurance training group ($SL\text{-}ET_{GRP}$) and double leg endurance training group ($DL\text{-}ET_{GRP}$) groups.

DL-HVY	$SL\text{-}ET_{GRP}$ (n= 17)			$DL\text{-}ET_{GRP}$ (n= 16)			Effect size	*P* values[a]		
End-exercise parameters	PRE	POST	Mean difference (95% CI)	PRE	POST	Mean difference (95% CI)	ηp^2	G	T	G x T
$\dot{V}O_{2p}$ ($mL \cdot min^{-1}$)	1712 (293)	1636 (249)†	76 (15; 137)	1665 (344)	1563 (351)†	103 (40; 166)	0.43	0,579	<0,0001	0,469
$\dot{V}CO_{2p}$ ($mL \cdot min^{-1}$)	1651 (263)	1554 (238)†	97 (37; 158)	1645 (334)	1478 (329)†	167(104; 229)	0.62	0,689	<0,0001	0,069
RER	0,97 (0,04)	0.95 (0.03)#	0.02 (0; 0.04)	0,99 (0,06)	0,95 (0,04)†	0.04 (0.02; 0.07)	0.40	0,540	<0,0001	0,067
O_2 cost ($mL \cdot Watt^{-1}$)	14,2 (1,2)	13,6 (1,4)	0.6 (-0.2; 1.4)	14,8 (2,9)	13.5 (1.7) †	1.3 (0.5; 2.1)	0.33	0.635	<0,0005	0.158
$\dot{V}_E$ ($L \cdot min^{-1}$)	70,3 (13,3)	62,8 (10,2)†	7.4 (2.9; 12)	68,0 (13,8)	56,5 (13,3)†	11.5 (6.8; 16.2)	0.60	0,313	<0,0001	0.158
$\dot{V}O_{2\text{-}RM}$	116 (27)	104 (24) †	12 (1.5; 22.7)	113 (29)	93 (24) †	20 (9; 31)	0.44	0.394	<0,0001	0.230
HR (bpm)	126 (24)	118 (22) †	8 (3; 13)	141 (18)	128 (19) †	13 (8; 18)	0.60	0.098	<0,0001	0.137
[La][b] ($mmol \cdot L^{-1}$)	6.2	5.2†	1 (0.1; 2)	6.6	4.6†	2 (1.1; 2.9)	0.52	0.881	<0,0001	0.088
$RPE_{dyspena}$	15,1 (1,5)	13.6 (1.6)†	1.5 (0.1; 2.9)	16,2 (1,6)	14.1 (1.8) †	2.1 (0.7; 3.4)	0.40	0.072	0.0001	0.523
$RPE_{leg\ pain}$	6,2 (1,6)	5.1 (1.7) †	1.1 (0.1; 2.2)	6,3 (1,4)	5.1 (1.9) †	1.2 (0.1; 2.2)	0.32	0.964	0.001	0.932
Power ($\dot{W}$)	95 (24)	95 (24)	-	94 (25)	94 (25)	-	-	-	-	-

Values are expressed as mean ± standard deviation.
***: *P* value from the two-way ANOVA are listed as group effect (G), time effect (T), groups x time effect (G x T).
†: Post-hoc test identifies significance ($P \leq 0.05$) in differences between PRE and POST.
#: Post-hoc test identifies significant trend ($0.05 < P \leq 0.1$) in differences between PRE and POST.

Table S1. Pulmonary O_2 uptake ($\dot{V}O_{2p}$) and heart rate (HR) kinetics parameters assessed during double leg moderate constant load exercise (DL-MOD) before (PRE) the endurance training period of the heart transplant (HTx) and non-cardiac transplant recipients (kidney and liver transplanted patients) .

$\dot{V}O_{2p}$ kinetics	HTx (n = 11)	Non-cardiac transplant recipients (n = 18)	P
O2 Def (mL O2)	804 ± 183	680 ± 244	0.160
MRT (sec)	55.8 ± 15.5	50.4 ± 14.8	0.353
*SCamp (mL O2)	214 ± 69	202 ± 73	0.629
HR kinetics	HTx (n = 10)	Non-cardiac transplant recipients (n = 17)	
Baseline	80.1 ± 15.1	74.8 ± 9.3	0.267
Amplitude	21.1 ± 6	25.4 ± 6.3	0.095
Time delay (sec)	20.3 ± 15.3	7.5 ± 6.4	0.005
Time constant (sec)	118.3 ± 79.5	42.4 ± 18.4	0.001
MRT (sec)	138.6 ± 88.1	49.9 ± 20.3	<0.001
95% CI for time constant	87 - 112	36 - 49	-

Values are expressed as mean ± standard deviation, note that $\dot{V}O_{2p}$ slow component amplitude (SCamp) refers to double leg heavy constant load exercise.
*: HTx (n = 13); non-cardiac transplant recipients (n = 20)

Table S2. Pulmonary O_2 uptake ($\dot{V}O_{2p}$) and heart rate (HR) kinetics parameters assessed during double leg moderate constant load exercise (DL-MOD) before (PRE) the endurance training period of the patients taking β-blockade medications and the patients not undergoing β-blockade therapy.

$\dot{V}O_{2p}$ kinetics	β-blockade (n= 12)	Not β-blockade (n= 17)	P
O2 Def (mL O2)	676 ± 157	711 ± 240	0.672
MRT (sec)	48.1 ± 11.8	51.2 ± 14.3	0.556
*SC_{amp} (mL O2)	219 ± 76	197 ± 66	0.370
HR kinetics	β-blockade (n= 10)	Not β-blockade (n= 17)	P
Baseline	69.7 ± 7.8	80.6 ± 12.3	0.019
Amplitude	21.9 ± 4.9	22.5 ± 7.6	0.193
Time delay (sec)	16.3 ± 16.2	10.3 ± 8.9	0.228
Time constant (sec)	71.2 ± 47.1	69.8 ± 68.7	0.955
MRT (sec)	87.5 ± 62.1	80.2 ± 74.1	0.795
95% CI for time constant	55 - 66	55 - 76	-

Values are expressed as mean ± standard deviation, note that $\dot{V}O_{2p}$ slow component amplitude (SC_{amp}) refers to double leg heavy constant load exercise.
*: β-blockade (n = 15); Not β-blockade (n = 18)

REFERENCE

1. Abbiss, C. R., Karagounis, L. G., Laursen, P. B., Peiffer, J. J., Martin, D. T., Hawley, J. A., Fatehee, N. N., Martin, J. C. (2011). Single-leg cycle training is superior to double-leg cycling in improving the oxidative potential and metabolic profile of trained skeletal muscle. *Journal of Applied Physiology*, *110*(5), 1248–1255. https://doi.org/10.1152/japplphysiol.01247.2010
2. Bell, C., Paterson, D. H., Kowalchuk, J. M., Moy, A. P., Thorp, D. B., Noble, E. G., Taylor, A. W., Cunningham, D. A. (2001). Determinants of oxygen uptake kinetics in older humans following single-limb endurance exercise training. *Experimental Physiology*, *86*(5), 659–665. https://doi.org/10.1113/eph8602209
3. Billat, V. L., Richard, R., Binsse, V. M., Koralsztein, J. P., & Haouzi, P. (1998). The V̇O2 slow component for severe exercise depends on type of exercise and is not correlated with time to fatigue. *Journal of Applied Physiology*, *85*(6), 2118–2124. https://doi.org/10.1152/jappl.1998.85.6.2118
4. Borg, G. A. V. (1982). Psychophysical bases of perceived exertion. Med Sci Sports Exerc. 1982;14(5):377-81. *Medicine and Science in Sports and Exercise*.
5. Buchheit, M., & Laursen, P. B. (2013). High-intensity interval training, solutions to the programming puzzle: Part I: Cardiopulmonary emphasis. *Sports Medicine*, *43*(5), 313–338. https://doi.org/10.1007/s40279-013-0029-x
6. Capelli, C., Antonutto, G., Kenfack, M. A., Cautero, M., Lador, F., Moia, C., Tam, E., Ferretti, G. (2006). Factors determining the time course of V̇O2max decay during bedrest: Implications for V̇O2max limitation. *European Journal of Applied Physiology*, *98*(2), 152–160. https://doi.org/10.1007/s00421-006-0252-3
7. Capelli, Carlo, Adami, A., Antonutto, G., Cautero, M., & Tam, E. (2009). Oxygen deficits and oxygen delivery kinetics during submaximal intensity exercise in humans after 14 days of

head-down tilt-bed rest. *European Journal of Applied Physiology*, *107*(1), 51–59. https://doi.org/10.1007/s00421-009-1098-2

8. Carra, J., Candau, R., Keslacy, S., Giolbas, F., Borrani, F., Millet, G. P., Varray, A., Ramonatxo, M. (2003). Addition of inspiratory resistance increases the amplitude of the slow component of O2 uptake kinetics. *Journal of Applied Physiology*, *94*(6), 2448–2455. https://doi.org/10.1152/japplphysiol.00493.2002
9. Ciolac, E. G., Mantuani, S. S., Neiva, C. M., Verardi, C. E. L., Pessôa-Filho, D. M., & Pimenta, L. (2015). Rating of perceived exertion as a tool for prescribing and self regulating interval training: A pilot study. *Biology of Sport*, *32*(2), 103–108. https://doi.org/10.5604/20831862.1134312
10. Colosio, A. L., Caen, K., Bourgois, J. G., Boone, J., & Pogliaghi, S. (2020). Bioenergetics of the VO2 slow component between exercise intensity domains. *Pflugers Archiv European Journal of Physiology*, *472*(10), 1447–1456. https://doi.org/10.1007/s00424-020-02437-7
11. de Souza, J. A. F., Araújo, B. T. S., de Lima, G. H. C., Dornelas de Andrade, A., Campos, S. L., de Aguiar, M. I. R., Carneiro, R. M. D., Brandão, D. C. (2019). Effect of exercise on endothelial function in heart transplant recipients: systematic review and meta-analysis. *Heart Failure Reviews*. https://doi.org/10.1007/s10741-019-09877-z
12. Del Vecchio, A., Casolo, A., Negro, F., Scorcelletti, M., Bazzucchi, I., Enoka, R., Felici, F., Farina, D. (2019). The increase in muscle force after 4 weeks of strength training is mediated by adaptations in motor unit recruitment and rate coding. *Journal of Physiology*, *597*(7), 1873–1887. https://doi.org/10.1113/JP277250
13. Duscha, B. D., Slentz, C. A., Johnson, J. L., Houmard, J. A., Bensimhon, D. R., Knetzger, K. J., & Kraus, W. E. (2005). Effects of exercise training amount and intensity on peak oxygen consumption in middle-age men and women at risk for cardiovascular disease. *Chest*, *128*(4),

2788–2793. https://doi.org/10.1378/chest.128.4.2788

14. Gordon, N., Abbiss, C. R., Maiorana, A. J., James, A. P., Clark, K., Marston, K. J., & Peiffer, J. J. (2019). High-Intensity Single-Leg Cycling Improves Cardiovascular Disease Risk Factor Profile. *Medicine and Science in Sports and Exercise*, *51*(11), 2234–2242. https://doi.org/10.1249/MSS.0000000000002053
15. Grassi, B., Marconi, C., Meyer, M., Rieu, M., & Cerretelli, P. (1997). Gas exchange and cardiovascular kinetics with different exercise protocols in heart transplant recipients. Journal of Applied Physiology, 82(6), 1952–1962. https://doi.org/10.1152/jappl.1997.82.6.1952
16. Grassi, B. (2001). Regulation of oxygen consumption at exercise onset: Is it really controversial? *Exercise and Sport Sciences Reviews*, *29*(3), 134–138. https://doi.org/10.1097/00003677-200107000-00009
17. Grassi B, Porcelli S, Marzorati M, Lanfranconi F, Vago P, Marconi C, Morandi L. Metabolic myopathies: functional evaluation by analysis of oxygen uptake kinetics. Med Sci Sports Exerc. 2009 Dec;41(12):2120-7. doi: 10.1249/MSS.0b013e3181aae96b. PMID: 19915508.
18. Hellsten, Y., & Nyberg, M. (2016). Cardiovascular adaptations to exercise training. *Comprehensive Physiology*, *6*(1), 1–32. https://doi.org/10.1002/cphy.c140080
19. Herman, T., Dall, C., Christensen, S., Goetze, J., Prescott, E., & Gustafsson, F. (2011). Effect of High Intensity Exercise on Peak Oxygen Uptake and Endothelial Function in Long-Term Heart. *American Journal of Transplantation*, *11*(3), 536–541. https://doi.org/10.1111/j.1600-6143.2010.03403.x
20. Holloszy, J. O. (1967). Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *The Journal of Biological Chemistry*, *242*(9), 2278–2282. https://doi.org/10.1016/j.cell.2007.03.044.Asymmetric

21. Jendzjowsky, N. G., Tomczak, C. R., Lawrance, R., Taylor, D. A., Tymchak, W. J., Riess, K. J., Warburton, D. E. R., Haykowsky, M. J. (2007). Impaired pulmonary oxygen uptake kinetics and reduced peak aerobic power during small muscle mass exercise in heart transplant recipients. *Journal of Applied Physiology*, *103*(5), 1722–1727. https://doi.org/10.1152/japplphysiol.00725.2007

22. Jones, A. M., & Carter, H. (2000). The effect of endurance training on parameters of aerobic fitness. / Effet de l'entrainement d'endurance sur les parametres de la capacite aerobie. *Sports Medicine*, *29*(6), 373–386. https://doi.org/10.2165/00007256-200029060-00001

23. Kempeneers, G., Noakes, T. D., van Zyl-Smit, R., Myburgh, K. H., Lambert, M., Adams, B., & Wiggins, T. (1990). Skeletal Muscle Limits the Exercise Tolerance of Renal Transplant Recipients: Effects of a Graded Exercise Training Program. *American Journal of Kidney Diseases*, *16*(1), 57–65. https://doi.org/10.1016/S0272-6386(12)80786-4

24. Klagsbrun, M., & D'Amore, P. (1991). Regulators of angiogenesis. *Annual Review of Physiology*, *53*, 217–239. https://doi.org/10.1146/annurev.physiol.53.1.217

25. Koga, S., Shiojiri, T., Shibasaki, M., Kondo, N., Fukuba, Y., & Barstow, T. J. (1999). Kinetics of oxygen uptake during supine and upright heavy exercise. *Journal of Applied Physiology*, *87*(1), 253–260. https://doi.org/10.1152/jappl.1999.87.1.253

26. Lampert, E., Mettauer, B., Hoppeler, H., Charloux, A., Charpentier, A., & Lonsdorfer, J. (1998). Skeletal muscle response to short endurance training in heart transplant recipients. *Journal of the American College of Cardiology*, *32*(2), 420–426. https://doi.org/10.1016/S0735-1097(98)00227-7

27. Lepretre, P. M., Koralsztein, J. P., & Billat, V. L. (2004). Effect of exercise intensity on relationship between V̇O2max and cardiac output. *Medicine and Science in Sports and Exercise*, *36*(8), 1357–1363. https://doi.org/10.1249/01.MSS.0000135977.12456.8F

28. MacPhee, S. L., Shoemaker, J. K., Paterson, D. H., & Kowalchuk, J. M. (2005). Kinetics of O2 uptake, leg blood flow, and muscle deoxygenation are slowed in the upper compared with lower region of the moderate-intensity exercise domain. *Journal of Applied Physiology*, *99*(5), 1822–1834. https://doi.org/10.1152/japplphysiol.01183.2004

29. Murias, J. M., Kowalchuk, J. M., & Paterson, D. H. (2011). Speeding of VO2 kinetics in response to endurance-training in older and young women. *European Journal of Applied Physiology*, *111*(2), 235–243. https://doi.org/10.1007/s00421-010-1649-6

30. Murias, J. M., Kowalchuk, J. M., & Peterson, D. H. (2010). Speeding of Vo2 kinetics with endurance training in old and young men is associated with improved matching of local O2 delivery to muscle O2 utilization. *Journal of Applied Physiology*, *108*(4), 913–922. https://doi.org/10.1152/japplphysiol.01355.2009

31. Nytrøen K, Rustad LA, Aukrust P, Ueland T, Hallén J, Holm I, Rolid K, Lekva T, Fiane AE, Amlie JP, Aakhus S, Gullestad L. High-intensity interval training improves peak oxygen uptake and muscular exercise capacity in heart transplant recipients. Am J Transplant. 2012 Nov;12(11):3134-42. doi: 10.1111/j.1600-6143.2012.04221.x. Epub 2012 Aug 17. PMID:

32. O'Connell, J. M., Weir, J. M., & MacIntosh, B. R. (2017). Blood lactate accumulation decreases during the slow component of oxygen uptake without a decrease in muscular efficiency. *Pflugers Archiv European Journal of Physiology*, *469*(10), 1257–1265. https://doi.org/10.1007/s00424-017-1986-y

33. Pepper, M. S., Vassalli, J. D., Orci, L., & Montesano, R. (1993). Biphasic effect of transforming growth factor-β1 on in vitro angiogenesis. *Experimental Cell Research*. https://doi.org/10.1006/excr.1993.1043

34. Petrakopoulou, P., Anthopoulou, L., Muscholl, M., Klauss, V., von Scheidt, W., Überfuhr, P., Meiser, B. M., Reichart, B., Weis, M. (2006). Coronary Endothelial Vasomotor Function and

Vascular Remodeling in Heart Transplant Recipients Randomized for Tacrolimus or Cyclosporine Immunosuppression. *Journal of the American College of Cardiology*, *47*(8), 1622–1629. https://doi.org/10.1016/j.jacc.2005.10.073

35. Poole, D. C., Schaffartzik, W., Knight, D. R., Derion, T., Kennedy, B., Guy, H. J., Prediletto, R., Wagner, P. D. (1991). Contribution of exercising legs to the slow component of oxygen uptake kinetics in humans. *Journal of Applied Physiology*, *71*(4), 1245–1253. https://doi.org/10.1152/jappl.1991.71.4.1245
36. Poole, David C., & Jones, A. M. (2012). Oxygen uptake kinetics. *Comprehensive Physiology*, *2*(2), 933–996. https://doi.org/10.1002/cphy.c100072
37. Ramzy, D., Rao, V., Tumiati, L. C., Xu, N., Miriuka, S., Delgado, D., & Ross, H. J. (2005). Tetrahydrobiopterin prevents cyclosporine-induced vasomotor dysfunction. *Transplantation*, *79*(8), 876–881. https://doi.org/10.1097/01.TP.0000157364.80712.45
38. Richard, R., Verdier, J., Doutreleau, S., & Piquard, F. (2005). Exercise Limitation in Trained Heart and Kidney Transplant Recipients : Central and Peripheral Limitations. *J Heart Lung Transplant.*, 1774–1780. https://doi.org/10.1016/j.healun.2005.03.017
39. Rud, B., Foss, Krustrup, P., Secher, N. H., & Hallén, J. (2012). One-legged endurance training: Leg blood flow and oxygen extraction during cycling exercise. *Acta Physiologica*, *205*(1), 177–185. https://doi.org/10.1111/j.1748-1716.2011.02383.x
40. Salvadego, D., Sartorio, A., Agosti, F., Tringali, G., Patrizi, A., Isola, M., LoMauro, A., Aliverti, A., Grassi, B. (2017). Respiratory muscle endurance training reduces the O2 cost of cycling and perceived exertion in obese adolescents. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *313*(4), R487–R495. https://doi.org/10.1152/ajpregu.00396.2016
41. Skattebo, Ø., Capelli, C., B.Rud, Auensen, M., Calbet, J. A. L., & Hallén, J. (2020). Increased

oxygen extraction and mitochondrial protein expression after small muscle mass endurance training. *Scandinavian Journal of Medicine & Science in Sports*, *30*(9), 1615–1631. https://doi.org/10.1111/sms.13707

42. Saltin, B., Nazar, K., Costill, D. L., Stein, E., Jansson, E., Essén, B. and Gollnick, P. D. (1976) The Nature of the Training Response; Peripheral and Central Adaptations to One-Legged Exercise, *Acta Physiol. Scand.*, 96(3), 289–305. doi: 10.1111/j.1748-1716.1976.tb10200.x.
43. Tomczak, C. R., Tymchak, W. J., & Haykowsky, M. J. (2013). Effect of exercise training on pulmonary oxygen uptake kinetics in heart transplant recipients. *American Journal of Cardiology*, *112*(9), 1489–1492. https://doi.org/10.1016/j.amjcard.2013.06.037
44. Tomczak, C. R., Warburton, D. E. R., Riess, K. J., Jendzjowsky, N. G., Esch, B. T., Liang, Y., Haennel, R. G., Haykowsky, M. J. (2008). Pulmonary oxygen uptake and heart rate kinetics during the six-minute walk test in transplant recipients. *Transplantation*, *85*(1), 29–35. https://doi.org/10.1097/01.tp.0000296056.00863.f0
45. Whipp, B. J., Davis, J. A., Torres, F., & Wasserman, K. (1981). A test to determine parameters of aerobic function during exercise. *Journal of Applied Physiology Respiratory Environmental and Exercise Physiology*, *50*(1), 217–221. https://doi.org/10.1152/jappl.1981.50.1.217
46. Williams, T. J., & McKenna, M. J. (2012). Exercise limitation following transplantation. *Comprehensive Physiology*, *2*(3), 1937–1979. https://doi.org/10.1002/cphy.c110021

GENERAL CONCLUSIONS

The findings from the first investigation showed that single and double leg cycling training are equally effective to improve the whole-body $\dot{V}O_{2max}$ in SOT; hoverer, even if no differences were found between the exercise typologies, the adaptive mechanisms behind the improvement might differ according to the training modality. Additionally, considering these results together with the one obtained from the multifactorial model of $\dot{V}O_{2max}$ limitation, it can be suggested that the limitation of whole-body $\dot{V}O_{2max}$ is equally distributed between central and peripheral factors in HTx, KTx and LTx; thus, contrary to the healthy condition, skeletal muscle might play a remarkable role in impairing maximal exercise capacity in this clinical population. The findings from the second investigation showed that single leg cycling training is as valid as double leg cycling to improve i) the tolerance to heavy exercise intensities, ii) to decrease the amplitude of $\dot{V}O_2$sc and iii) to accelerate MRT and reducing the O_2Def during moderate intensity exercise. Unfortunately, since HR kinetics were faster after the training program and no difference were reported in the magnitude of the improvement between the two groups, it is not possible to make inferences whether the ameliorations originated by an enhanced O_2 availability or an improved O_2 utilization at muscular level.

In conclusion, small muscle mass endurance training has been showed to be as valid as large muscle mass endurance training to improve maximal and submaximal exercise capacities in HTx, LTx and KTx; therefore, it may represent a useful training modality in this clinical population. Finally, these findings taken together, further points towards the hypothesis that skeletal muscle, when negatively affected by deconditioning and/or the side effects of pharmacological therapies, likely provide a remarkable limitation to the exercise capacity in HTx. LTx and KTx.

CHAPTER III

Aim of chapter III

1) The first work related to this section of the thesis evaluated the reliability between two non-invasive methods for the determination of cardiac output during exercise. Furthermore, the repeatability of the two devices at submaximal and supramaximal cycling exercise intensities was investigated, since it is pivotal in order to studythe cardiovascular adaptations in response to training, detraining and/or pharmacological therapies. The two non-invasive techniques at stake are the new cardio-impedance, by means of Physioflow^{TM}, and the inert-gas rebreathing, through Innocor^{TM}.

2) The second work related to this section of the thesis aimed to apply the repeated occlusions method to determine noninvasively, by near-infrared spectroscopy, for the assessment of the skeletal muscle $\dot{V}O2$ recovery kinetics following standard cycle ergometer exercise of different intensities. Moreover, the pulmonary $\dot{V}O2$ and muscle $\dot{V}O2$ kinetics of the recovery phase were compared in order to disclose a possible dissociation between these two parameters (i.e. faster muscle $\dot{V}O2$ vs pulmonary $\dot{V}O2$ kinetics).

3) The third investigation regarding this section of the thesis sought to evaluate the level of agreement, of cardiac output measured non-invasively with Physioflow^{TM}, between three different exhaustive cycling exercises, namely incremental, supramaximal constant load and very heavy constant load tests. Furthermore, the repeatability of the cardiac output assessment, in the exhaustive cycling exercises at stake, was investigated.

Main results of chapter III

1) The Bland-Altman plot revealed that difference (i.e. the bias) between the cardiac output assessed by Physioflow^{TM} minus the corresponding value obtained by Innocor^{TM}), amounted to -0.65 L and it was significantly different from zero. Moreover, the differences at stake were

moderately correlated with their corresponding means, indicating a linear relationship between the amplitude of the error and the absolute values of the measurements so that Innocor™ tended to overestimate cardiac output in comparison with Physioflow™ for values of cardiac output ranging between 10.0 and 15.0 $L \cdot min^{-1}$ and to underestimate it for larger values corresponding o higher sub-maximal exercise intensities. In addition, the grand averages of the slopes of the individual linear regressions between sub-maximal, steady–state $\dot{V}O_2$ and $\dot{Q}$ were significantly different and amounted to 5.1 ± 1.0 $L \cdot min^{-1}$ and 3.4 ± 0.6 $L \cdot min^{-1}$ for Physioflow™ and Innocor™, respectively. The slopes reported for the cardio-impedance are very similar to the one reported for the gold-standard, invasive cardiac output assessment (i.e. 4.5 to 6 $L \cdot min^{-1}$), on the contrary the ones calculated for Innocor™ seems to be inferior to the reference values The determination of cardiac output with Physioflow™was characterized by a good repeatability compared to Innocor™, since it was reported a coefficient of variability by about 8% regardless the intensity of exercise. Indeed, for Innocor™ the coefficient of variability amounted to 7.7 for submaximal workload and to 27.7% for supramaximal intensity. Therefore, Physioflow™ might represent a good alternative to the non-invasive techniques for the determination of cardiac output in healthy population, at least during exercise at submaximal intensity (For further details the reader is kindly referred to the published article located after the *"REFRENCES"* section from page 135)

2) The results indicate the feasibility of the determination of muscle $\dot{V}O_2$ kinetics of the recovery phase following cycling exercise test at moderate, heavy and maximal intensities; providing a valuable functional assessment technique of skeletal muscle oxidative metabolism. Furthermore, the results suggest an intensity domain dependency of the time constant of muscle $\dot{V}O_2$ kinetics of the recovery phase (For further details the reader is kindly referred to the published article located after the *"REFRENCES"* section from page 136).

3) The collected data are still under supervision and analysis, thus it would be premature to draw any suggestion and/ or conclusion about the reliability and the repeatability of Physioflow™ used to

determine cardiac output during incremental, supramaximal constant load and very heavy constant load tests.

REFERENCES

1. Andersen, P., & Henriksson, J. (1977). Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. *The Journal of Physiology*, *270*(3), 677–690.
2. Arora, S., Aukrust, P., Andreassen, A., Simonsen, S., Gude, E., Grov, I., … Gullestad, L. (2009). The prognostic importance of modifiable risk factors after heart transplantation. *American Heart Journal*, *158*(3), 431–436. https://doi.org/10.1016/j.ahj.2009.05.036
3. Åstrand, P. O., Rodahl, K., Dahl, H. A., &Strømme, S. B. (2003). Textbook of work physiology. Physiologicalbases of exercise, 4th ed., Human Kinetics, Champaign.
4. Barker, A. R., Welsman, J. R., Fulford, J., Welford, D., & Armstrong, N. (2010). Quadriceps muscle energeticsduring incremental exercise in children and adults. *Medicine and Science in Sports and Exercise*, *42*(7),1303–1313. https://doi.org/10.1249/MSS.0b013e3181cabaeb
5. Bassett, D., & Howley, E. T. (2000). Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Medicine & Science in Sports & Exercise*, *32*(1), 70–84. https://doi.org/10.1097/00005768-200001000-00012
6. Bjørgen, S., Hoff, J., Husby, V. V. S., Richardson, R. S., & Helgerud, J. (2009). Aerobic high intensity one and two legs interval cycling in chronic obstructive pulmonary disease : The sum of the parts is greater than the whole Aerobic high intensity one and two legs interval cycling in chronic obstructive pulmonarydisease : the sum. *Eur J Appl Physiol*, *106*(4), 501–507. https://doi.org/10.1007/s00421-009-1038-1
7. Bodziak, K. A., & Hricik, D. E. (2009). New-onset diabetes mellitus after solid organ transplantation. *Transplant International*, *22*(5), 519–530. https://doi.org/10.1111/j.1432-2277.2008.00800.x
8. Borst, S. E., Conover, C. F., & Bagby, G. J. (2005). Association of resistin with visceral fat and

muscle insulinresistance. *Cytokine*, *32*(1), 39–44. https://doi.org/10.1016/j.cyto.2005.07.008

9. Braith, R. W., Magyari, P. M., Pierce, G. L., Edwards, D. G., Hill, J. A., White, L. J., & Aranda, J. M. (2005). Effect of resistance exercise on skeletal muscle myopathy in heart transplant recipients. *American Journal of Cardiology*, *95*(10), 1192–1198. https://doi.org/10.1016/j.amjcard.2005.01.048
10. Bringard, A., Pogliaghi, S., Adami, A., De Roia, G., Lador, F., Lucini, D., … Ferretti, G. (2010). Cardiovascular determinants of maximal oxygen consumption in upright and supine posture at the end of prolonged bed rest in humans. *Respiratory Physiology and Neurobiology*, *172*(1–2), 53–62. https://doi.org/10.1016/j.resp.2010.03.018
11. Brunzell, J. D., Davidson, M., Furberg, C. D., Goldberg, R. B., Howard, B. V., Stein, J. H., & Witztum, J. L. (2008). Lipoprotein Management in Patients With Cardiometabolic Risk. Consensus Conference Report From the American Diabetes Association and the American College of Cardiology Foundation. *Journal of the American College of Cardiology*, *51*(15), 1512–1524. https://doi.org/10.1016/j.jacc.2008.02.034
12. Bussières, L. M., Pflugfelder, P. W., Taylor, A. W., Noble, E. G., & Kostuk, W. J. (1997). Changes in skeletal muscle morphology and biochemistry after cardiac transplantation. *American Journal of Cardiology*, *79*(5), 630–634. https://doi.org/10.1016/S0002-9149(96)00829-6
13. Capelli, C., Antonutto, G., Kenfack, M. A., Cautero, M., Lador, F., Moia, C., … Ferretti, G. (2006). Factors determining the time course of V̇ O2max decay during bedrest: Implications for V̇ O2max limitation. *European Journal of Applied Physiology*, *98*(2), 152–160. https://doi.org/10.1007/s00421-006-0252-3
14. Cerretelli, P. (1976). Limiting factors to oxygen transport on Mount Everest. *Journal of Applied Physiology*, *40*(5), 658–667. https://doi.org/10.1152/jappl.1976.40.5.658
15. Costa, E. C., Hay, J. L., Kehler, D. S., Boreskie, K. F., Arora, R. C., Umpierre, D., … Duhamel, T. A. (2018). Effects of High-Intensity Interval Training Versus Moderate-Intensity

Continuous Training On Blood Pressure in Adults with Pre- to Established Hypertension: A Systematic Review and Meta-Analysis of Randomized Trials. *Sports Medicine*, *48*(9), 2127–2142. https://doi.org/10.1007/s40279-018-0944-y

16. Costill, D. L., Daniels, J., Evans, W., Fink, W., Krahenbuhl, G., & Saltin, B. (1976). Skeletal muscle enzymes and fiber composition in male and female track athletes. *Journal of Applied Physiology*, *40*(2), 149–154.https://doi.org/10.1152/jappl.1976.40.2.149
17. Cruz-Jentoft, A. J., Landi, F., Schneider, S. M., Zúñiga, C., Arai, H., Boirie, Y., … Cederholm, T. (2014). Prevalence of and interventions for sarcopenia in ageing adults: A systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS). *Age and Ageing*, *43*(6), 48–759.https://doi.org/10.1093/ageing/afu115
18. Cruz-Jentoft, A. J., & Sayer, A. A. (2019). Sarcopenia. *The Lancet*, *393*(10191), 2636–2646.https://doi.org/10.1016/S0140-6736(19)31138-9
19. Dempsey, J. A., Hanson, P. G., & Henderson, K. S. (1984). Exercise-induced arterial hypoxaemia in healthy human subjects at sea level. *The Journal of Physiology*, *355*(1), 161–175.
20. Dempsey, J. A. (1986). Is the lung built for exercise? Med. Sci. Sports Exerc, 18(2), 143–155.
21. di Prampero, P. E. (2003). Factors limiting maximal performance in humans. *European Journal of AppliedPhysiology*, *90*(3–4), 420–429. https://doi.org/10.1007/s00421-003-0926z
22. di Prampero, P. E., & Ferretti, G. (1990). Factors limiting maximal oxygen consumption in humans. *Respiration Physiology*, *80*(2–3), 113–128. https://doi.org/10.1016/0034-5687(90)90075-A
23. Dolmage, T. E., & Goldstein, R. S. (2008). Effects of one-legged exercise training of patients with COPD. *Chest*, *133*(2), 370–376. https://doi.org/10.1378/chest.07-1423
24. Duncan, G. E., Howley, E. T., & Johnson, B. N. (1997). Applicability of V'O2max criteria: discontinuous versus continuous protocols. Med. Sci. Sports Exerc, 29(2), 273-278.
25. Englesbe, M. J., Patel, S. P., He, K., Lynch, R. J., Schaubel, D. E., Harbaugh, C., …

Sonnenday, C. J. (2010). Sarcopenia and mortality after liver transplantation. *Journal of the American College of Surgeons*, *211*(2), 271–278. https://doi.org/10.1016/j.jamcollsurg.2010.03.039

26. Esposito, F, Reese, V., Shabetai, R., Wagner, P., & Richardson, R. (2011). Isolated quadriceps training increases maximal exercise capacity in chronic heart failure: the role of skeletal muscle convective and diffusive oxygen transport. *J Am Coll Cardiol*, *58*(13), 1353–1362. https://doi.org/10.1038/jid.2014.371

27. Esposito, Fabio, Mathieu-Costello, O., Shabetai, R., Wagner, P. D., & Richardson, R. S. (2010). Limited maximal exercise capacity in patients with chronic heart failure: Partitioning the contributors. *Journal of the American College of Cardiology*, *55*(18), 1945–1954. https://doi.org/10.1016/j.jacc.2009.11.086

28. Ferretti, G. (2014). Maximal oxygen consumption in healthy humans: theories and facts. *European Journal of Applied Physiology*, *114*(10), 2007–2036. https://doi.org/10.1007/s00421-014-2911-0

29. Ferretti, G., & Capelli, C. (2009a). Maximal O 2 consumption: Effects of gravity withdrawal and resumption. *Respiratory Physiology and Neurobiology*, *169*(SUPPL.), 50–54. https://doi.org/10.1016/j.resp.2009.03.012

30. Ferretti, G., & Capelli, C. (2009b). Maximal O2 consumption: Effects of gravity withdrawal and resumption. *Respiratory Physiology and Neurobiology*, *169*(SUPPL.), 50–54. https://doi.org/10.1016/j.resp.2009.03.012

31. Fontana, L., Eagon, J. C., Trujillo, M. E., Scherer, P. E., & Klein, S. (2007). Systemic Inflammation in Obese Humans. *Diabetes*, *56*(April), 1010–1013. https://doi.org/10.2337/db06-1656.CRP

32. Fontana, L., Villareal, D. T., Weiss, E. P., Racette, S. B., Steger-May, K., Klein, S., & Holloszy, J. O. (2007). Calorie restriction or exercise: Effects on coronary heart disease risk factors. A randomized, controlled trial. *American Journal of Physiology - Endocrinology and Metabolism*, *293*(1), 197–202. https://doi.org/10.1152/ajpendo.00102.2007

33. Gill, J. S. (2008). Cardiovascular disease in transplant recipients: Current and future treatment strategies. *Clinical Journal of the American Society of Nephrology*, *3*(SUPPL. 2), 29–37. https://doi.org/10.2215/CJN.02690707

34. Gledhill, N. (1982). Blood doping and related issues: A brief review. *Medicine and Science in Sports and Exercise*, *14*(3), 183–189. https://doi.org/10.1249/00005768-198203000-00005

35. Global Recommendations on Physical Activity for Health. Geneva: World Health Organization; 2010. Available from: https://www.ncbi.nlm.nih.gov/books/NBK305057/

36. Gordon, D., Mehter, M., Gernigon, M., Caddy, O., Keiller, D., & Barnes, R. (2012). The effects of exercise modality on the incidence of plateau at V·O2max. *Clinical Physiology and Functional Imaging*, *32*(5), 394–399. https://doi.org/10.1111/j.1475-097X.2012.01142.x

37. Gordon, N., Abbiss, C. R., Maiorana, A. J., James, A. P., Clark, K., Marston, K. J., & Peiffer, J. J. (2019). High-Intensity Single-Leg Cycling Improves Cardiovascular Disease Risk Factor Profile. *Medicine and Science in Sports and Exercise*, *51*(11), 2234–2242. https://doi.org/10.1249/MSS.0000000000002053

38. Grassi, B., Marconi, C., Meyer, M., Rieu, M., & Cerretelli, P. (1997). Gas exchange and cardiovascular kineticswith different exercise protocols in heart transplant recipients. *Journal of Applied Physiology*, *82*(6), 1952–1962. https://doi.org/10.1152/jappl.1997.82.6.1952

39. Green, S., & Askew, C. (2018). VO2peak is an acceptable estimate of cardiorespiratory fitness but not VO2max. J Appl Physiol, 125(1), 229–232. https://doi.org/10.1152/japplphysiol.00850.2017

40. Hawkins, M. N., Raven, P. B., Snell, P. G., Stray-Gundersen, J., & Levine, B. D. (2007). Maximal oxygen uptake as a parametric measure of cardiorespiratory capacity. *Medicine and Science in Sports and Exercise*, *39*(1), 103–107. https://doi.org/10.1249/01.mss.0000241641.75101.64

41. Heisel, O., Heisel, R., Balshaw, R., & Keown, P. (2004). New Onset Diabetes Mellitus in Patients Receiving Calcineurin Inhibitors: A Systematic Review and Meta-Analysis. *American Journal of Transplantation*,*4*(4), 583–595. https://doi.org/10.1046/j.1600-6143.2003.00372.x

42. Hellsten, Y., & Nyberg, M. (2016). Cardiovascular adaptations to exercise training. *Comprehensive Physiology*, *6*(1), 1–32. https://doi.org/10.1002/cphy.c140080

43. Helmrich, S. P., Ragland, D. R., & Paffenbarger, R. S. (1994). Prevention of non—insulin-dependent diabetesmellitus with physical activity. *Medicine & Science in Sports & Exercise*, *26*(7), 824–830.

44. Herman, T., Dall, C., Christensen, S., Goetze, J., Prescott, E., & Gustafsson, F. (2011). Effect of High IntensityExercise on Peak Oxygen Uptake and Endothelial Function in Long-Term Heart. *American Journal of Transplantation*, *11*(3), 536–541. https://doi.org/10.1111/j.1600-6143.2010.03403.x

45. Hill, A. V., & Lupton, H. (1923). Muscular exercise, lactic acid, and the supply and utilization of oxygen. *QJM: An International Journal of Medicine*, *os-16*(62), 135–171. https://doi.org/10.1093/qjmed/os- 16.62.135

46. Hokanson, J. F., Mercier, J. G., & Brooks, G. A. (1995). Cyclosporine A decreases rat skeletal muscle mitochondrial respiration in vitro. *American Journal of Respiratory and Critical Care Medicine*, *151*(6),1848–1851. https://doi.org/10.1164/ajrccm.151.6.7767529

47. Hoppeler, H. (1990). The different relationship of VO2max to muscle mitochondria in humans and quadrupedal animals. *Respiration Physiology*, *80*(2–3), 137–145.

48. Hoppeler, H., & Weibel, E. R. (2000). Structural and functional limits for oxygen supply to to muscle. *Acta Physiologica Scandinavica*, *168*(4), 445–456. https://doi.org/10.1046/j.1365-201X.2000.00696.x

49. Howley, E. T., Bassett, D. R., & Welch, H. G. (1995). Criteria for maximal oxygen uptake: review and commentary. *Medicine and Science in Sports and Exercise*. https://doi.org/10.1249/00005768- 199509000-00009

50. Iannetta, D., Azevedo, R. D. A., Ingram, C. P., Keir, D. A., & Murias, J. M. (2020). Evaluating the suitability of supra-POpeak verification trials after ramp-incremental exercise to confirm the attainment of maximum O2 uptake. Am J Physiol Regul Integr Comp Physiol, 319(3), 315–322. https://doi.org/10.1152/ajpregu.00126.2020

51. Jenssen, T., & Hartmann, A. (2019). Post-transplant diabetes mellitus in patients with solid organ transplants. *Nature Reviews Endocrinology*, *15*(3), 172–188. https://doi.org/10.1038/s41574-018-0137-7

52. Johnston SD, Morris JK, Cramb R, Gunson BK, Neuberger J. Cardiovascular morbidity and mortality after orthotopic liver transplantation. Transplantation. 2002 Mar 27;73(6):901-6. doi: 10.1097/00007890- 200203270-00012. PMID: 11923689.

53. Joyner, M. J., & Casey, D. P. (2015). Regulation of Increased Blood Flow (Hyperemia) to Muscles During Exercise: A Hierarchy of Competing Physiological Needs. *Physiol Rev*, *95*(2), 549–601. https://doi.org/10.1152/physrev.00035.2013

54. Kaiser, P., Tesch, P., Frisk-Holmberg, M., Juhlin-Dannfelt, A., & Kaijser, L. (1986). Effect of β1-selective and non-selective β-blockade on work capacity and muscle metabolism. *Clinical Physiology*, *6*(2), 197–207. https://doi.org/10.1111/j.1475-097X.1986.tb00069.x

55. Kallwitz, E. R. (2015). Sarcopenia and liver transplant: The relevance of too little muscle mass. *World Journal of Gastroenterology*, *21*(39), 10982–10993. https://doi.org/10.3748/wjg.v21.i39.10982

56. Kempeneers, G., Noakes, T. D., van Zyl-Smit, R., Myburgh, K. H., Lambert, M., Adams, B., & Wiggins, T. (1990). Skeletal Muscle Limits the Exercise Tolerance of Renal Transplant Recipients: Effects of a Graded Exercise Training Program. *American Journal of Kidney Diseases*, *16*(1), 57–65. https://doi.org/10.1016/S0272-6386(12)80786-4

57. Kenney, M. J., & Seals, D. R. (1993). Postexercise Hypotension Key Features, Mechanisms, and Clinical Significance. *Hypertension*, *22*(5), 653–664.

58. Kilroe SP, Fulford J, Jackman SR, VAN Loon LJC, Wall BT. Temporal Muscle-specific Disuse Atrophy during One Week of Leg Immobilization. Med Sci Sports Exerc. 2020 Apr;52(4):944-954. doi: 10.1249/MSS.0000000000002200. PMID: 31688656.

59. Klausen, K., Andersen, L. B., & Pelle, I. (1981). Adaptive changes in work capacity, skeletal muscle capillarization and enzyme levels during training and detraining. *Acta Physiologica Scandinavica*, *113*(1), 9–16. https://doi.org/10.1111/j.1748-1716.1981.tb06854.x

60. Komi, P. V, & Karlson, J. (1978). Skeletal muscle fibre types , enzyme activities and physical performance inyoung males and females. *Acta Physiologica*, *103*(2), 210–218.

61. Lampert, E., Mettauer, B., Hoppeler, H., Charloux, A., Charpentier, A., & Lonsdorfer, J. (1996). Structure of skeletal muscle in heart transplant recipients. *Journal of the American College of Cardiology*, *28*(4), 980–984. https://doi.org/10.1016/S0735-1097(96)00272-0

62. Lampert, E., Mettauer, B., Hoppeler, H., Charloux, A., Charpentier, A., & Lonsdorfer, J. (1998). Skeletal muscle response to short endurance training in heart transplant recipients. *Journal of the American College of Cardiology*, *32*(2), 420–426. https://doi.org/10.1016/S0735-1097(98)00227-7

63. Leal, J. M., Galliano, L. M., & Del Vecchio, F. B. (2020). Effectiveness of High-Intensity Interval Training Versus Moderate-Intensity Continuous Training in Hypertensive Patients: a Systematic Review and Meta-Analysis. *Current Hypertension Reports*, *22*(3). https://doi.org/10.1007/s11906-020-1030-z

64. Levine, B. D. (2008). VO2,max: What do we know, and what do we still need to know? *The Journal of Physiology Online*, *586*(1), 25–34. https://doi.org/10.1113/jphysiol.2007.147629

65. MacPhee, S. L., Shoemaker, J. K., Paterson, D. H., & Kowalchuk, J. M. (2005). Kinetics of O2 uptake, leg blood flow, and muscle deoxygenation are slowed in the upper compared with lower region of the moderate-intensity exercise domain. *Journal of Applied Physiology*, *99*(5), 1822–1834. https://doi.org/10.1152/japplphysiol.01183.2004

66. Malina, R., Bouchard, C., & Bar-Or, O. (2004). Growth, maturation and physical activity., 2nd ed., United States, Champaign: Human Kinetics.

67. McArdle, W. D., Katch, F. I., Katch, V. L. (2010). Exercise physiology, nutrition, energy, and human performance., 7th ed., Philadelphia: Wolters Kluwer - Lippincott Williams and Wilkins.

68. Mercier, J. G., Hokanson, J. F., & Brooks, G. A. (1995). Effects of cyclosporine A on skeletal

muscle mitochondrial respiration and endurance time in rats. *American Journal of Respiratory and Critical Care Medicine*, *151*(5), 1532–1536. https://doi.org/10.1164/ajrccm.151.5.7735611

69. Migliavacca, E., Tay, S.K.H., Patel, H.P. et al. Mitochondrial oxidative capacity and NAD+ biosynthesis are reduced in human sarcopenia across ethnicities. Nat Commun 10, 5808 (2019). https://doi.org/10.1038/s41467-019-13694-1
70. Miller, W., Koceja, D., & Hamilton, E. (1997). A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention. *International Journal of Obesity*, *21*(10), 941–947.
71. Moya-Nájera, D., Moya-Herraiz, Á., Compte-Torrero, L., Hervás, D., Borreani, S., Calatayud, J., … Colado, J. C. (2017). Combined resistance and endurance training at a moderate-to-high intensity improves physical condition and quality of life in liver transplant patients. *Liver Transplantation*, *23*(10), 1273– 1281. https://doi.org/10.1002/lt.24827
72. Mulder E, Clément G, Linnarsson D, Paloski WH, Wuyts FP, Zange J, Frings-Meuthen P, Johannes B, Shushakov V, Grunewald M, Maassen N, Buehlmeier J, Rittweger J. Musculoskeletal effects of 5 days of bed rest with and without locomotion replacement training. Eur J Appl Physiol. 2015 Apr;115(4):727-38. doi: 10.1007/s00421-014-3045-0. Epub 2014 Nov 26. PMID: 25425257; PMCID: PMC4359292.
73. Munch, G. W., Iepsen, U. W., Ryrsø, C. K., Rosenmeier, J. B., Pedersen, B. K., & Mortensen, S. P. (2018). Effect of 6 wk of high-intensity one-legged cycling on functional sympatholysis and ATP signaling in patients with heart failure. *American Journal of Physiology - Heart and Circulatory Physiology*, *314*(3),H616–H626. https://doi.org/10.1152/ajpheart.00379.2017
74. Murias, J. M., Pogliaghi, S., & Paterson, D. H. (2018). Measurement of a true V·O2max during a ramp incremental test is not confirmed by a verification phase. *Frontiers in Physiology*, *9*(143), 1–8. https://doi.org/10.3389/fphys.2018.00143
75. Myers, J., Prakash, M., Froelicher, V., Do, D., Partington, S., & Atwood, E. J. (2002).

Exercise Capacity and Mortality among Men Referred for Exercise Testing. *The New England Jpurnal of Medicine*, *346*(11), 793–801.

76. Myers J, Kaykha A, George S, Abella J, Zaheer N, Lear S, Yamazaki T, Froelicher V. Fitness versus physical activity patterns in predicting mortality in men. Am J Med. 2004 Dec 15;117(12):912-8. doi: 10.1016/j.amjmed.2004.06.047. PMID: 15629729.

77. Nytrøen, K., Rustad, L. A., Aukrust, P., Ueland, T., Hallén, J., Holm, I., … Gullestad, L. (2012). High-intensity interval training improves peak oxygen uptake and muscular exercise capacity in heart transplant recipients. *American Journal of Transplantation*, *12*(11), 3134–3142. https://doi.org/10.1111/j.1600- 6143.2012.04221.x

78. Olsen, R. H., Krogh-Madsen, R., Thomsen, C., Booth, F. W., & Pedersen, B. K. (2008). Metabolic Responsesto Reduced Daily Steps. *JAMA*, *299*(11), 1261–1263.

79. Painter, P., Taylor, J., Wolcott, S., Krasnoff, J., Adey, D., Tomlanovich, S., … Topp, K. (2003). Exercise capacity and muscle structure in kidney recipient and twin donor. *Clinical Transplantation*, *17*(3), 225–230. https://doi.org/10.1034/j.1399-0012.2003.00032.x

80. Possamai, L. T., Campos, F. D. S., Cesar, P., Aguiar, R. A. De, Guilherme, L., Guglielmo, A., & de Lucas, R. D. (2020). Similar maximal oxygen uptake assessment from a step cycling incremental test and verification tests on the same or different day. Appl Physiol Nutr Metab, 45(4), 357–361.

81. Pescatello, L. S., & Kulikowich, J. M. (2001). The aftereffects of dynamic exercise on ambulatory blood pressure. *Medicine and Science in Sports and Exercise*, *33*(11), 1855–1861.https://doi.org/10.1097/00005768-200111000-00009

82. Pescatello, Linda S., MacDonald, H. V., Lamberti, L., & Johnson, B. T. (2015). Exercise for Hypertension: A Prescription Update Integrating Existing Recommendations with Emerging Research. *Current Hypertension Reports*, *17*(11). https://doi.org/10.1007/s11906-015-0600-y

83. Petrakopoulou, P., Anthopoulou, L., Muscholl, M., Klauss, V., von Scheidt, W., Überfuhr, P., … Weis, M. (2006). Coronary Endothelial Vasomotor Function and Vascular Remodeling in Heart Transplant Recipients Randomized for Tacrolimus or Cyclosporine

Immunosuppression. *Journal of the American College of Cardiology*, *47*(8), 1622–1629. https://doi.org/10.1016/j.jacc.2005.10.073

84. Poole, D. C., & Jones, A. M. (2012). Oxygen uptake kinetics. *Comprehensive Physiology*, *2*(2), 933–996. https://doi.org/10.1002/cphy.c100072

85. Ramzy, D., Rao, V., Tumiati, L. C., Xu, N., Miriuka, S., Delgado, D., & Ross, H. J. (2005). Tetrahydrobiopterin prevents cyclosporine-induced vasomotor dysfunction. *Transplantation*, *79*(8), 876–881. https://doi.org/10.1097/01.TP.0000157364.80712.45

86. Richard, R., Verdier, J., Doutreleau, S., & Piquard, F. (2005). Exercise Limitation in Trained Heart and Kidney Transplant Recipients : Central and Peripheral Limitations. *J Heart Lung Transplant.*, 1774–1780. https://doi.org/10.1016/j.healun.2005.03.017

87. Riess, K. J., Haykowsky, M., Lawrance, R., Tomczak, C. R., Welsh, R., Lewanczuk, R., … Gourishankar, S. (2014). Exercise training improves aerobic capacity , muscle strength , and quality of life in renal transplant recipients, *39*(5), 566–571.

88. Rud, B., Foss, Krustrup, P., Secher, N. H., & Hallén, J. (2012). One-legged endurance training: Leg blood flow and oxygen extraction during cycling exercise. *Acta Physiologica*, *205*(1), 177–185. https://doi.org/10.1111/j.1748-1716.2011.02383.x

89. Saltin B. Hemodynamic adaptations to exercise. Am J Cardiol. 1985 Apr 26;55(10):42D-47D. doi: 10.1016/0002-9149(85)91054-9. PMID: 3993550.

90. Shin, J., Syme, C., Wang, D., Richer, L., Pike, G. B., Gaudet, D., … Pausova, Z. (2019). Novel Genetic Locus of Visceral Fat and Systemic Inflammation. *Journal of Clinical Endocrinology and Metabolism*, *104*(9),3735–3742. https://doi.org/10.1210/jc.2018-02656

91. Skattebo, Ø., Capelli, C., B.Rud, Auensen, M., Calbet, J. A. L., & Hallén, J. (2020). Increased oxygen extraction and mitochondrial protein expression after small muscle mass endurance training. *Scandinavian Journal of Medicine & Science in Sports*, *30*(9), 1615–1631. https://doi.org/10.1111/sms.13707

92. Stephenson, A. L., Yoshida, E. M., Abboud, R. T., Fradet, G., & Levy, R. D. (2001). Impaired exercise performance after successfull liver transplantation. *Transplantation*, *72*(6),

1161–1164. https://doi.org/10.5860/rusq.50n1.102

93. Stratom, J. R., Kemp, G. J., Daly, R. C., Yacoub, M., & Rajagopalan, B. (1994). Effects of cardiac transplantation on bioenergetic abnormalities of skeletal muscle in congestive heart failure. *Circulation*,*89*(4), 1624–1631. https://doi.org/10.1161/01.cir.89.4.1624

94. Suzuki Y, Murakami T, Haruna Y, Kawakubo K, Goto S, Makita Y, Ikawa S, Gunji A. Effects of 10 and 20 days bed rest on leg muscle mass and strength in young subjects. Acta Physiol Scand Suppl. 1994;616:5-18. PMID: 8042525.

95. Talbot, L. a, Metter, E. J., & Fleg, J. L. (2000). Leisure-time physical activities and their relationship to cardiorespiratory fitness in healthy men and women 18-95 years old. *Medicine and Science in Sports and Exercise*, *32*(2), 417–425. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10694126 Tanner, R., & Gore, C. (2013). Physiological tests for elite athletes: Human Kinetics.

96. Tomczak, C. R., Warburton, D. E. R., Riess, K. J., Jendzjowsky, N. G., Esch, B. T., Liang, Y., … Haykowsky, M. J. (2008). Pulmonary oxygen uptake and heart rate kinetics during the six-minute walk test in transplant recipients. *Transplantation*, *85*(1), 29–35. https://doi.org/10.1097/01.tp.0000296056.00863.f0

97. Tyni-Lenné, R., Gordon, A., Jansson, E., Bermann, G., & Sylvén, C. (1997). Skeletal muscle endurance training improves peripheral oxidative capacity, exercise tolerance, and health-related quality of life in women with chronic congestive heart failure secondary to either ischemic cardiomyopathy or idiopathic dilated cardiomyopat. *American Journal of Cardiology*, *80*(8), 1025–1029. https://doi.org/10.1016/S0002-9149(97)00597-3

98. Tyni-Lenné, R., Gordon, A., Jensen-Urstad, M., Dencker, K., Jansson, E., & Sylvén, C. (1999). Aerobic training involving a minor muscle mass shows greater efficiency than training involving a major muscle mass in chronic heart failure patients. *Journal of Cardiac Failure*, *5*(4), 300–307. https://doi.org/10.1016/S1071-9164(99)91334-9

99. Urs, S., Vissing, S. F., J, M. B., Rollins, J. A., Tindall, R. S., Ring, S., … Victor, R. G. (1990). Cyclosporine- Induced Sympathetic Activation and Hypertension after Heart

Transplantation. *The New English Journalof Medicine*, *323*(16), 1120–1123.

100. Van Den Ham, E. C. H., Kooman, J. P., Schols, A. M. W. J., Nieman, F. H. M., Does, J. D., Franssen, F. M. E., … Van Hooff, J. P. (2005). Similarities in skeletal muscle strength and exercise capacity between renal transplant and hemodialysis patients. *American Journal of Transplantation*, *5*(8), 1957–1965. https://doi.org/10.1111/j.1600-6143.2005.00944.x
101. Zuccarelli L, Porcelli S, Rasica L, Marzorati M, Grassi B. Comparison between Slow Components of HR and V˙O2 Kinetics: Functional Significance. Med Sci Sports Exerc. 2018 Aug;50(8):1649-1657. doi: 10.1249/MSS.0000000000001612. PMID: 29570539.
102. Wagner, J., Niemeyer, M., Infanger, D., Hinrichs, T., Guerra, C., Klenk, C., Karsten, K., Cajochen, C., Schmidt-Trucksass, A., & Knaier, R. (2021). Verification-phase tests show low reliability and add little value in determining VO2 max in young trained adults. PLoS ONE, 16(1), 1–14. https://doi.org/10.1371/journal.pone.0245306
103. Warburton, D. E. R., Nicol, C. W., & Bredin, S. S. D. (2006). Health benefits of physical activity: the evidence. *Family Medicine and Primary Care Review*, *8*(3), 1110–1115.
104. Warden, B. A., & Duell, P. B. (2019). Management of dyslipidemia in adult solid organ transplant recipients. *Journal of Clinical Lipidology*, *13*(2), 231–245. https://doi.org/10.1016/j.jacl.2019.01.011
105. Wehrlin, J. P., & Hallén, J. (2006). Linear decrease in VO2max and performance with increasing altitude in endurance athletes. *European Journal of Applied Physiology*, *96*(4), 404–412. https://doi.org/10.1007/s00421-005-0081-9 Williams, T. J., & McKenna, M. J. (2012). Exercise limitation following transplantation. *Comprehensive Physiology*, *2*(3), 1937–1979. https://doi.org/10.1002/cphy.c110021
106. Wilmore, J. H., Costill, D. L., & Kenny, W. L. (2011). Physiology of Sport and Exercise., 5th ed., UnitedStates, Champaign: Human Kinetics.

https://www.ncbi.nlm.nih.gov/books/NBK305057/

https://www.who.int/chp/chronic_disease_report/media/Factsheet1.pdf

https://www.who.int/dietphysicalactivity/factsheet_inactivity/en/

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ORIGINAL ARTICLE

Cardiac output with modified cardio-impedance against inert gas rebreathing during sub-maximal and maximal cycling exercise in healthy and fit subjects

Alessio del Torto[1,2,3] · Øyvind Skattebo[2] · Jostein Hallén[2] · Carlo Capelli[2]

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Abstract

Purpose We measured cardiac output ($\dot{Q}$) during sub-maximal and supra-maximal exercise with inert gas rebreathing ($\dot{Q}_{IN}$) and modified cardio-impedance ($\dot{Q}_{PF}$) and we evaluated the repeatability of the two methods.

Methods $\dot{V}O_2$ and $\dot{Q}$ were measured twice in parallel with the two methods at sub-maximal (50–250 W) and supra-maximal exercise in 7 young subjects (25 ± 1 years; 74.4 ± 5.2 kg; 1.84 ± 0.07 m).

Results $\dot{Q}_{IN}$ and $\dot{Q}_{PF}$ increased by 3.4 L·min^{-1} and by 5.1 L·min^{-1} per 1 L·min^{-1} of increase in $\dot{V}O_2$, respectively. Mean $\dot{Q}_{PF}$ (23.3 ± 2.5 L·min^{-1}) was 9% lower than $\dot{Q}_{IN}$ (25.8 ± 2.2 L·min^{-1}) during supra-maximal exercise. Bland–Altman analysis showed that: (i) bias ($\dot{Q}_{PF}$–$\dot{Q}_{IN}$) was significantly different from zero (− 0.65 ± 2.61 L·min^{-1}) and; (ii) the ratios $\dot{Q}_{PF} \div \dot{Q}_{IN}$ were linearly related with $\dot{Q}$, indicating that $\dot{Q}_{IN}$ tended to overestimate $\dot{Q}$ in comparison with $\dot{Q}_{PF}$ for values ranging from 10.0 to 15.0 L·min^{-1} and to underestimate it for larger values. The coefficient of variation was similar for sub-maximal values (8.6% vs. 7.7%; 95% CL: ×/÷1.31), but lower for $\dot{Q}_{PF}$ (7.6%; 95% CL: ×/÷ 2.05) than for $\dot{Q}_{IN}$ (27.7%; 95% *CL*: ×/÷2.54) at supra-maximal intensity.

Conclusions $\dot{Q}_{PF}$ seems to represent a valuable alternative to invasive methods for assessing $\dot{Q}$ during sub-maximal exercise. The $\dot{Q}_{PF}$ underestimation with respect to $\dot{Q}_{IN}$ during supra-maximal exercise suggests that $\dot{Q}_{PF}$ might be less optimal for supra-maximal intensities.

Keywords Cycling exercise · Oxygen uptake · Cardiac output · Repeatability · Reliability

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✉ Alessio del Torto
deltorto.alessio@spes.uniud.it;
alessio.deltorto12@gmail.com

Øyvind Skattebo
oyvind.skattebo@nih.no

Jostein Hallén
jostein.hallen@nih.no

Carlo Capelli
carlo.capelli@nih.no

[1] Department of Molecular and Translational Medicine, University of Brescia, Viale Europa 11, 25123 Brescia, Italy

[2] Department of Physical Performance, Norwegian School of Sport Sciences, Sognsveien 220, 0806 Oslo, Norway

[3] Department of Medical Area (DAME), University of Udine, Udine, Italy

Abbreviations

BW	Body weight
C_aO_2	Arterial blood concentration of O_2
(C_aO_2–$C\bar{v}O_2$)	Artero-to-mixed venous blood difference in O_2 concentrations
CV	Coefficient of variation
ΔWL	Net increment of workload during incremental test to exhaustion
ES	Effect size
H	Height
Hb	Haemoglobin
HR	Heart rate
HR_{max}	Maximal heart rate
N_2O	Nitrous oxide
$\dot{Q}$	Cardiac output
$\dot{Q}_{IN}$	Cardiac output assessed by using inert gas rebreathing
$\dot{Q}_{max}$	Maximal cardiac output
$\dot{Q}_{PF}$	Cardiac output assessed using cardio impedance (Physioflow™)

$\dot{Q}_aO_2$	Systemic oxygen delivery
$\dot{Q}_aO_{2max}$	Maximal systemic oxygen delivery
RC	Repeatability coefficient
SF_6	Sulfur hexafluoride
TE	Typical error
TTE	Time to exhaustion
$\dot{V}CO_2$	Carbon dioxide output
$\dot{V}_E$	Pulmonary ventilation
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2s}$	Oxygen uptake at steady state
$\dot{V}O_{2max}$	Maximal oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake
WL	Workload
WL_{max}	Maximal workload

Introduction

$\dot{Q}_aO_2$ is the product of $\dot{Q}$ times C_aO_2 and it is mainly set by $\dot{Q}$, as C_aO_2 is usually hardly affected by exercise intensity in normoxia. $\dot{Q}_aO_{2max}$ is the main limiting factor of $\dot{V}O_{2max}$ if one exercises using large muscle mass at sea level (di Prampero and Ferretti 1990; Mortensen et al. 2005). Therefore, the assessment of $\dot{Q}$ is of paramount importance if one wants to quantify the determinants of individual maximal aerobic exercise capacity.

In addition, the evaluation of the $\dot{Q}$ during exercise is essential to quantify the improvements in maximal aerobic power observed after physical training, which usually elicits a remarkable increase of $\dot{Q}_aO_{2max}$. By the same token, the evaluation of $\dot{Q}$ allows us also to gain insightful information on the adaptations brought about by sedentary lifestyle, disuse or aging (Montero et al. 2015; Warburton et al. 1999a).

To assess $\dot{Q}$ in humans, a variety of invasive and non-invasive methods have been proposed and evaluated (Warburton et al. 1999a, b). The formers are usually considered as the most accurate when compared with the latter ones (Warburton et al. 1999a, b).

Despite their high validity and reliability, invasive methods in exercise physiology are not broadly diffused especially because of their practical—technical limitations, costs and the high training level required by the operators (Reuter et al. 2010; Warburton et al. 1999a). These drawbacks have led to the proposal of several non-invasive approaches for assessing $\dot{Q}$ during exercise: the modified cardio-impedance, the inert gas rebreathing methods and the pulse contour analysis, just to cite a few of them (Tam et al. 2004; Warburton et al. 1999a, b).

However, issues concerning their accuracy, reliability and validity have emerged, especially during maximal exercise. Indeed, as shown by Siebenmann et al. (2015), the determination of $\dot{Q}$ during exercise through most popular non-invasive methods (e.g. modified impedance cardiography, inert gas rebreathing and pulse contour analysis) usually generate significantly different values as compared with the ones obtained using the direct Fick's method. Thus, the values of $\dot{Q}$ measured during exercise depend on the applied non-invasive method (Siebenmann et al. 2015). Nonetheless, considering the problems inherent in the invasive determination of $\dot{Q}$ with invasive procedures during exercise, it would be important to evaluate the reliability and repeatability of different non-invasive methods. With regards to this, the determination of $\dot{Q}$ during exercise using inert gas rebreathing with Innocor™ (Innovision, DK) has been compared to the direct Fick method in healthy subjects (Siebenmann et al. 2015). However, repeatability was not investigated and needs further elucidation.

Furthermore, the assessment of $\dot{Q}$ by the modified cardio-impedance has been validated against gold-standard methods in both patients and healthy volunteers during light to severe exercise and at maximal intensity (Charloux et al. 2000; Siebenmann et al. 2015). On the other hand, the determination of $\dot{Q}$ during maximal efforts by using this technology seems to be spoiled by artefacts induced by the excessive movements of the subject (Charloux et al. 2000; Richard et al. 2001).

Hence, we performed the current investigation to compare first inert gas rebreathing by means of Innocor™ (Innovision, DK) and the modified cardio-impedance through Physioflow™ (Manatec Biomed., F) for measuring steady-state $\dot{Q}$ during cycling exercise at sub-maximal intensity in young, fit subjects.

Furthermore, since $\dot{Q}$ could be underestimated and/or overestimated by non-invasive approaches during intense exercise (Siebenmann et al. 2015), we also measured $\dot{Q}$ during cycling exercise of severe and maximal intensity.

Finally, the repeatability of the two methods in the two exercise conditions was evaluated, as "good" repeatability is crucial to evaluate the cardiovascular adaptations induced by interventions and cardiovascular therapies.

Materials and methods

Subjects

Seven young, active and healthy, non-smoker male were investigated after screening for cardiopulmonary diseases, (age: 25 ± 1 years; BW: 74.4 ± 5.2 kg; *H*: 1.84 ± 0.07 m; $\dot{V}O_{2peak}$: 4643 ± 369 mL·min^{-1}).

The experimental design, methods and procedures of the investigation followed the Declaration of Helsinki and approved by the Ethics Committee of The Norwegian School of Sport Sciences (04-020517) and The Norwegian Centre

for Research Data (54117). The subjects gave their written informed consent before participation.

Experimental design

The subjects were investigated in three occasions and they did not perform heavy physical exercise the day before the tests. During the first visit to the laboratory, *BW* (Seca 877, Seca, Hamburg, Germany), *H* (Seca 217, Seca, Hamburg, Germany) and $\dot{V}O_{2peak}$ of the subjects were measured.

On the second day, after a familiarization session with the rebreathing maneuver, $\dot{Q}$ at steady state was measured during four-five submaximal cycling tests between 50 and 250 W (50 W increments). Thereafter, after 5 min of active recovery, and 5 min of rest sitting on the ergometer, $\dot{Q}_{max}$ was measured during supra-maximal exercise. During the third session, performed after a few days, the very same protocol was repeated. All the experiments were performed keeping the environmental conditions strictly controlled (average temperature = 21 °C; average relative humidity = 55–65% and a cooling fan was placed behind the subjects).

Experimental protocol

$\dot{V}O_{2peak}$ determination

In their first visit the subjects performed four sub-maximal exercise tests at the WL of 50, 100, 150 and 200 W pedaling for 6 min at 80–85 revolution-per-minute (rpm) so that the individual linear relationships between WL and: (i) $\dot{V}O_{2s}$; (ii) HR, both averaged in the last 2 min of each step, were obtained.

Then, the linear relationship between WL and HR was extrapolated to the individual HR_{max} (Tanaka et al. 2001) to obtain WL_{max}. Afterwards, we subtracted from WL_{max} the WL of the warm-up preceding the ramp test to obtain ΔWL, which, once divided by 10, yielded the increment in watt per minute (W min^{-1}) of the maximal, ramp test.

The incremental test started with 3 min of warm up at 100 W followed by a stepwise increase in WL every minute until exhaustion and it was terminated when the subject was not able to keep the selected pedaling rate of 80 rpm. The rates of increase of the WL was able to induce exhaustion in all the subjects in 10–12 min. HR, $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ were measured continuously and averaged on a 30-s base. Peak oxygen uptake ($\dot{V}O_{2peak}$) was defined as the highest $\dot{V}O_2$ measured in a 30-s epoch.

Cardiac output during sub-maximal exercise

The subject started pedaling at 50 W maintaining a fixed pedaling frequency of 80–85 rpm. The sub-maximal steps were carried out at the workloads indicated above and, if feasible, also at 250 W. After 5 min of exercise at each step, $\dot{Q}$ at steady state was measured using inert gas rebreathing ($\dot{Q}_{IN}$). Afterwards, the WL was increased and the procedure repeated.

$\dot{Q}$ and HR with Physioflow™ were recorded throughout experiment; $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$, were assessed from the third to the fifth minute at each WL until the beginning of the rebreathing maneuver. Once the subject had completed the last sub-maximal step, he cooled down pedaling for 5 min at 50 W and then remained seated for 5 min on the saddle.

Cardiac output during supra-maximal exercise

Knowing the relationship between WL and $\dot{V}O_{2s}$ and the $\dot{V}O_{2peak}$, the WL corresponding to 105% of individual maximal aerobic mechanical power was calculated for the supra-maximal test. The subject started to pedal for 3 min at 50 W, thereafter the WL was quickly increased to the calculated supra-maximal WL and the subject kept pedaling until exhaustion.

Also during this test, $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ were measured. Approximately one minute before exhaustion, the mouthpiece of the metabolic cart was removed and $\dot{Q}_{IN}$ was assessed during the last 30 s of exercise before exhaustion. As during sub-maximal exercise, $\dot{Q}_{PF}$ with Physioflow™ was continuously recorded.

Methods

$\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ were measured every 30 s using mixing chamber, open-circuit indirect calorimetry (Oxycon Pro; Jaeger Instrument, Hoechberg, Germany) during all the experiments. Before each test, the gas analyzers and ventilatory flow transducer were calibrated according to the instruction manual. $\dot{Q}_{IN}$ at steady state during sub-maximal and at maximal exercise was assessed via the inert gases rebreathing using Innocor™ (Innovision, DK) (Clemensen et al. 1994), which was calibrated according to the instruction manual. During the rebreathing (5–6 breaths, 10 to 15 s), the subject rebreathes a gas mixture from a rubber bag filled with 5.0% of N_2O, blood soluble gas, 1% SF_6, blood non-soluble gas used to calculate the variations of the volume of the closed system, 94% of O_2 and ambient air so that at the beginning of the rebreathing an O_2 enriched mixture containing abut 0.5% N_2O and 0.1% sulfur hexafluoride SF_6 in the filled bag exists. The volume of the ambient air diluting the concentrated mixture, and hence the volume of the bag, are calculated based on tidal volume and $\dot{V}O_2$ preceding rebreathing. Pulmonary N_2O transfer is estimated from the rate of disappearance of N_2O over three expirations after a stable SF_6 concentration is attained. The rate of disappearance of

N_2O gas from alveoli is proportional to the blood flow of the ventilated parts of the lungs.

Beat-by-beat $\dot{Q}_{PF}$ and HR were measured using a modified cardio-impedance method using Physioflow™ (Manatec Biomed., F) (Charloux et al. 2000). Electrode placement and skin preparation were carried out according to the instructions of the operating manual. Moreover, the subjects wore a tight mesh t-shirt to avoid the displacement of the electrodes. After instrumentation, the subject sat quietly on the bike and after 3–5 min blood pressure was measured three times (Spot Vital Signs® LXi, Welch Allyn, USA). The mean values of systolic and diastolic pressures were fed to the software to calibrate the Physioflow™.

To reposition the electrodes in the very same positions in the following experimental session, skin landmarks were traced on a transparent plastic sheet.

Statistics and data analysis

Data are reported as mean ± standard deviation. The averages of the beat-by-beat $\dot{Q}_{PF}$ measured during the fifth and sixth minutes of sub-maximal exercise were computed and compared with the ones assessed in parallel with rebreathing. During supra-maximal exercise, $\dot{Q}_{PF}$ recorded during the rebreathing was averaged. ($C_aO_2 - C\bar{v}O_2$) was calculated as the ratio between $\dot{V}O_2$ and $\dot{Q}$; values of (C_aO_2–$C\bar{v}O_2$) larger than 200 mL·L^{-1} were considered not plausible and this criterion was utilized to identify impossible $\dot{Q}$ values (Siebenmann et al. 2015).

Linear regressions were computed by means of least–square methods and the significance between of the differences between slopes and intercepts were evaluated (Zar 1999). Paired data were analyzed using a Student's *t* test; effect size (ES) for paired data was also calculated (Cohen 1988).

The agreement between the two sets of $\dot{Q}$ data (cardio-impedance and inert gas rebreathing) was evaluated by using Bland–Altman analysis (Bland and Altman 2003) plotting the differences ($\dot{Q}_{PF}$–$\dot{Q}_{IN}$) and the ratios ($\dot{Q}_{PF} \div \dot{Q}_{IN}$) against their corresponding averages.

The repeatability was evaluated by computing the typical error (TE) and the coefficient of variation (CV) as measures of the absolute and relative error, respectively (Hopkins 2000). TE and CV can be considered as the variation one can expect from one trial to another if subjects perform multiple trials.

Repeatability coefficient (RC), namely the difference that will be exceeded by only 5% of pairs of measurements performed on the same subject, was also calculated (Bland and Altman 2003).

Correlation analyses were conducted with Pearson's product-moment correlation and correlation coefficients (r) were classified as small ($0.1 \le r < 0.3$), moderate ($0.3 \le r < 0.5$), high ($0.5 \le r < 0.7$), very high ($0.7 \le r < 0.9$), and almost perfect ($r \ge 0.9$) (Hopkins et al. 2009).

Data were analyzed by using MedCalc Ver 17.6 (MedCalc Software bvba, Ostend, Belgium) and a Microsoft Office Excel spreadsheet prepared for the purpose (MO, Microsoft, Seattle, USA).

Results

One subject was not able to perform the supra-maximal workload in one of the two experimental sessions. In another subject, two replicated measurements of $\dot{Q}$ during the supra-maximal protocol using inert gas rebreathing were lost due to technical errors.

$\dot{V}O_2$ during sub-maximal exercise increased linearly as a function of WL from 1.21 L·min^{-1} ± 0.04 at 50 W, 1.78 L·min^{-1} ± 0.05 at 100 W, 2.39 L·min^{-1} ± 0.05 at 150 W, 3.05 L·min^{-1} ± 0.08 at 200 W and 3.62 L·min^{-1} ± 0.06 at 250 W.

Likewise, $\dot{Q}$ increased linearly as a function of exercise intensity: $\dot{Q}_{PF}$ from 10.4 ± 1.7 L·min^{-1} at 50 W to 22.9 ± 2.5 L·min^{-1} at 250 W and $\dot{Q}_{IN}$ from 12.6 ± 1.0 L·min^{-1} at 50 W to 20.8 ± 1.4 L·min^{-1} at 250 W.

The individual linear regressions between sub-maximal, steady–state $\dot{Q}$ and $\dot{V}O_2$ are reported for all the subjects in Fig. 1. Both $\dot{Q}_{PF}$ and $\dot{Q}_{IN}$ increased linearly as a function of $\dot{V}O_2$. However, the average slopes of the regressions obtained using the two methods were significantly different. In the first experimental run, $\dot{Q}_{PF}$ increased, on the average, by 4.7 ± 0.7 L·min^{-1} per L·min^{-1} of increase in $\dot{V}O_2$, whereas $\dot{Q}_{IN}$ increased by 3.3 ± 0.6 L·min^{-1} per L·min^{-1} ($n = 7$; $P < 0.01$; large ES). In the second run, the slope for $\dot{Q}_{PF}$ turned out to be 5.7 ± 1.17 L·min^{-1} per L·min^{-1} of increase in $\dot{V}O_2$, whereas it was 3.4 ± 0.6 L·min^{-1} per L·min^{-1} for $\dot{Q}_{IN}$ ($n = 7$; $P < 0.01$; large *ES*). The grand averages were 5.1 ± 1.0 L·min^{-1} and 3.4 ± 0.6 L·min^{-1} per L·min^{-1} for $\dot{Q}_{PF}$ and $\dot{Q}_{IN}$, respectively ($n = 14$; $P < 0.001$; ES = 1.33).

Average WL during the supra-maximal tests was 343 ± 34 W, which corresponded to about ~ 106% of mean maximal aerobic mechanical power (324 ± 32 W). The mean time to exhaustion (TTE) was 220 ± 41 s for the supra-maximal test. $\dot{V}O_2$ attained the value of 4.32 ± 0.30 L·min^{-1} during supra-maximal exercise. These $\dot{V}O_2$ values were about 7% smaller ($P = 0.013$, large *ES*) than the $\dot{V}O_{2peak}$ assessed during the preliminary maximal test (4.64 ± 0.37 L·min^{-1}), due to the switch of mouthpieces ~ 1 min before exhaustion (from indirect calorimetry to inert-gas rebreathing).

During supra-maximal test, inert gas rebreathing generated only one implausible value of $\dot{Q}_{IN}$; modified cardio impedance generated three implausible values of $\dot{Q}_{PF}$. When only the plausible values were considered, $\dot{Q}_{PF}$ during

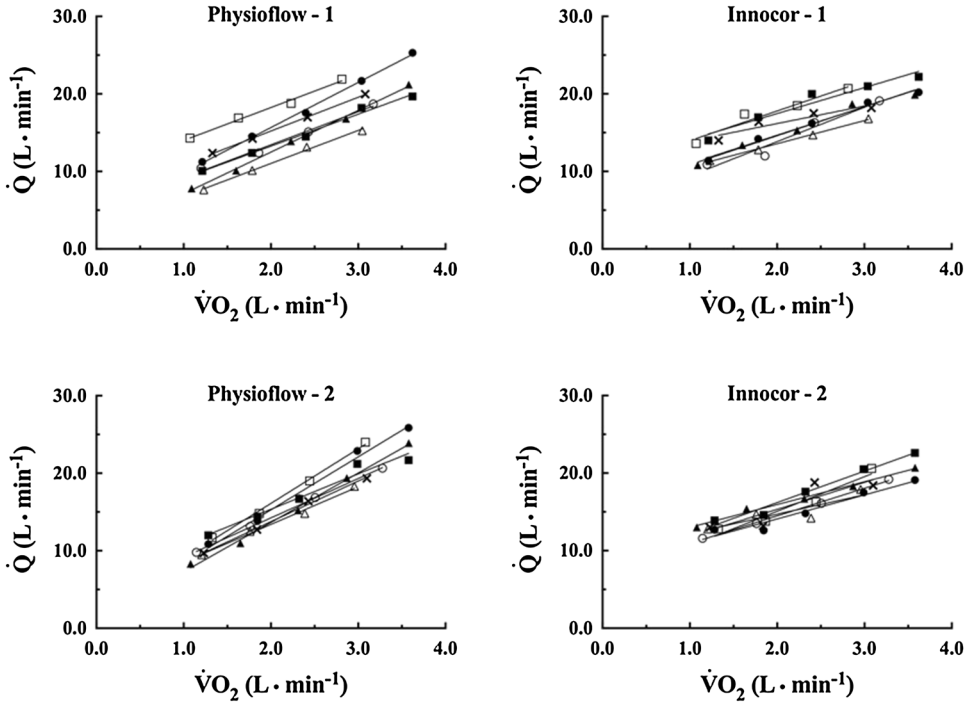

Fig. 1 The individual steady-state $\dot{Q}$ values of the seven subjects measured during sub-maximal cycling exercise with modified cardio impedance (left column) and inert gas rebreathing (right column) in the first (top diagrams) and in the second (bottom diagrams) experimental trial are represented as a function of the corresponding $\dot{V}O_{2s}$. The graphs also report the corresponding individual regression lines. Please see text for further details

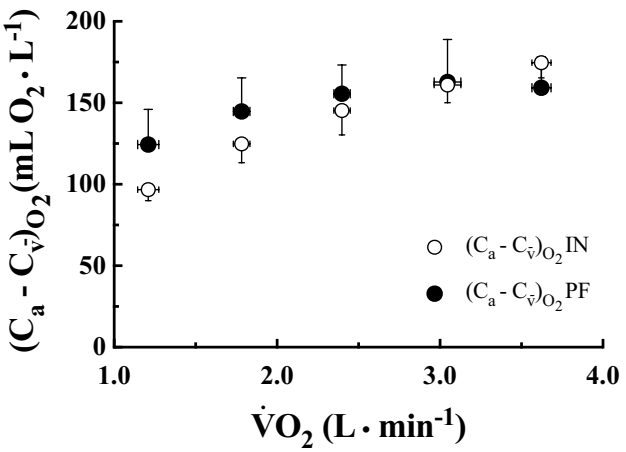

Fig. 2 Steady-state values of the artero-mixed venous O_2 difference calculated form the corresponding values of $\dot{Q}$ and $\dot{V}O_{2s}$. White dots refer $\dot{Q}_{IN}$, and black dots refer to $\dot{Q}_{PF}$

supra-maximal test was equal to 23.3 ± 2.5 L·min^{-1} and $\dot{Q}_{IN}$ to 25.8 ± 2.2 L·min^{-1}, i.e. about 9% larger, on the average, than the one assessed with cardio impedance.

During sub-maximal exercise, (C_aO_2–$C\bar{v}O_2$) calculated as the ratio between $\dot{V}O_2$ and $\dot{Q}_{PF}$ increased on the average from 124 ± 22 mL·L^{-1} at 50 W to 159 ± 19 mL·L^{-1} at 250 W; the one obtained from $\dot{Q}_{IN}$ increased from 96.8 ± 6.8 mL·L^{-1} to 174.7 ± 9.3 mL·L^{-}1 within the same WL range (Fig. 2). During supra-maximal test, when only the plausible values of $\dot{Q}$ were considered, (C_aO_2–$C\bar{v}O_2$) calculated from $\dot{Q}_{PF}$ was 182.3 ± 10.3 mL·L^{-1} and the one corresponding to $\dot{Q}_{IN}$ was 169.3 ± 17.3 mL·L^{-1}.

The results of the Bland–Altman plot are shown in Fig. 3 where the average of the differences ($\dot{Q}_{PF}$–$\dot{Q}_{IN}$) obtained at all the exercise intensities are plotted against their corresponding means (top diagram). Average $\dot{Q}_{PF}$–$\dot{Q}_{IN}$ amounted to − 0.65 L·min^{-1} (bias) and was significantly different from zero ($P = 0.044$,); SD (precision) was 2.61 L·min^{-1} and the 95% limits of agreement ranged from − 5.8 to 4.5 L·min^{-1}. Since the distribution of the difference was markedly heteroscedastic—the absolute values of $\dot{Q}_{PF}$–$\dot{Q}_{IN}$ increased with the average of the two measurements—we also plotted the ratios ($\dot{Q}_{PF} \div \dot{Q}_{IN}$) vs. the corresponding means. Mean $\dot{Q}_{PF} \div \dot{Q}_{IN}$ amounted to 0.95 and it was significantly different from 1 ($P = 0.0157$). SD was 0.16 and the 95% limits of agreement of the discrepancy of the ratio form 1 were 0.68/1.36).

($\dot{Q}_{PF}$–$\dot{Q}_{IN}$) were moderately and linearly related with their corresponding means ($n = 68$; $y = -3.95 + 0.19 \cdot \times$; $P = 0.057$; $r^2 = 0.11$, $F = 8.16$). Similarly, also ($\dot{Q}_{PF} \div \dot{Q}_{IN}$) were moderately and linearly related to the means ($n = 68$; $y = 0.68 + 0.017 \cdot \times$; $P < 0.0001$; $r^2 = 0.21$, $F = 20.06$). This indicates a linear relationship between the amplitude of the error and the absolute values of the measurements so that $\dot{Q}_{IN}$ tended to overestimate $\dot{Q}$ in comparison with $\dot{Q}_{PF}$ for values of $\dot{Q}$ ranging between 10.0 and 15.0 L·min^{-1} and to underestimate it for larger values corresponding o higher sub-maximal exercise intensities.

For $\dot{Q}_{PF}$, *TE* was 1.17 L·min^{-1} (95% confidence limits, CL: ×/÷ 1.29; $n = 31$) and 1.64 L·min^{-1} (95% CL: ×/÷1.98;

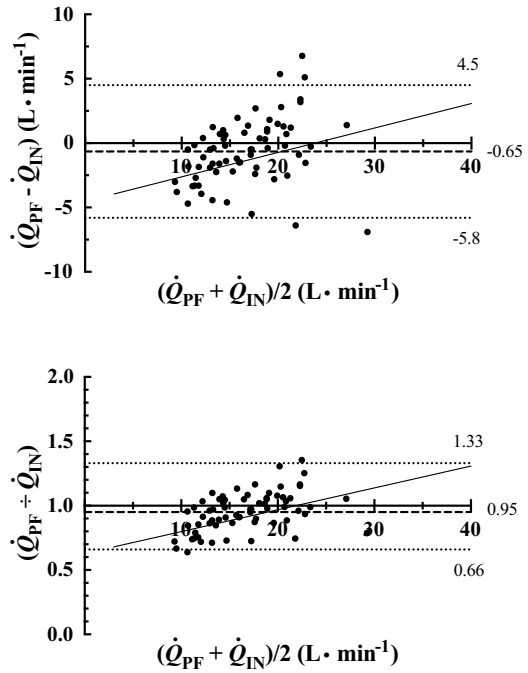

Fig. 3 Top: Bland–Altman diagram of the differences $\dot{Q}_{PF}$–$\dot{Q}_{INt}$ plotted as a function of their averages. Bottom: Bland–Altman diagram of the ratios $\dot{Q}_{PF} \div \dot{Q}_{INt}$ plotted as a function of their averages. Dotted lines in the two diagrams refer to biases and to errors, i.e. 95% CI of the bias. Continuous thin lines describe the linear relationships between the variables at stake. Please see text for more details

$n = 6$) at sub-maximal and supra-maximal workloads, respectively. This corresponded to CVs of 8.6% (95% CL: ×/÷1.31) and 7.6% (95% CL: ×/÷2.05), respectively. For $\dot{Q}_{IN}$, *TE* was 1.10 L·min^{-1} (95% CL: ×/÷1.29; $n = 31$) and 5.90 L·min^{-1} (95% CL: ×/÷2.19; $n = 5$) at sub-maximal and supra-maximal workloads, respectively. This corresponded to *CV*s of 7.7% (95% CL: ×/÷ 1.31) and 27.7% (95% CL: ×/÷2.54), respectively. Finally, RC amounted to 3.79 L·min^{-1} and to 4.81 L·min^{-1} for $\dot{Q}_{PF}$ and $\dot{Q}_{IN}$, respectively.

Discussion

$\dot{Q}$ measured with cardio impedance and inert gas rebreathing increased during sub-maximal exercise by 5.1 L·min^{-1} and 3.4 L·min^{-1} per L·min^{-1} of increase in $\dot{V}O_2$, respectively.

Second, Bland–Altman analysis showed that: (i) bias between $\dot{Q}_{PF}$ and $\dot{Q}_{IN}$ amounted to − 0.65 L·min^{-1} and was significantly different from zero; (ii) ($\dot{Q}_{PF}$–$\dot{Q}_{IN}$) and ($\dot{Q}_{PF} \div \dot{Q}_{IN}$) were correlated with the absolute values of $\dot{Q}$, implying that $\dot{Q}_{IN}$ overestimated $\dot{Q}$ in comparison with $\dot{Q}_{PF}$ when $\dot{Q}$ was lower than about 15.0 L·min^{-1}, whereas it underestimated $\dot{Q}$ for values above 15.0 L·min^{-1}.

Finally, $\dot{Q}_{PF}$ was characterized by a good repeatability in comparison with $\dot{Q}_{IN}$, since it showed a coefficient of variation close to 8% regardless the intensity of the exercise.

The $\dot{Q}$ determination by the Physioflow™ has been validated against the Fick method during exercise in patients and in healthy subjects (Charloux et al. 2000; Richard et al. 2001). $\dot{Q}$ is expected to increase by ~ 5–6 L·min^{-1} per L·min^{-1} increase in $\dot{V}O_2$ when it is measured with invasive, gold-standard methods. Therefore, the average slope of the linear $\dot{Q}_{PF}$ vs. $\dot{V}O_2$ relationship (5.1 ± 1.0 L·min^{-1}) is within the range of values mentioned above and it is also practically identical to the one measured with direct Fick method during sub-maximal cycling (4.9 ± 0.3 L·min^{-1}) (Siebenmann et al. 2015). However, the slope of the $\dot{Q}_{PF}$ vs. $\dot{V}O_2$ regression line appeared to be lower when compared to other studies: Siebenmann et al. (2015) have reported an increase of $\dot{Q}_{PF}$, determined by Physioflow of 6.0 ± 0.4 L·min^{-1} per L·min^{-1}; $\dot{Q}_{PF}$ increased by 7.2 L·min^{-1} per L·min^{-1} increase in $\dot{V}O_2$ in patients with chronic obstructive pulmonary disease and sleep apnea syndrome likely because of the lower (C_aO_2–$C\bar{v}O_2$) prevailing in these patients (Charloux et al. 2000).

$\dot{Q}$ has been simultaneously assessed with Innocor™ and the direct Fick method in healthy subjects during sub-maximal and maximal cycling exercise (Siebenmann et al. 2015). In that occasion, the average increase of $\dot{Q}$ per L·min^{-1} increase in $\dot{V}O_2$ turned out to be 3.9 ± 0.2 L·min^{-1}, lower than expected and in agreement with the findings of the present investigation. Moreover, also in studies where Innocor™ was used in patients (Agostoni et al. 2005), this method underestimated $\dot{Q}$ when compared to invasive method, as $\dot{Q}$ increased by 3.5 L·min^{-1} per L·min^{-1} of increase in $\dot{V}O_2$. In conclusion, a bulk of evidence seems to suggest that Innocor™ may underestimate $\dot{Q}$ during cycling exercise in healthy, young subjects by about 20%.

Bland–Altman plots confirmed the poor level of agreement between $\dot{Q}_{PF}$ and $\dot{Q}_{IN}$ during sub-maximal exercise. First, bias was significantly different from 0; second, $\dot{Q}_{PF} \div \dot{Q}_{IN}$ were moderately and linearly related with the absolute values of $\dot{Q}$ indicating that $\dot{Q}_{IN}$ in respect to $\dot{Q}_{PF}$ overestimated $\dot{Q}$ below values of approximately 15 L·min^{-1}, corresponding to a $\dot{V}O_2$ of 2.4–2.5 L·min^{-1} (about 55% of $\dot{V}O_{2max}$ in our subjects), and underestimated $\dot{Q}$ at higher exercise intensities.

During supra-maximal exercise, $(C_aO_2–C\bar{v}O_2)_{PF}$ turned out to be, on the average, remarkably high: 182.3 ± 10.8 mL·L^{-1}. In addition, three $(C_aO_2–C\bar{v}O_2)_{PF}$ values out of seven indicated not plausible $\dot{Q}$. These findings suggest that modified cardio impedance may underestimate $\dot{Q}$ in this condition. Indeed, strenuous cycling exercise against a constant supra-maximal WL (~ 106% of maximal aerobic mechanical power) may have negatively affected cardio

impedance recoding because of excessive movements, respiratory artefacts due the broad excursion of the thoracic cage due to hyperventilation and to accumulation of fluid in the lungs, thereby causing the underestimation of $\dot{Q}_{PF}$ at maximal exercise (Charloux et al. 2000; Kemps et al. 2008; Warburton et al. 1999b).

Recent data (Siebenmann et al. 2015) tended to show that also inert gas rebreathing underestimated $\dot{Q}$ during maximal cycling exercise in young, active men: Innocor™ generated a higher number of not plausible $\dot{Q}$ values than Physioflow™ and maximal $(C_aO_2–C\bar{v}O_2)_{IN}$ was about 180 mL·L^{-1} (Fig. 1a in Siebenmann et al. 2015). The results reported in the present investigation partially agree with the ones summarised above: maximal $(C_aO_2–C\bar{v}O_2)_{IN}$ calculated using $\dot{V}O_{2peak}$ was indeed about 4% larger than the value measured using direct Fick method (170 ± 20 mL·L^{-1}) at the end of maximal cycling exercise in active, young men (Siebenmann et al. 2015), but only one $\dot{Q}$ during supra-maximal exercise turned out to be frankly unrealistic.

As reported by Jarvis et al. (2007), Innocor™ may underestimate $\dot{Q}$ because of N_2O recirculation that occurs already after 8.5 s at a $\dot{V}O_2$ of 2.5 L·min^{-1} and in less than 8 s at a $\dot{V}O_2$ of 3 L·min^{-1} (Rigatto et al. 1968). Therefore, recirculation of N_2O may prevent further uptake of the soluble gas, contributing to the observed underestimation of $\dot{Q}$. Moreover, as N_2O only dissolves into the liquid phase of the whole blood, the progressive hemoconcentration occurring from rest to maximal exercise and resulting in a 5–10% increase in Hb concentration and in a reduction of plasma volume, may limit the uptake of N_2O.

So, it seems that during the supra-maximal tests both the two non-invasive techniques may underestimate $\dot{Q}_{max}$ and that $\dot{Q}_{PF}$ may be characterized by a larger underestimation of $\dot{Q}$, whereby few implausible values of $(C_aO_2–C\bar{v}O_2)_{PF}$ were observed at supra-maximal intensity. It is worth noting that, by performing ramp exercise to exhaustion, and not constant work rate of supra-maximal intensity, some of the causes of artifacts that undermine the measurements obtained with $\dot{Q}_{PF}$ may be avoided, especially if the subjects keep their thorax as still as possible approaching exhaustion. This is also partially confirmed by the findings of other investigators (Siebenmann et al. 2015) who found higher values of $\dot{Q}_{max}$ with Physioflow™ by using an incremental test to exhaustion.

The modified cardio-impedance benefits from "good" repeatability. Therefore, when multiple $\dot{Q}$ assessments are required on the same subject, $\dot{Q}_{PF}$ would vary within an "acceptable" range due to random errors. Regarding Innocor™, its *TE, CVs* and *RC* suggest that this method may be not suitable when $\dot{Q}$ has to be measured repeatedly during maximal exercise. However, these results should be interpreted with a pinch of salt because of the limited number of subjects evaluated during supra-maximal exercise with inert gas rebreathing, and of the short duration of the exercise bout. Indeed, it has been shown that Innocor™ was characterized by a CV of 7.0%, 95% CL: 5.5 to 9.5 (Fontana et al. 2009) during repetitions of maximal exercise in a group of 30 young men and women.

A clear limitation of the study was the low number of tested subjects (7 healthy males) and the absence of a gold-standard method as a reliable comparison. Furthermore, during the supra-maximal test the participants might have interrupted the exercise before attaining maximal $\dot{Q}$. Furthermore, as already outlined, the change of mouthpiece necessary for carrying out the measurement of $\dot{Q}$ by Innocor™ prevented us to measure $\dot{V}O_2$ using mixing chamber during the last minute of the supra-maximal effort.

Conclusions

The modified cardio-impedance might be a non-invasive alternative to the invasive approaches for the determination of $\dot{Q}$ in healthy population, at least during exercise at sub-maximal intensity. In addition, it is attractive because its implementation does not need trained personnel. However, the application of Physioflow™ during supra-maximal effort seems to be less indicated.

Moreover, the "good" repeatability showed by the Physioflow™ makes this technique suitable when the changes brought about by interventions such as training/de-training and the efficacy of specific therapies on the cardiovascular responses have to be evaluated and quantified.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

References

Agostoni P, Cattadori G, Apostolo A, Contini M, Palermo P, Marenzi G, Wasserman K (2005) Noninvasive measurement of cardiac output during exercise by inert gas rebreathing technique: a new tool for heart failure evaluation. J Am Coll Cardiol 46:1779–1781

Bland JM, Altman DG (2003) Applying the right statistics: analyses of measurement studies. Ultrasound Obstet Gynecol 22:85–93

Charloux A, Lonsdorfer-Wolf E, Richard R, Lampert E, Oswald-Mammosser M, Mettauer B, Geny B, Lonsdorfer J (2000) A new impedance cardiograph device for the non-invasive evaluation of

cardiac output at rest and during exercise: comparison with the "direct" Fick method. Eur J Appl Physiol 82:313–320
Clemensen P, Christensen P, Norsk P, Grønlund JA (1994) Modified photo- and magnetoacoustic multigas analyzer applied in gas exchange measurements. J Appl Physiol 76:2832–2839
Cohen J (1988) Statistical power analysis for behavioral science, 2nd edn. Lawrence Earlbaum Associates, Hillsdale, pp 20–27
di Prampero PE, Ferretti G (1990) Factors limiting maximal oxygen consumption in humans. Respir Physiol 811:113–128
Fontana P, Boutellier U, Tolgo M (2209) Reliability of measurements with Innocor™ during exercise. Int J Sports Med 30:747–753
Hopkins WG (2000) Measures of reliability in sports medicine and science. Sports Med 30:1–15
Hopkins WG, Marshall SW, Batterham AM, Hanin J (2009) Progressive statistics for studies in sports medicine and exercise science. Med Sci Sports Exerc 41:3–13
Jarvis SS, Levine BD, Prisk GK, Shykoff BE, Elliott AR, Rosow E, Blomqvist CG, Pawelczyk JA (2007) Simultaneous determination of the accuracy and precision of closed-circuit cardiac output rebreathing techniques. J Appl Physiol 103:867–874
Kemps HM, Thijssen EJ, Schep G, Sleutjes BT, De Vries WR, Hoogeveen AR, Wijn PF, Doevendans PA (2008) Evaluation of two methods for continuous cardiac output assessment during exercise in chronic heart failure patients. J Appl Physiol 105:1822–1829
Montero D, Diaz-Cañestro C, Lundby C (2015) Endurance training and O_{2max}: role of maximal cardiac output and oxygen extraction. Med Sci Sports Exerc 47:2024–2033
Mortensen SP, Dawson EA, Yoshiga CC, Dalsgaard MK, Damsgaard R, Secher NH, González-Alonso J (2005) Limitations to systemic and locomotor limb muscle oxygen delivery and uptake during maximal exercise in humans. J Physiol 566:273–285
Reuter DA, Huang C, Edrich T, Shernan SK, Eltzschig HK (2010) Cardiac output monitoring using indicator-dilution techniques: basics, limits, and perspectives. AnesthAnalg 110:799–811
Richard R, Lonsdorfer-Wolf E, Charloux A, Doutreleau S, Buchheit M, Oswald-Mammosser M, Lampert E, Mettauer B, Geny B, Lonsdorfer J (2001) Non-invasive cardiac output evaluation during a maximal progressive exercise test, using a new impedance cardiograph device. Eur J Appl Physiol 85:202–207
Rigatto M, Jones NL, Campbell EJ (1968) Pulmonary recirculation time: influence of posture and exercise. Clin Sci 35:183–195
Siebenmann C, Rasmussen P, Sørensen H, Zaar M, Hvidtfeldt M, Pichon A, Secher NH, Lundby C (2015) Cardiac output during exercise: a comparison of four methods. Scand J Med Sci Sports 25:e20–e27
Tam E, AzabjiKenfack M, Cautero M, Lador F, Antonutto G, di Prampero PE, Ferretti G, Capelli C (2004) Correction of cardiac output obtained by Modelflow from finger pulse pressure profiles with a respiratory method in humans. Clin Sci 106:371–376
Tanaka H, Monahan KD, Seals DR (2001) Age-predicted maximal heart rate rivisited. J Am Colloid Cardiol 37:153–156
Warburton D, Haykowsky M, Quinney HA, Humen DP, Teo KK (1999a) Reliability and validity of measures of cardiac output during incremental to maximal aerobic exercise—part I: conventional techniques. Sports Med 27:23–41
Warburton D, Haykowsky M, Quinney HA, Humen DP, Teo KK (1999b) Reliability and validity of measures of cardiac output during incremental to maximal aerobic exercise—part II: novel techniques and new advances. Sports Med 27:241–260
Zar J (1999) Chap. 18: Comparing simple linear regression equations. In: Biostatistical analysis, 4th edn. Prentice-Hall, Upper Saddle River, NJ, pp 360–377

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RESEARCH ARTICLE

Skeletal muscle $\dot{V}O_2$ kinetics by the NIRS repeated occlusions method during the recovery from cycle ergometer exercise

Lucrezia Zuccarelli,[1]* Paulo Cesar do Nascimento Salvador,[1,2]* Alessio Del Torto,[1] Riccardo Fiorentino,[1] and Bruno Grassi[1]

[1]*Department of Medicine, University of Udine, Udine, Italy; and* [2]*Sport Center, Federal University of Santa Catarina, Florianopolis, Brazil*

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Zuccarelli L, do Nascimento Salvador PC, Del Torto A, Fiorentino R, Grassi B. Skeletal muscle $\dot{V}O_2$ kinetics by the NIRS repeated occlusions method during the recovery from cycle ergometer exercise. *J Appl Physiol* 128: 534–544, 2020. First published January 23, 2020; doi:10.1152/japplphysiol.00580.2019.—Near-infrared spectroscopy (NIRS) has been utilized as a noninvasive method to evaluate skeletal muscle mitochondrial function in humans, by calculating muscle $\dot{V}O_2$ ($\dot{V}O_2m$) recovery (off-) kinetics following short light-intensity plantar flexion exercise. The aim of the present study was to determine $\dot{V}O_2m$ off- kinetics following standard cycle ergometer exercise of different intensities. Fifteen young physically active healthy men performed an incremental exercise (INCR) up to exhaustion and two repetitions of constant work-rate (CWR) exercises at 80% of gas exchange threshold (GET; MODERATE) and at 40% of the difference between GET and peak pulmonary $\dot{V}O_2$ ($\dot{V}O_2p$; HEAVY). $\dot{V}O_2p$ and vastus lateralis muscle fractional O_2 extraction by NIRS (Δ[deoxy(Hb+Mb)]) were recorded continuously. Transient arterial occlusions were carried out at rest and during the recovery for $\dot{V}O_2m$ calculation. All subjects tolerated the repeated occlusions protocol without problems. The quality of the monoexponential fitting for $\dot{V}O_2m$ off-kinetics analysis was excellent ($0.93 \leq r^2 \leq 0.99$). According to interclass correlation coefficient, the test-retest reliability was moderate to good. $\dot{V}O_2m$ values at the onset of recovery were ~27, ~38, and ~35 times higher (in MODERATE, HEAVY, and INCR, respectively) than at rest. The time constants (τ) of $\dot{V}O_2m$ off-kinetics were lower ($P < 0.001$) following MODERATE (29.1 ± 6.8 s) vs. HEAVY (40.8 ± 10.9) or INCR (42.9 ± 10.9), suggesting an exercise intensity dependency of $\dot{V}O_2m$ off-kinetics. Only following MODERATE the $\dot{V}O_2m$ off-kinetics were faster than the $\dot{V}O_2p$ off-kinetics. $\dot{V}O_2m$ off-kinetics, determined noninvasively by the NIRS repeated occlusions technique, can be utilized as a functional evaluation tool of skeletal muscle oxidative metabolism also following conventional cycle ergometer exercise.

NEW & NOTEWORTHY This is the first study in which muscle $\dot{V}O_2$ recovery kinetics, determined noninvasively by near-infrared spectroscopy (NIRS) by utilizing the repeated occlusions method, was applied following standard cycle ergometer exercise of different intensities. The results demonstrate that muscle $\dot{V}O_2$ recovery kinetics, determined noninvasively by the NIRS repeated occlusions technique, can be utilized as a functional evaluation tool of skeletal muscle oxidative metabolism also following conventional cycle ergometer exercise, overcoming significant limitations associated with the traditionally proposed protocol.

cycling; mitochondria; NIRS; oxygen consumption; skeletal muscle oxidative metabolism

INTRODUCTION

The functional evaluation of skeletal muscle oxidative metabolism is crucially important in the assessment of exercise tolerance in normal subjects, athletes, and patients affected by many chronic diseases such as heart failure and chronic obstructive pulmonary disease (16, 30, 32). Since the pioneering studies by Meyer (see, e.g., Ref. 28), the rate of phosphocreatine (PCr) recovery following exercise, as determined by ^{31}P nuclear magnetic resonance spectroscopy (^{31}P-MRS), has been traditionally considered a valuable functional evaluation tool of skeletal muscle oxidative performance (23). The main limitations of this approach are represented by the cost of the equipment and by the limited exercise paradigms which can be performed in the NMR magnet.

Since PCr resynthesis is accomplished solely by oxidative phosphorylation (23); however, the kinetics of PCr recovery (off-kinetics) should be closely related to the off-kinetics of muscle O_2 uptake ($\dot{V}O_2m$) decrease during the same recovery period. The importance of the $\dot{V}O_2m$ off-kinetics following exercise as a functional evaluation tool is underlined by the fact that in single muscle fibers the velocity constant of this variable is directly related to maximal O_2 uptake ($\dot{V}O_{2max}$) (43). Direct measurements of $\dot{V}O_2m$ off-kinetics, however, have been traditionally difficult to perform in humans, and implied invasive measurements (see e.g., Ref. 24). A proxy of $\dot{V}O_2m$ kinetics could be pulmonary $\dot{V}O_2$ ($\dot{V}O_2p$) kinetics. Although the two variables appear reasonably similar during the on (rest-to-exercise)-transition (14), however, different results have been seen during the off (exercise-to-recovery)-transition (24).

A reliable and noninvasive method to determine $\dot{V}O_2m$ off-kinetics in exercising humans would therefore be highly desirable. An answer to this need could derive from a method recently proposed by two groups (3, 34), based on a concept originally developed by Hamaoka et al. (17) and by Van Beekvelt et al. (40): in ischemic conditions, the linear rate of increase in deoxy-(hemoglobin+myoglobin), or the linear rate of decrease of oxy-(hemoglobin+myoglobin), as determined by near-infrared spectroscopy (NIRS) (6, 15), represents an index of $\dot{V}O_2m$. By performing a series of repeated short ischemia (blood flow occlusions induced by rapid inflation and subsequent deflation of a pneumatic cuff with suprasystolic

* L. Zuccarelli and P. C. do Nascimento Salvador contributed equally to this work.

Address for reprint requests and other correspondence: B. Grassi, Dept. of Medicine, Univ. of Udine, Piazzale M. Kolbe 4, Udine (UD) 33100, Italy (e-mail: bruno.grassi@uniud.it).

pressure) during the recovery from exercise, $\dot{V}O_2m$ measurements have been obtained with a temporal resolution allowing performance of a reliable $\dot{V}O_2m$ off-kinetics analysis (3, 34). The method has been validated against other approaches of functional evaluation of skeletal muscle oxidative metabolism, such as [PCr] (squared brackets denote concentrations) recovery kinetics (35) and high-resolution respirometry of permeabilized skeletal muscle fibers (33).

In a recent review, Adami and Rossiter (3) have summarized the key methodological issues of the repeated ischemia approach to determine $\dot{V}O_2m$ off-kinetics. Some limitations, in our opinion, should be acknowledged in the proposed protocols. According to Adami and Rossiter, for the method to effectively evaluate skeletal muscle oxidative metabolism, mitochondrial enzymes should be "maximally activated." In our opinion it is not clear if this actually happens with the proposed protocol (3, 34). A short (~15 s) cyclical plantar flexion/relaxation exercise against a "manually applied resistance" (1) is usually performed. Fifteen seconds of contractions are not enough to reach a $\dot{V}O_2m$ steady state. No measurement of external work rate can be performed. No inferences on exercise intensity can be made. The extent of the $\dot{V}O_2m$ increase during exercise, with respect to the resting baseline, is not known. Since the involved muscle mass is relatively small, and the exercise is very short, no systemic measurements of exercise intensity [to identify exercise intensity domains, which characterize the physiological responses to exercise (16, 30, 32)] can be made. Moreover, no comparisons with $\dot{V}O_2p$ kinetics (on- and off-transients) are possible. No inferences on O_2 availability (another prerequisite for the measurement, see Ref. 3), or on its consequences, can be directly made, although some cautionary rules have been proposed by the authors (3).

The aim of the present study was to provide some answers to the issues raised above. More specifically, we applied the repeated ischemia approach to determine $\dot{V}O_2m$ off-kinetics during the recovery from standard cycle ergometer exercise, carried out for several minutes at moderate intensity, below the gas exchange threshold (GET), at heavy intensity above GET, and during the recovery from an incremental exercise. $\dot{V}O_2p$ off-kinetics were concurrently determined, as well as muscle deoxygenation off-kinetics by NIRS (see Ref. 15) in the opposite leg compared with the one in which the repeated ischemia protocol was performed. We hypothesized an exercise intensity dependency of $\dot{V}O_2m$ off-kinetics (slower kinetics as a function of exercise intensity), paralleled by an exercise intensity dependency of $\dot{V}O_2p$ off-kinetics. Faster $\dot{V}O_2m$ vs. $\dot{V}O_2p$ off-kinetics were expected at all exercise intensities.

The obtained results, besides clarifying some of the issues/doubts discussed above, would allow insights into basic physiological mechanisms during metabolic transitions, and would allow putting the repeated ischemia approach to determine $\dot{V}O_2m$ kinetics in the "real life" context of cycle ergometer exercise in different intensity domains.

MATERIALS AND METHODS

Subjects. Fifteen healthy, habitually active men (age 25 ± 4 yr; height 180 ± 6 cm; body mass 77 ± 8 kg; body mass index 23.8 ± 2.1 kg/m^2) were tested. All participants were moderately trained and attained the American College of Sports Medicine (ACSM) exercise recommendations for adults (at least 150 min/wk). Subjects were instructed to arrive at the laboratory in a rested and fully hydrated state and to avoid strenuous exercise in the 24 h preceding each testing session. Subjects abstained from drinking alcohol (24 h) and caffeine (5 h) before the exercise test and had their last meal at least 3 h before each testing session. The procedures used in this study were approved by the local Institutional Review Board and were conducted in accordance with the Declaration of Helsinki. The subjects were fully informed of any potential risk associated with the experiments before verbal and written consents were obtained.

Exercise protocols. Exercise tests were carried out in a well-ventilated laboratory at 19–21°C, under continuous medical supervision and 12-lead electrocardiography (Quark C12x, Cosmed). The participants were required to report to the laboratory on three separate occasions over a 2-wk period. On their first visit, anthropometric measurements were performed and subjects completed a ramp incremental exercise (INCR; 30 W/min) up to voluntary exhaustion on an electronically braked cycle ergometer (Ergomedic 839 E, Monark) to determine $\dot{V}O_{2peak}$ and GET. Pedaling frequency was digitally displayed to the subjects, who were asked to keep a constant cadence throughout the tests at their preferred value (between 70 and 90 rpm). Voluntary exhaustion was defined as the incapacity to maintain the imposed load and pedaling frequency despite vigorous encouragement by the researchers. Peak values of the main variables were taken as the highest 15-s mean values attained before the subject's voluntary exhaustion. $\dot{V}O_2p$ at GET was determined by two independent investigators by standard methods (7). To identify the work rate corresponding to $\dot{V}O_2p$ at GET, the effect of the delayed $\dot{V}O_2p$ adjustment to the increased work rate during the incremental test was corrected by shifting the linear $\dot{V}O_2p$ vs. time (and work rate) relationship to the left, by an amount corresponding to the individual mean response time of the $\dot{V}O_2p$ kinetics (21.8 ± 8.9 s) (41).

After the first visit, the subjects performed on 2 different days two repetitions of 6-min constant work rate (CWR) submaximal exercise corresponding to 80% of GET (MODERATE) and 40% of the difference between GET and $\dot{V}O_2p$ peak (HEAVY). MODERATE was always carried out before HEAVY. HEAVY exercise was performed when subjects reached again baseline values of the main investigated variables (~30 min of recovery).

Measurements. Pulmonary ventilation ($\dot{V}E$), $\dot{V}O_2$, and CO_2 output ($\dot{V}CO_2$) were determined breath-by-breath by a metabolic cart (Quark PFTergo, Cosmed, Rome, Italy). Expiratory flow measurements were performed by a turbine flow meter calibrated before each experiment by a 3-L syringe at different flow rates. $\dot{V}O_2p$ and $\dot{V}CO_2$ were determined by continuously monitoring PO_2 and PCO_2 at the mouth throughout the respiratory cycle and from established mass balance equations. Calibration of O_2 and CO_2 analyzers was performed before each experiment by utilizing gas mixtures of known composition. Gas exchange ratio (R) was calculated as $\dot{V}CO_2/\dot{V}O_2p$. HR was determined from the electrocardiogram signal.

Oxygenation changes in a superficial portion of vastus lateralis muscles of both limbs were evaluated by NIRS. The main advantages and limitations of this technology have been recently discussed in reviews (6, 15). Portable continuous-wave, spatially resolved near-infrared (NIR) light photometers (PortaLite, Artinis Medical Systems) were utilized. The PortaLite probe consists of three light transmitters (each emitted two wavelengths of 760 nm and 850 nm) separated by 3, 3.5, and 4 cm from the receiving optode. The deepest signal (4 cm) was taken into account for the analysis. Thus the light penetration depth can be estimated to be at least 2 cm [i.e., at least about half of the source detector distance (18)]. The instruments provide measurements of micromolar (μM) changes in deoxygenated hemoglobin (Hb)+myoglobin (Mb) concentrations (Δ[deoxy(Hb+Mb)]) and in oxygenated (Hb+Mb; Δ[oxy(Hb+Mb)]). The sum between the two variables (Δ[deoxy(Hb+Mb)+oxy(Hb+Mb)]) is related to changes in the total Hb volume (blood volume in the investigated tissue). An increased Δ[deoxy(Hb+Mb)] or a decreased Δ[oxy(Hb+Mb)], would indicate an increased fractional O_2 extraction (ratio between $\dot{V}O_2m$ and O_2 delivery in the investigated tissue (see Ref. 15) only when

Δ[deoxy(Hb+Mb)+oxy(Hb+Mb)] is constant. This is unlikely in exercising muscles. In the past the problem was circumvented, at least in part, by taking as an index of deoxygenation the Δ[deoxy(Hb+Mb)] variable, which is relatively insensitive to blood volume changes, and has been demonstrated to nicely correlate with other variables related to fractional O_2 extraction (15). In the present study, the problem was solved by utilizing, for values during exercise, the method proposed by Ryan et al. (34), which allows correction of the Δ[deoxy(Hb+Mb)] variable for changes in blood volume.

The probes were firmly attached to the skin overlying the lower third of vastus lateralis muscles (~10 cm above the knee joint) of the right and left limbs, parallel to the major axis of the thigh, by a belt secured by Velcro straps and adhesive tape. The skin was carefully shaven before the experimentation. The places where the probes were attached were recorded using a skin marker and reproduced throughout the tests. Black clothes were put around the probes and the skin to prevent contamination from ambient light. The sampling frequency was set at 10 Hz. Skinfold thicknesses at the sites of application of the NIR probes were determined by a caliper (Gima, Milan, Italy) to estimate adipose tissue thickness (ATT). The averaged values of skin and subcutaneous tissue thickness were 3.6 ± 1.0 and 4.1 ± 1.0 mm for the right and left limb, respectively. The NIRS probe on the left leg was utilized to determine muscle deoxygenation changes during recovery, whereas the NIRS probe on the right leg was utilized to determine muscle oxygenation changes during exercise and for the repeated ischemia protocol and the determination of the $\dot{V}O_2m$ off-kinetics during the recovery (see below).

Δ[Deoxy(Hb+Mb)] values with respect to an initial value arbitrarily set equal to zero, were calculated and expressed in arbitrary units. Before the exercise period, an ischemic/hyperemia calibration of the right limb (i.e., physiological normalization) was utilized to normalize Δ[deoxy(Hb+Mb)] values (27) by inflating a pressure cuff (~300 mmHg) positioned at the inguinal crease of the thigh (subjects in the sitting position on the cycloergometer) for a few minutes (from 2 to 4) until a signal plateau (indicating maximal deoxygenation) was reached. Δ[deoxy(Hb+Mb)] values obtained during exercise were then expressed as a percentage of the values obtained during the ischemic calibration. All subjects were seated on the cycle ergometer during the recovery period. They were instructed to place the leg on which the occlusions were performed on a wooden platform (height 10 cm), with the foot fixed to the pedal, and to keep the other leg relaxed, with the foot fixed to the pedal.

$\dot{V}O_2p$ and HR kinetics. $\dot{V}O_2p$ kinetics were mathematically evaluated during transitions from rest to low (MODERATE) and high (HEAVY) intensity CWR exercises (on-kinetics) and during the recovery from MODERATE, HEAVY, and INCR exercises (off-kinetics). Breath-by-breath $\dot{V}O_2p$ values were initially examined to exclude outlier values caused by sighs, swallowing, and coughs, time aligned, and then superimposed for each subject (25). Average $\dot{V}O_2p$ values every 10 s were calculated. Data obtained during the first 20 s of the on-transition [cardiodynamic phase (42)] were excluded from analysis. Thus on-$\dot{V}O_2p$ kinetics analysis dealt mainly with *phase 2* (or fundamental component) of the response. To evaluate mathematically the $\dot{V}O_2p$ kinetics, data were first fitted by the function:

$$y(t) = y_{BAS} + A_f\left[1 - e^{(t-TD_f)/\tau_f}\right] \quad (1)$$

and parameter values (TD_f, τ_f) were determined that yielded the lowest sum of squared residuals. In *Eq. 1*, t is the time, y_{BAS} indicates the baseline, A_f is the amplitude between the y_{BAS} and the steady state during the fundamental component, TD_f is the time delay, and τ_f is the time constant of the function for the fundamental component. To check the presence of a slow component (42) of the kinetics, data were also fitted by other two functions. For details, please see Zuccarelli et al. (45).

Average HR values every 5 s were calculated. HR kinetics were analyzed by applying the same equations described above for $\dot{V}O_2p$, as suggested by previous authors (see e.g., Refs. 11 and 45).

For the $\dot{V}O_2p$ off-kinetics, a monoexponential function based on previous literature (29) was utilized:

$$y(t) = y_{END} - A\left[1 - e^{(t-TD)/\tau}\right] \quad (2)$$

where $y(t)$ represents the $\dot{V}O_2p$ value at a given time (t), y_{END} is the average value over the last 60 s of exercise, A is the amplitude of the exponential term describing changes in $\dot{V}O_2p$ from exercise to its asymptote during the recovery, τ is the time constant, and TD is the time delay of the function.

Equation 2 was also used for the analysis of skeletal muscle reoxygenation off-kinetics (Δ[deoxy(Hb+Mb)]) in the leg without occlusions.

$\dot{V}O_2m$ off-kinetics. Following the method proposed by Ryan et al. (34) and Adami and Rossiter (3), $\dot{V}O_2m$ was estimated by calculating the slope of the initial linear increase (~3 s) in NIRS-measured Δ[deoxy(Hb+Mb)] during short (5–10 s) bouts of ischemia induced by rapid (<1 s) inflation and deflation (DN 200/10/5, Stanley) of a pneumatic cuff during the recovery from MODERATE, HEAVY, and INCR exercises. A repeated arterial occlusion method (see Refs. 3 and 34) was carried out at the end of each exercise protocol (i.e., INCR, MODERATE, and HEAVY). When the muscle reached a desaturation target of 50% of the physiological normalization (1) (see below), several intermittent arterial occlusions were performed: six occlusions lasting 5 s each, separated by 10 s, and subsequently six occlusions lasting 10 s separated by 30–60 s. When the target of 50% was not reached at the end of the exercise protocol, the first arterial occlusion was performed after 10 s.

$\dot{V}O_2m$ values were then fit by a monoexponential function according to *Eq. 3* (33):

$$y(t) = y_{END} - A \times e^{-kt} \quad (3)$$

where $y(t)$ represents the value of $\dot{V}O_2m$ at a given time (t), y the $\dot{V}O_2m$ immediately after the cessation of the exercise, A is the amplitude of the response, k is the exponential recovery rate constant ($k = [1/\tau]$; expressed in min^{-1}), and t is time. Resting $\dot{V}O_2m$ values were estimated by the same approach, described above, on the data obtained during an arterial occlusion carried out at rest before the physiological normalization procedure (see above).

Statistical analysis. Results are expressed as means ± SD. Data fitting by exponential functions was performed by the least-squared residuals method. The statistical significance of differences between HR and $\dot{V}O_2p$ slow component amplitudes was checked by a two-tailed Student's t test for paired data. A one-way ANOVA with repeated measures was used to analyze the differences of $\dot{V}O_2m$, $\dot{V}O_2p$, and muscle deoxygenation parameters at the different exercise intensities. Assumptions of sphericity were assessed using the Mauchly test, and any violations were corrected using the Geisser-Greenhouse correction factor. When significant effects were observed, a Tukey's post hoc test was used to determine the exact location of the difference. The level of significance was set at $P < 0.05$. Statistical analyses were carried out with a commercially available software package (Prism 7.0; GraphPad). A coefficient of variation (CV) and intraclass correlation coefficient (ICC) were utilized to analyze test-retest reliability. ICC estimates were calculated using SPSS statistical package version 23 (SPSS, Chicago, IL) based on a mean-rating ($K = 2$), absolute-agreement, two-way mixed-effects model. The Bland-Altman test for repeated measurements was used to assess the agreement between the two evaluations of the $\dot{V}O_2m$ off-kinetics.

RESULTS

All subjects completed the entire protocol, with no adverse events. Adipose tissue thickness was not significantly different in the right compared with the left limb ($P = 0.30$). Main respiratory, cardiovascular, and metabolic end-exercise or steady-state values, determined during INCR (peak values) and

Table 1. *Main respiratory, cardiovascular, and metabolic end-exercise values or steady-state values, determined during incremental exercise (INCR) and constant work rate exercises (MODERATE and HEAVY)*

	INCR	MODERATE	HEAVY
Work rate, W	315 ± 45*#	118 ± 28§#	201 ± 36§*
$\dot{V}O_2p$, L/min	3.596 ± 0.442*#	2.067 ± 0.297§#	3.183 ± 0.356§*
$\dot{V}O_2p$, ml·kg^{-1}·min^{-1}	47.5 ± 6.7*#	28.0 ± 5.4§#	41.0 ± 6.0§*
$\dot{V}CO_2$, L/min	4.351 ± 0.428*#	1.994 ± 0.243§#	3.145 ± 0.313§*
R	1.21 ± 0.06*#	0.93 ± 0.06§#	1.00 ± 0.04§*
$\dot{V}E$, L/min	143.6 ± 28.5*#	52.8 ± 7.5§#	88.7 ± 11.1§*
V_T, L	2.95 ± 0.33*#	2.04 ± 0.28§#	2.61 ± 0.29§*
fR, breaths/min	49.4 ± 11.3*#	26.4 ± 4.5§#	34.2 ± 5.8§*
$P_{ET_{O_2}}$, mmHg	114.5 ± 4.8*#	100.1 ± 4.2§#	104.7 ± 4.2§*
$P_{ET_{CO_2}}$, mmHg	36.6 ± 5.2*#	43.8 ± 2.3§#	41.80 ± 3.3§*
HR, beats/min	181 ± 10*#	124 ± 17§#	162 ± 14§*
RPE, 6–20	19 ± 1.06*#	8 ± 2.03§#	16 ± 1.60§*
Δ[Deoxy(Hb+Mb)], %ischemia	68.3 ± 31.9*	20.7 ± 19.6§#	71.2 ± 19.9*

Values are means ± SD. $\dot{V}O_2p$, pulmonary oxygen uptake; $\dot{V}CO_2$, CO_2 output; R, gas exchange ratio; $\dot{V}E$, pulmonary ventilation; V_T, tidal volume; fR, breathing frequency; $P_{ET_{O_2}}$, end-tidal O_2 partial pressure; $P_{ET_{CO_2}}$, end-tidal CO_2 partial pressure; HR, heart rate; RPE, rate of perceived exertion; Δ[deoxy(Hb+Mb)], changes in deoxygenated Hb and myoglobin Mb concentrations, muscle oxygenation index obtained by near-infrared spectroscopy. *$P < 0.05$ vs. MODERATE. #$P < 0.05$ vs. HEAVY. §$P < 0.05$ vs. INCR.

MODERATE and HEAVY CWR exercises are shown in Table 1. $\dot{V}O_2p$ peak values were typical for young physically active subjects. R peak, HR peak [corresponding to 96% of the age-predicted maximum (calculated as 208 - 0.7 × age) (39)], and RPE peak values indirectly confirm that the INCR exercise was maximal. GET occurred at 58% of $\dot{V}O_2p$ peak. Work rates for MODERATE and HEAVY were 37 ± 6 and 64 ± 5% of peak work rate, respectively. Mean values of $\dot{V}O_2p$ and HR determined during the last 30 s of MODERATE were 58 ± 6% of $\dot{V}O_2p$ peak and 69 ± 9% of HR peak, respectively, whereas during HEAVY values were 89 ± 6% of $\dot{V}O_2$peak and 90 ± 6% of HR peak. Skeletal muscle fractional O_2 extraction (as indicated by Δ[deoxy(Hb+Mb)]) during the last 10 s of MODERATE, HEAVY, and INCR was ~21, 71, and 68% of the ischemic/hyperemia calibration, respectively. Δ[Deoxy(Hb+Mb)] values were significantly lower in MODERATE compared with INCR and HEAVY ($F = 39.80$; $P < 0.01$). No significant differences were observed between HEAVY and INCR.

In Fig. 1, $\dot{V}O_2p$ and HR on-kinetics obtained in a typical subject for the two investigated intensities are shown. No slow component for $\dot{V}O_2p$ was observed in MODERATE, whereas a slow component was observed for HR. In HEAVY slow components were detected both for $\dot{V}O_2p$ and HR. In Table 2, parameters deriving from the fitting of $\dot{V}O_2p$ and HR on-kinetics are presented. For MODERATE, *Eq. 1* represented the best fit for the $\dot{V}O_2p$ data in all subjects with the exception of one, who did show a slow component with an amplitude relative to the entire responses (A'_s/A_{tot}) equal to 7.8%. For HR, confirming the data obtained in a recent study by our group (45) a slow component with a relative amplitude of 21.4 ± 13.0% of A'_s/A_{tot} was detected in 12 subjects of 15. For HEAVY, a slow component was observed in all subjects, for both $\dot{V}O_2p$ and HR. The relative amplitude of the HR slow component was greater than the relative amplitude of the $\dot{V}O_2p$ slow component (23.0 ± 11.0 and 13.3 ± 6.4%, respectively); also, these data confirm those obtained in a previous study by our group.

Representative $\dot{V}O_2p$ and $\dot{V}O_2m$ off-kinetics curves for a typical subject following MODERATE, HEAVY, and INCR are shown in Fig. 2. A monoexponential decrease was observed in all conditions for both variables. For $\dot{V}O_2m$, individual values of the coefficient of determination (r^2) ranged between 0.93 and 0.99. For $\dot{V}O_2p$, the r^2 range was 0.96–0.99. In the panel with the $\dot{V}O_2m$ off-data, values obtained at rest before the exercise are also shown (dashed horizontal line). Δ[Deoxy(Hb+Mb)] values during the first occlusion following MODERATE, HEAVY, and INCR were ~11, 49, and 48%, respectively, of the ischemic/hyperemia calibration. $\dot{V}O_2m$ values at the onset of recovery (extrapolated to time = 0 s according to the fitted monoexponential curve) were ~27, 38, and 35 times higher than those determined at rest (dashed horizontal line) for MODERATE, HEAVY, and INCR, respectively.

As mentioned above, following MODERATE and HEAVY each subject performed two repetitions of the protocol for $\dot{V}O_2m$ off-kinetics determination: individual test-retest reproducibility was moderate and good for MODERATE and HEAVY, respectively [interclass correlation coefficient (ICC) = 0.65; coefficient of variation (CV) = 43.5% for MODERATE and ICC = 0.76, CV = 29.9% for HEAVY]. A corresponding Bland-Altman plot revealed a mean bias of −2.76 s and a 95% confidence interval of −24.91, 19.38 s.

Parameters of the $\dot{V}O_2p$ and $\dot{V}O_2m$ off-kinetics are reported in Table 3. For both $\dot{V}O_2p$ off- and $\dot{V}O_2m$-off, τ and mean response time (MRT = τ+time delay) values were significantly lower in MODERATE (see statistical details in Table 3) vs. HEAVY and INCR, whereas no significant differences were observed between HEAVY and INCR. τ of $\dot{V}O_2m$-off and $\dot{V}O_2p$-off values are also presented in Fig. 3. τ $\dot{V}O_2m$ off- was significantly lower (faster kinetics) than the τ $\dot{V}O_2p$ off- following MODERATE, whereas no significant differences were observed following HEAVY or INCR. The same conclusions applied to k ($k = [1/\tau]$). A significant correlation between individual values of the τ $\dot{V}O_2p$ off- and the τ of $\dot{V}O_2m$ off- was observed following MODERATE, but not following HEAVY or INCR (Fig. 4). In Fig. 4, the identity lines ($y = x$) are also shown. Following MODERATE, all experimental points (with the exception of one) lay above the identity line, confirming that the τ $\dot{V}O_2p$ off- overestimated the τ $\dot{V}O_2m$ off-. A significant correlation ($r = 0.43$, $P = 0.003$) between individual values of k of $\dot{V}O_2m$ off- and $\dot{V}O_2p$ peak values during INCR, HEAVY, and MODERATE was found (Fig. 5).

For $\dot{V}O_2p$ off-, the asymptotic values of the monoexponential functions were higher in INCR (0.673 ± 0.076 L/min) vs. HEAVY (0.516 ± 0.053 L/min) and MODERATE (0.411 ± 0.061 L/min), and were higher in HEAVY vs. MODERATE. The same trend was also observed for $\dot{V}O_2m$ off-values.

The kinetics of vastus lateralis deoxygenation (Δ[deoxy (Hb+Mb)]) off-kinetics in the leg which did not undergo the repeated occlusions protocol, fitted by a monoexponential function, were significantly faster following MODERATE (TD = 11.3 ± 4.9 s; τ = 22.3 ± 14.0 s; MRT = 33.6 ± 17.7 s) vs. following HEAVY (TD = 19.8 ± 8.1 s; τ = 48.5 ± 24.9 s; MRT = 68.3 ± 28.2 s) and INCR (TD = 17.2 ± 11.5 s; τ = 59.4 ± 25.8 s; MRT = 76.4 ± 30.8 s). No significant dif-

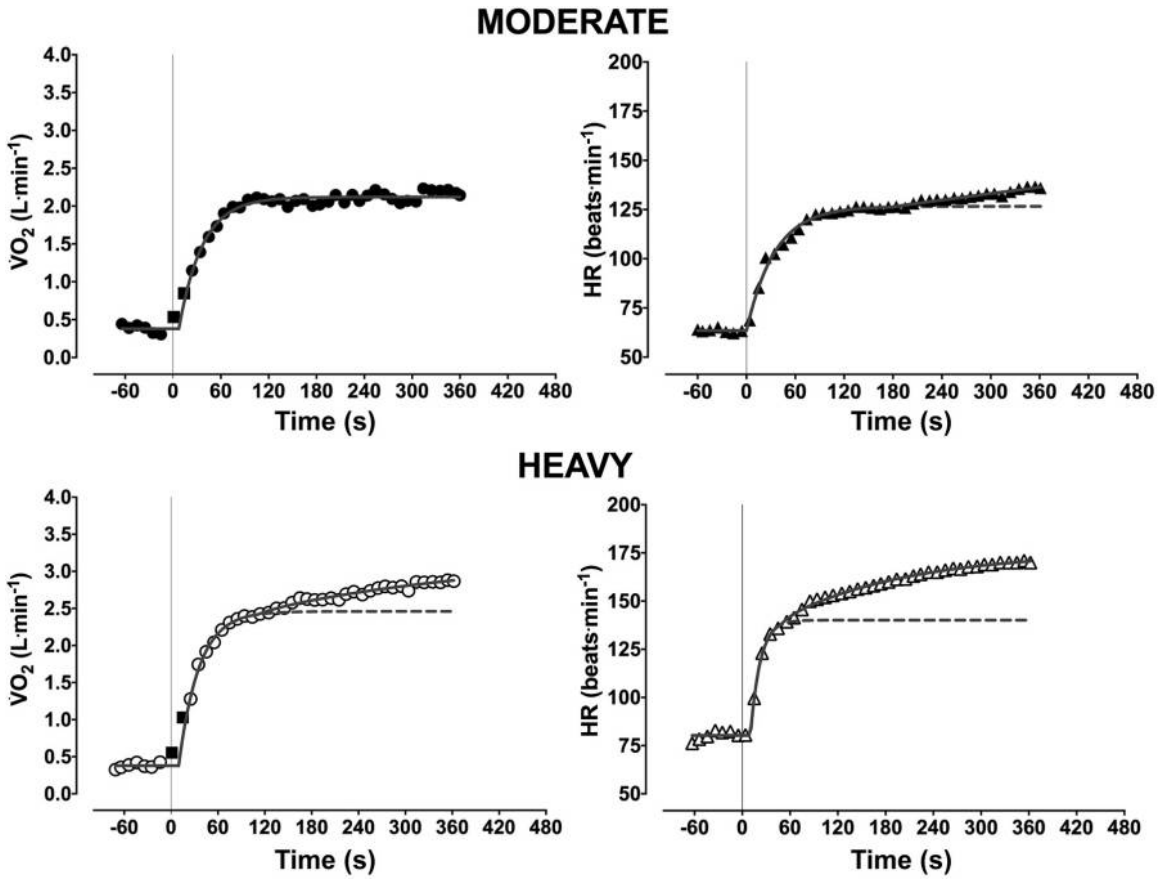

Fig. 1. Pulmonary O_2 uptake ($\dot{V}O_2p$; *left*) and heart rate (HR; *right*) on-kinetics for a representative subject during constant work rate (CWR) exercise at 2 investigated intensity domains, MODERATE and HEAVY. The fitted functions are also shown. The dashed lines indicate the continuation of the monoexponential fitting; vertical distances between experimental data and the dashed lines indicate the amplitude of the slow components of the responses. The first two $\dot{V}O_2p$ data points (cardiodynamic phase) were excluded from the fitting. The vertical lines indicate the transitions from rest to the imposed work rate. See text for further details.

ferences were observed between HEAVY and INCR. The MRT of the Δ[deoxy(Hb+Mb)] off-kinetics was not different from the τ of $\dot{V}O_2m$ off- following MODERATE, whereas it was substantially slower following HEAVY and INCR.

In Fig. 6, typical examples of the off-kinetics of Δ[deoxy (Hb+Mb)], Δ[oxy(Hb+Mb)], and Δ[oxy+deoxy(Hb+Mb)] obtained on the vastus lateralis muscles of the leg undergoing (*top* panels) and not undergoing (*bottom* panels) the repeated occlusions protocol are shown. As expected, in the leg undergoing the repeated occlusions, during each occlusion (vertical lines) an increase in Δ[deoxy(Hb+Mb)] and a decrease in the Δ[oxy(Hb+Mb)], with no significant change in Δ[oxy+deoxy(Hb+Mb)], were observed. The pattern of the three variables is typical for an increased fractional O_2 extraction, induced by the occlusion of blood flow. Interestingly, in the contralateral leg, not undergoing the repeated occlusion protocol, in the period corresponding to the occlusions occurring in the contralateral leg Δ[deoxy(Hb+Mb)] decreased, whereas Δ[oxy (Hb+Mb)] and Δ[oxy+deoxy(Hb+Mb)] increased, following a pattern typical for vasodilation. In other words, the

Table 2. *Pulmonary O_2 uptake ($\dot{V}O_2p$) and heart rate (HR) kinetics parameters determined during constant work rate (CWR) exercises*

Intensity Domain	Work Rate, W	$\dot{V}O_2p_{bas}$, L/min	$\dot{V}O_2p$, L/min	A_f, L/min	TD_f, s	T_f, s	TD_s, s	A_s', L/min	A_s'/A_{tot}, %
MODERATE	118 ± 28	0.420 ± 0.069	2.067 ± 0.297	1.647 ± 0.301	10.3 ± 6.2	19.4 ± 4.9			
HEAVY	201 ± 36	0.470 ± 0.096	3.183 ± 0.356	2.361 ± 0.403	10.2 ± 3.4	23.5 ± 7.1	109.8 ± 38.3	0.489 ± 0.208	13.3 ± 6.4
Intensity Domain	Work Rate, W	HR_{bas}, beats/min	HR, beats/min	A_f, beats/min	TD_f, s	τ_f, s	TD_s, s	A_s', beats/min	A_s'/A_{tot}, %
MODERATE	118 ± 28	72 ± 11	124 ± 17	44 ± 12	3.1 ± 4.5*	15.5 ± 11.1*	105.1 ± 51.5	12 ± 9	21.4 ± 13.0
HEAVY	201 ± 36	78 ± 12	162 ± 14	65 ± 12	2.8 ± 3.4*	23.8 ± 11.0	81.4 ± 44.9*	20 ± 10	23.0 ± 11.0*

Values are means ± SD. MODERATE, moderate CWR exercise; HEAVY, heavy CWR exercise; $\dot{V}O_{2bas}$, oxygen uptake baseline; $\dot{V}O_2$, end-exercise oxygen uptake; HR_{bas}, heart rate baseline; HR, end-exercise heart rate; A_f, amplitude of the fundamental component; TD_f, time delay fundamental; τ_f, time constant fundamental; TD_s, time delay slow component; A_s', actual amplitude of the slow component; A_s'/A_{tot}, total amplitude of the response. *$P < 0.05$, significantly different from HR values.

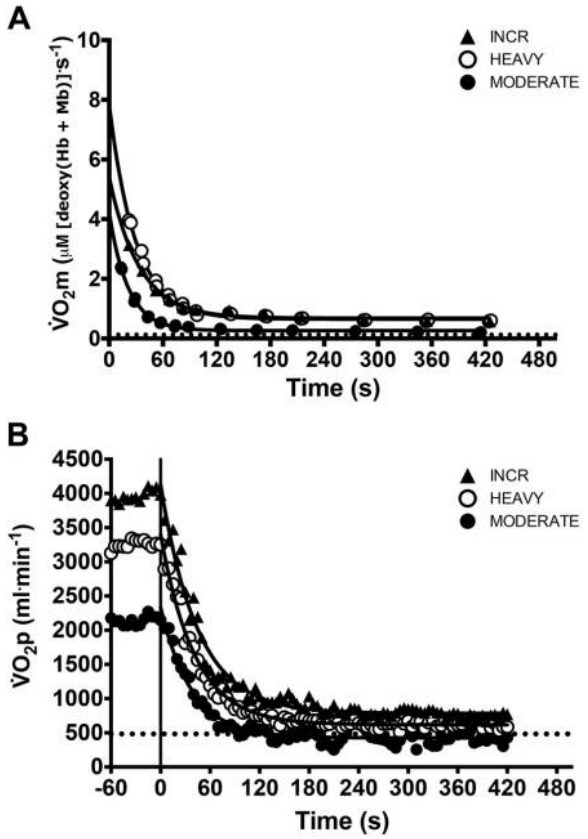

Fig. 2. *A*: muscle $\dot{V}O_2$ ($\dot{V}O_2m$) recovery kinetics for a representative subject following incremental (INCR), MODERATE, and HEAVY exercises are shown. *B*: pulmonary $\dot{V}O_2$ ($\dot{V}O_2p$) recovery kinetics following INCR, MODERATE, and HEAVY exercises for a representative subject. In *A* and *B*, experimental data and fitted functions are shown. The dotted horizontal lines indicate the resting baseline values. See text for further details.

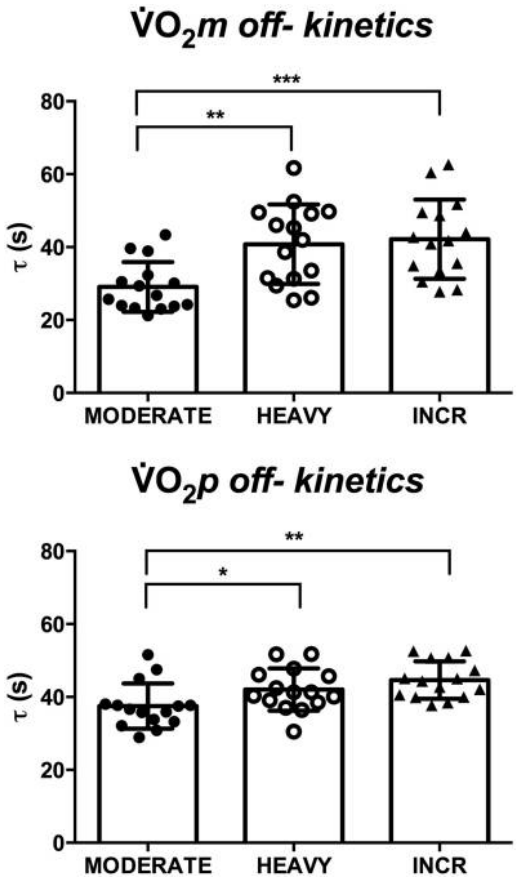

Fig. 3. Time constant (τ) values (individual values and means ± SD values) of muscle $\dot{V}O_2$ ($\dot{V}O_2m$) and pulmonary $\dot{V}O_2$ ($\dot{V}O_2p$) recovery kinetics following MODERATE, HEAVY, and INCR. *Significant difference ($P < 0.05$). See text for further details.

occlusions in one leg induced a reflex vasodilation in the contralateral leg.

DISCUSSION

To the best of our knowledge, this is the first study to analyze $\dot{V}O_2m$ off-kinetics by NIRS, by applying the repeated occlusions approach, during the recovery of CWR cycle ergometer exercise carried out at moderate intensity (MODERATE), at heavy intensity (HEAVY), and during the recovery from an incremental exercise (INCR). The study demonstrates the feasibility of the proposed approach and negates the need to perform a specific protocol of plantar flexion exercise, as proposed by Ryan et al. (34) and by Adami and Rossiter (3). In other words, $\dot{V}O_2m$ off-kinetics can be effectively determined following standard cycle ergometer exercises carried out for other purposes ($\dot{V}O_2p$ peak, GET, $\dot{V}O_2p$ on-kinetics evaluation). As discussed in the INTRODUCTION, $\dot{V}O_2m$ off-kinetics represents a valuable functional evaluation tool of skeletal muscle oxidative metabolism, which can be utilized in normal subjects, athletes, and patient populations.

Table 3. *Muscle ($\dot{V}O_2m$) and pulmonary O_2 uptake ($\dot{V}O_2p$) kinetics parameters determined in the recovery from incremental exercise (INCR) and constant work rate (CWR) exercises*

	MODERATE (95%CI)		HEAVY (95%CI)		INCR (95%CI)	
	$\dot{V}O_2m$ (95%CI)	$\dot{V}O_2p$ (95%CI)	$\dot{V}O_2m$ (95%CI)	$\dot{V}O_2p$ (95%CI)	$\dot{V}O_2m$ (95%CI)	$\dot{V}O_2p$ (95%CI)
TD, s		1.08 ± 1.35 (0.33–1.84)		1.6 ± 2.0 (0.47–2.58)		1.9 ± 1.7 (0.93–2.84)
τ, s	29.1 ± 6.8*# (25.3–32.9)	37.5 ± 6.2# (34.0–40.9)	40.8 ± 10.9 (34.8–46.8)	42.1 ± 6.0 (38.8–45.2)	42.2 ± 10.9 (36.2–48.2)	44.7 ± 5.1 (41.8–47.5)
MRT, s		38.6 ± 7.1# (34.7–42.5)		43.5 ± 6.9 (39.7–47.4)		46.5 ± 5.0 (43.8–49.3)
k, min^{-1}	2.16 ± 0.45* (1.91–2.41)	1.64 ± 0.25# (1.50–1.77)	1.58 ± 0.44 (1.33–1.82)	1.45 ± 0.22 (1.34–1.57)	1.51 ± 0.38 (1.30–1.72)	1.36 ± 0.15 (1.28–1.44)

Values are means ± SD. TD, time delay; τ, time constant; MRT, mean response time; k, recovery rate constant; CI, confidence interval. *Significantly different ($P < 0.05$) from corresponding value for $\dot{V}O_2p$.#$P < 0.05$ vs. HEAVY and INCR.

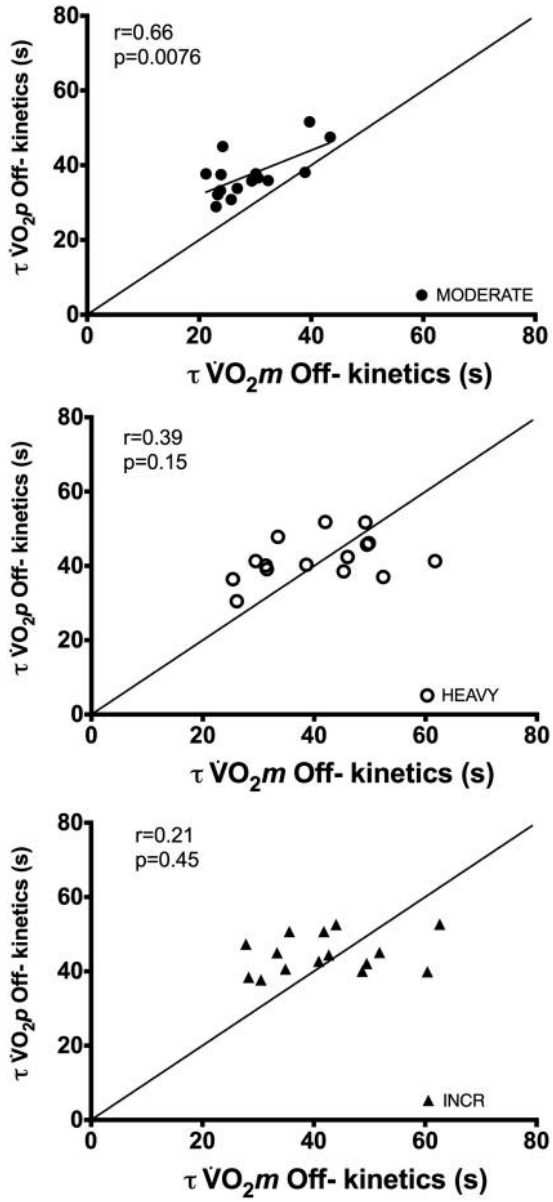

Fig. 4. Individual values of the time constant (τ) of the pulmonary $\dot{V}O_2$ ($\dot{V}O_2p$) recovery kinetics as a function of the τ of the muscle $\dot{V}O_2$ ($\dot{V}O_2m$) recovery kinetics, following MODERATE, HEAVY, and INCR. The identity lines ($y = x$) are also shown. Only following MODERATE a significant linear correlation (shown in the figure) was observed between the 2 variables. See text for further details.

More specifically, the quality of the monoexponential fitting utilized for $\dot{V}O_2m$ off-kinetics analysis was excellent (r^2 values between 0.93 and 0.99). All subjects tolerated the repeated occlusions protocol without significant problems, even following the intense or exhaustive exercise, and the study had no drop-outs. Based on the ICC results, the test-retest reliability was moderate to good. The values of the time constant (τ) of the $\dot{V}O_2m$ off-kinetics were significantly lower (indicating a faster kinetics) following MODERATE compared with HEAVY and INCR. No significant differences were observed between τ values in HEAVY and INCR. The mechanisms responsible for the slower $\dot{V}O_2m$ off-kinetics at higher work rates could be similar to those responsible for the slow component of $\dot{V}O_2p$ on-kinetics: the recruitment of intrinsically slower fibers, in terms of oxidative metabolism; the presence of acidosis; reduced efficiency/fatigue; and a relative lack of O_2 (16, 21). In the present study, however, no significant correlation was observed between the relative amplitude of the slow component of $\dot{V}O_2p$ on-kinetics and the difference in τ of the $\dot{V}O_2m$ off-kinetics determined following MODERATE and HEAVY. The individual values of the skeletal muscle rate constant (k) correlated with $\dot{V}O_2p$ peak, confirming the data obtained by Wüst et al. (43) in an animal model, as mentioned in the INTRODUCTION. The observation confirms the role of $\dot{V}O_2m$ off-kinetics as a functional evaluation tool of oxidative metabolism.

In terms of O_2 availability during the recovery phase, our study allows to make some indirect inferences. According to Adami and Rossiter (3), adequate O_2 availability is a prerequisite for a reliable functional evaluation of oxidative metabolism by the determination of the $\dot{V}O_2m$ off-kinetics. Following the recommendations by Adami and Rossiter (3), to ensure adequate O_2 availability the repeated occlusions protocol was initiated at specific percentages of the physiological calibration (the range between Δ[deoxy(Hb+Mb)] at the end of the sustained arterial occlusion and the peak value reached during the reactive hyperemia; see MATERIALS AND METHODS). Whereas the occlusions following MODERATE exercise were conducted under conditions of almost maximum O_2 availability (first occlusion occurring at ~10% of the distance between maximum oxygenation and maximum deoxygenation; the following occlusions at even lower percentages), for HEAVY and INCR the availability of O_2 was relatively minor (first occlusion at ~50% of the physiological calibration range). According to Adami and Rossiter (3), this percentage should correspond to an adequate O_2 availability, but no experimental data have been provided to support this concept. Thus in strict terms it cannot be excluded that the slower $\dot{V}O_2m$ off-kinetics observed following HEAVY and INCR, with respect to MODERATE, could be attributable, at least in part, to a reduced availability of O_2. In any case, the exercise-intensity dependency of the τ of $\dot{V}O_2m$ off- observed in the present study underscores the need to quantify the absolute and relative (i.e., with respect to GET, critical power, $\dot{V}O_2p$ peak) intensity of the exercise preceding the recovery phase during which the $\dot{V}O_2m$

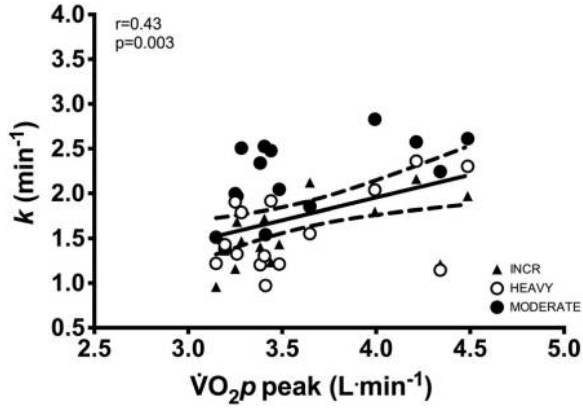

Fig. 5. Individual values of the recovery rate constant (k) of muscle $\dot{V}O_2$ ($\dot{V}O_2m$) recovery kinetics following MODERATE, HEAVY, and INCR as a function of pulmonary $\dot{V}O_2$ peak. Dashed lines indicate the 95% confidence intervals.

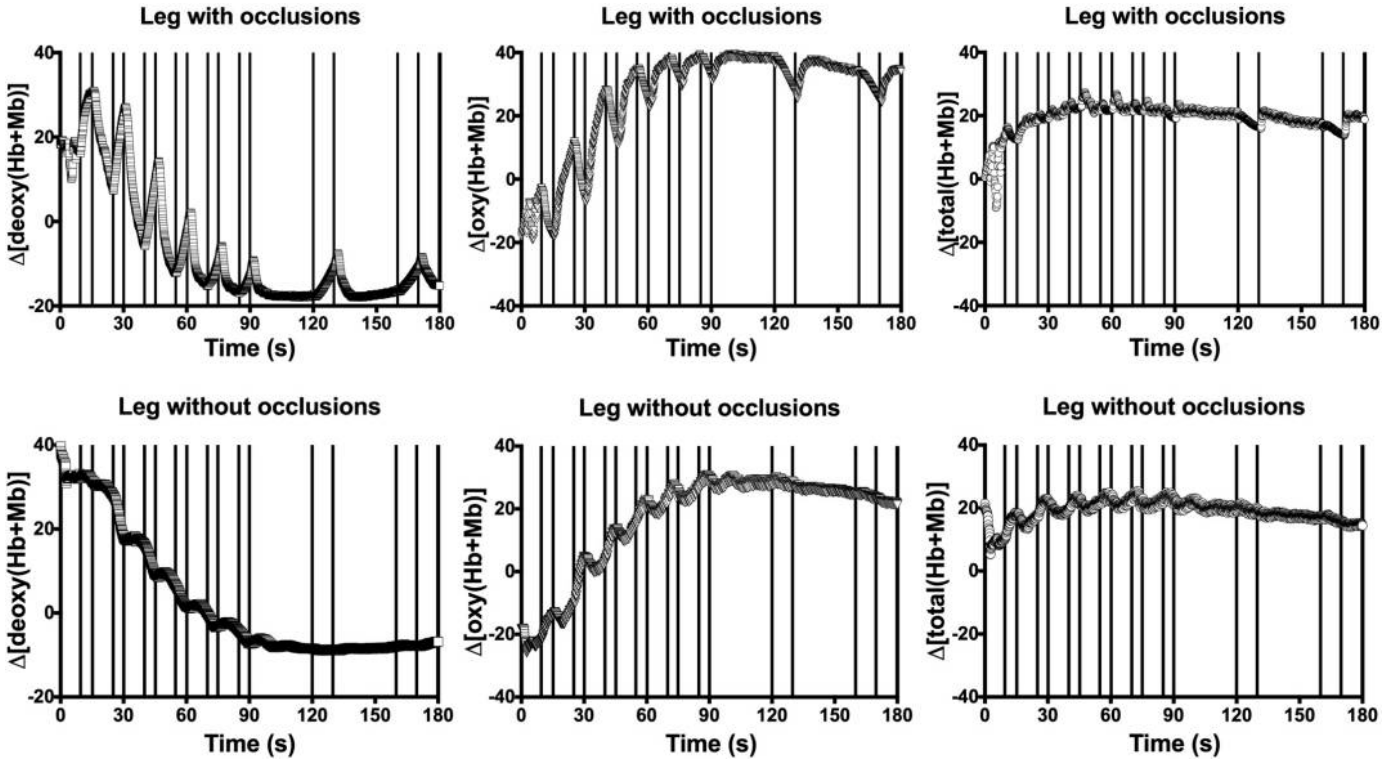

Fig. 6. Typical individual examples of Δ[deoxy(Hb+Mb)], Δ[oxy(Hb+Mb)], and Δ[oxy+deoxy(Hb+Mb)] near-infrared spectroscopy (NIRS)-derived signals during the recovery from HEAVY exercise in the leg in which the ischemia protocol was performed (leg with occlusions) and in the leg in which the occlusions were not performed. The vertical lines indicate the beginning and the cessation of each period of ischemia. See text for further details.

off-kinetics is determined. This quantification was impossible following the experimental approach proposed by Adami and Rossiter (3) and by Ryan et al. (34), whereas it was feasible by following the approach utilized in the present study.

Another methodological aspect which remained unresolved with the approach proposed by Ryan et al. (34) and by Adami and Rossiter (3) is the following: how much, in quantitative terms, was $\dot{V}O_2m$ increased (vs. rest) during the exercise preceding the recovery phase? The issue is critical, since, as suggested by Adami and Rossiter (3), only in the presence of a significant activation of oxidative metabolism the determination of the $\dot{V}O_2m$ off-kinetics would represent a valid functional evaluation tool. The data of the present study allow an indirect answer to this question, based on two assumptions. *1*) The backextrapolation to *time 0* of the monoexponential function describing $\dot{V}O_2m$ off-kinetics represents a reliable estimate of the $\dot{V}O_2m$ at the end of exercise. Considering the very precise fitting of $\dot{V}O_2m$ data, and since the adopted monoexponential functions substantially yield no time delay, the mentioned assumptions appear legitimate. *2*) $\dot{V}O_2m$ values obtained during the ischemia carried out with the subject in resting conditions (see MATERIALS AND METHODS) represent a reliable estimate of the resting oxidative metabolism of the muscle. When it was calculated for MODERATE, HEAVY, and INCR, the ratio between the $\dot{V}O_2m$ extrapolated to the end of exercise and the resting $\dot{V}O_2m$ (horizontal dashed lines in Fig. 2*A*), values equal to ~27, 35, and 38 were obtained. In other words, at the end of the exercise $\dot{V}O_2m$ values were ~25–40 times higher than at rest. These values appear to be compatible with the literature (4, 5, 24) and confirm the significant increase in oxidative energy expenditure during the exercise preceding the off-kinetics. The resting $\dot{V}O_2m$ values indicated by the dashed line in Fig. 2 allow us to make a further observation. At the end of the recovery phase considered in the present study (~7 min), the $\dot{V}O_2m$ values (see the asymptote of the function describing $\dot{V}O_2m$ off-kinetics) were still significantly higher than the $\dot{V}O_2m$ values at rest. In other words, a very slow component of the $\dot{V}O_2m$ off-kinetics was presumably present, and it could not be considered by our analysis (26).

By utilizing the velocity constant k as the parameter to evaluate the $\dot{V}O_2m$ off-kinetics, we were able to compare the data obtained in the present study, following the three investigated work rates (2.16 min^{-1} following MODERATE, 1.58 min^{-1} following HEAVY, 1.51 min^{-1} following INCR), with literature obtained in different populations by utilizing the plantar flexion exercise protocol (see INTRODUCTION), and summarized by Adami and Rossiter (3). Since $k = 1/\tau$, a faster kinetics is indicated by a lower value of τ (time constant) or by a higher value of k (velocity constant). The k data of the present study (see above) substantially correspond to the higher (MODERATE) and lower (HEAVY and INCR) ends of the spectrum for "normal" subjects reported by Adami and Rossiter (3). As expected, the $\dot{V}O_2m$ off-kinetics of the present study were significantly slower than those observed in endurance athletes (9), and significantly faster than those observed in patient populations [chronic heart failure (38, 44), spinal cord injury (12, 13), chronic obstructive pulmonary disease (1, 2)]. As mentioned above, our data underscore the exercise intensity dependency of the $\dot{V}O_2m$ off-kinetics. On the other hand, an exercise intensity dependency could not be identified by the

plantar flexion protocol proposed by Ryan et al. (34) and by Adami and Rossiter (3), in which exercise intensity cannot be quantified.

A slightly slower $\dot{V}O_2m$ off-kinetics following sprint vs. moderate running was described by Buchheit et al. (10) by utilizing an experimental approach similar to that of the present study. On the other hand, no difference in $\dot{V}O_2m$ off-kinetics, determined by a different method (invasive measurements and the Fick equation to calculate $\dot{V}O_2m$ across the exercising muscles), was described by Krustrup et al. (24) following moderate- vs. heavy-intensity knee extension exercise. Rossiter et al. (31) observed no difference in τ for the PCr off-kinetics (considered a close proxy of $\dot{V}O_2m$ off-; see INTRODUCTION) following moderate- vs. heavy-intensity knee extension exercise. After consideration that following heavy-intensity exercise a slow component of PCr off- was observed in the study by Rossiter et al. (31), the results of the present study (slower $\dot{V}O_2m$ off- following heavy-intensity exercise) appear in substantial agreement with those obtained by Rossiter et al. (31). Ryan et al. (35) observed no differences in $\dot{V}O_2m$ off-kinetics (NIRS+repeated occlusions, as in the present study) following plantar flexion exercises carried out with increasing contraction frequencies; the difference with the results of the present study could relate, at least in part, to the very short duration (15 s) of the exercise employed by Ryan et al. (35), which obviously precluded the reaching of a steady state for $\dot{V}O_2m$.

Regarding the $\dot{V}O_2p$ off-kinetics, they followed a pattern similar to that described above for $\dot{V}O_2m$ off-: the kinetics were faster following MODERATE vs. following HEAVY or INCR, with no significant difference between these last two conditions. As far as the comparison between the $\dot{V}O_2m$ off- and the $\dot{V}O_2p$ off-kinetics, no significant differences were described following HEAVY and INCR, whereas following MODERATE the $\dot{V}O_2m$ off-kinetics was faster. On the other hand, only following MODERATE a significant correlation between the τ of $\dot{V}O_2p$ off- and the τ of $\dot{V}O_2m$ off- was observed. In other words, following MODERATE the τ of $\dot{V}O_2p$ off- was correlated with, but overestimated, the τ of $\dot{V}O_2m$ off-. Following HEAVY and INCR, no correlations between the two variables were observed. Thus following all exercise intensities the $\dot{V}O_2p$ off-kinetics cannot be utilized as a proxy for the $\dot{V}O_2m$ off-kinetics. This confirms the conclusions by the study of Krustrup et al. (24), in which $\dot{V}O_2m$ off-kinetics were determined, by a different method, following knee extension exercise. The mechanisms responsible for the discrepancies between the two kinetics are likely attributable to the influence of cardiocirculatory adjustments (cardiac output is exponentially decreasing in the period taken into consideration by the off-kinetics analysis) and/or of changes in O_2 stores between skeletal muscles and the subject's mouth. The faster of $\dot{V}O_2m$ off- compared with the $\dot{V}O_2p$ off-kinetics described in the present study following MODERATE confirms the observations by Krustrup et al. (24) following knee extension exercise.

As illustrated in Fig. 6, during the recovery following exercise, ischemia applied to one leg induced a reflex vasodilation in the contralateral leg. To the best of our knowledge, this represents a novel observation. It has been described before that voluntary contraction in another limb (19), mental stress (8), immobile alerting and fighting behavior, and imagery of voluntary exercise (19) induce vasodilation and increase blood flow in another limb. The mechanism(s) responsible for the metaboreflex observed in the present study are not clear. Increased shear stress and increased nitric oxide (NO) and prostacyclin release by endothelial cells (37) must be excluded, since the metabolic signal(s) arose from an ischemic maneuver. β_2-Mediated vasodilatory effects, as a result of adrenaline release by the adrenal medulla (37), must be excluded as well, since the vasodilation occurred almost immediately during the ischemia in the other limb. It can be hypothesized that metabolic signals induced by the ischemia in one limb, superimposed on metabolic signals associated with the recovery phase following exercise, reached the cardiovascular control center through group III and IV afferent fibers. The efferent arm of the metaboreflex, responsible for the neurogenic vasodilation, is less clear. Conflicting evidence is present in the literature in favor or against the occurrence of a withdrawal of sympathetic vasoconstriction (see discussion in Ref. 19). The idea of a sympathetic cholinergic vasodilation (presumably involving NO) has been around for decades (see Refs. 22 and 37) and would be supported by the observation that the initial vasodilation in the nonexercising limb is blocked by atropine but not by propanolol (36). A strong argument against this possibility, however, lies in the lack of anatomic or histochemical evidence for any cholinergic neural pathway in human skeletal muscles (22, 37). In any case, the metaboreflex was present only transiently, since it disappeared after ~90 s into the recovery (see Fig. 6).

In conclusion, $\dot{V}O_2m$ off-kinetics determination by the NIRS repeated occlusions approach, carried out following standard cycle ergometer exercise at different intensities, is a feasible and useful functional evaluation tool for skeletal muscle oxidative metabolism. With respect to the original approach (plantar flexion exercise) proposed by Ryan et al. (34) and by Adami and Rossiter (3), the approach proposed in the present study can be applied during the recovery following standard cycle ergometer exercises conducted for the evaluation of other relevant variables of oxidative metabolism ($\dot{V}O_2p$ peak, gas exchange threshold, critical power, $\dot{V}O_2p$ kinetics), without the need of performing an additional protocol.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

L.Z., P.C.d.N.S., and B.G. conceived and designed research; L.Z., P.C.d.N.S., A.D.T., and R.F. performed experiments; L.Z., P.C.d.N.S., A.D.T., and R.F. analyzed data; L.Z., P.C.d.N.S., R.F., and B.G. interpreted results of experiments; L.Z., P.C.d.N.S., and R.F. prepared figures; L.Z., P.C.d.N.S., and B.G. drafted manuscript; L.Z., P.C.d.N.S., A.D.T., R.F., and B.G. edited and revised manuscript; L.Z., P.C.d.N.S., A.D.T., R.F., and B.G. approved final version of manuscript.

REFERENCES

1. **Adami A, Cao R, Porszasz J, Casaburi R, Rossiter HB.** Reproducibility of NIRS assessment of muscle oxidative capacity in smokers with and without COPD. *Respir Physiol Neurobiol* 235: 18–26, 2017. doi:10.1016/j.resp.2016.09.008.
2. **Adami A, Corvino RB, Casaburi R, Cao R, Calmelat R, Porszasz J, Rossiter HB.** Low oxidative capacity in skeletal muscle of both the upper and lower limbs in COPD patients. *FASEB J* 31: 1020.9, 2017.
3. **Adami A, Rossiter HB.** Principles, insights, and potential pitfalls of the noninvasive determination of muscle oxidative capacity by near-infrared spectroscopy. *J Appl Physiol (1985)* 124: 245–248, 2018. doi:10.1152/japplphysiol.00445.2017.
4. **Andersen P, Saltin B.** Maximal perfusion of skeletal muscle in man. *J Physiol* 366: 233–249, 1985. doi:10.1113/jphysiol.1985.sp015794.
5. **Bangsbo J, Krustrup P, González-Alonso J, Boushel R, Saltin B.** Muscle oxygen kinetics at onset of intense dynamic exercise in humans. *Am J Physiol Regul Integr Comp Physiol* 279: R899–R906, 2000. doi:10.1152/ajpregu.2000.279.3.R899.
6. **Barstow TJ.** Understanding near infrared spectroscopy and its application to skeletal muscle research. *J Appl Physiol (1985)* 126: 1360–1376, 2019. doi:10.1152/japplphysiol.00166.2018.
7. **Beaver WL, Wasserman K, Whipp BJ.** A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol (1985)* 60: 2020–2027, 1986. doi:10.1152/jappl.1986.60.6.2020.
8. **Blair DA, Glover WE, Greenfield AD, Roddie IC.** Excitation of cholinergic vasodilator nerves to human skeletal muscles during emotional stress. *J Physiol* 148: 633–647, 1959. doi:10.1113/jphysiol.1959.sp006312.
9. **Brizendine JT, Ryan TE, Larson RD, McCully KK.** Skeletal muscle metabolism in endurance athletes with near-infrared spectroscopy. *Med Sci Sports Exerc* 45: 869–875, 2013. doi:10.1249/MSS.0b013e31827e0eb6.
10. **Buchheit M, Ufland P, Haydar B, Laursen PB, Ahmaidi S.** Reproducibility and sensitivity of muscle reoxygenation and oxygen uptake recovery kinetics following running exercise in the field. *Clin Physiol Funct Imaging* 31: 337–346, 2011. doi:10.1111/j.1475-097X.2011.01020.x.
11. **Engelen M, Porszasz J, Riley M, Wasserman K, Maehara K, Barstow TJ.** Effects of hypoxic hypoxia on O_2 uptake and heart rate kinetics during heavy exercise. *J Appl Physiol (1985)* 81: 2500–2508, 1996. doi:10.1152/jappl.1996.81.6.2500.
12. **Erickson ML, Ryan TE, Backus D, McCully KK.** Endurance neuromuscular electrical stimulation training improves skeletal muscle oxidative capacity in individuals with motor-complete spinal cord injury. *Muscle Nerve* 55: 669–675, 2017. doi:10.1002/mus.25393.
13. **Erickson ML, Ryan TE, Young HJ, McCully KK.** Near-infrared assessments of skeletal muscle oxidative capacity in persons with spinal cord injury. *Eur J Appl Physiol* 113: 2275–2283, 2013. doi:10.1007/s00421-013-2657-0.
14. **Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK, Wagner PD.** Muscle O_2 uptake kinetics in humans: implications for metabolic control. *J Appl Physiol (1985)* 80: 988–998, 1996. doi:10.1152/jappl.1996.80.3.988.
15. **Grassi B, Quaresima V.** Near-infrared spectroscopy and skeletal muscle oxidative function in vivo in health and disease: a review from an exercise physiology perspective. *J Biomed Opt* 21: 091313, 2016. doi:10.1117/1.JBO.21.9.091313.
16. **Grassi B, Rossiter HB, Zoladz JA.** Skeletal muscle fatigue and decreased efficiency: two sides of the same coin? *Exerc Sport Sci Rev* 43: 75–83, 2015. doi:10.1249/JES.0000000000000043.
17. **Hamaoka T, Iwane H, Shimomitsu T, Katsumura T, Murase N, Nishio S, Osada T, Kurosawa Y, Chance B.** Noninvasive measures of oxidative metabolism on working human muscles by near-infrared spectroscopy. *J Appl Physiol (1985)* 81: 1410–1417, 1996. doi:10.1152/jappl.1996.81.3.1410.
18. **Hamaoka T, McCully KK, Quaresima V, Yamamoto K, Chance B.** Near-infrared spectroscopy/imaging for monitoring muscle oxygenation and oxidative metabolism in healthy and diseased humans. *J Biomed Opt* 12: 062105, 2007. doi:10.1117/1.2805437.
19. **Ishii K, Liang N, Oue A, Hirasawa A, Sato K, Sadamoto T, Matsukawa K.** Central command contributes to increased blood flow in the noncontracting muscle at the start of one-legged dynamic exercise in humans. *J Appl Physiol (1985)* 112: 1961–1974, 2012. doi:10.1152/japplphysiol.00075.2012.
20. **Jones AM, Grassi B, Christensen PM, Krustrup P, Bangsbo J, Poole DC.** Slow component of VO2 kinetics: mechanistic bases and practical applications. *Med Sci Sports Exerc* 43: 2046–2062, 2011. doi:10.1249/MSS.0b013e31821fcfc1.
21. **Jones AM, Krustrup P, Wilkerson DP, Berger NJ, Calbet JA, Bangsbo J.** Influence of exercise intensity on skeletal muscle blood flow, O_2 extraction and O_2 uptake on-kinetics. *J Physiol* 590: 4363–4376, 2012. doi:10.1113/jphysiol.2012.233064.
22. **Joyner MJ, Dietz NM.** Sympathetic vasodilation in human muscle. *Acta Physiol Scand* 177: 329–336, 2003. doi:10.1046/j.1365-201X.2003.01090.x.
23. **Kent JA, Fitzgerald LF.** In vivo mitochondrial function in aging skeletal muscle: capacity, flux, and patterns of use. *J Appl Physiol (1985)* 121: 996–1003, 2016. doi:10.1152/japplphysiol.00583.2016.
24. **Krustrup P, Jones AM, Wilkerson DP, Calbet JA, Bangsbo J.** Muscular and pulmonary O_2 uptake kinetics during moderate- and high-intensity sub-maximal knee-extensor exercise in humans. *J Physiol* 587: 1843–1856, 2009. doi:10.1113/jphysiol.2008.166397.
25. **Lamarra N, Whipp BJ, Ward SA, Wasserman K.** Effect of interbreath fluctuations on characterizing exercise gas exchange kinetics. *J Appl Physiol (1985)* 62: 2003–2012, 1987. doi:10.1152/jappl.1987.62.5.2003.
26. **Margaria R, Edwards HT, Dill DB.** The possible mechanisms of contracting and paying the oxygen debt and the role of lactic acid in muscular contraction. *Am J Physiol* 106: 689–715, 1933. doi:10.1152/ajplegacy.1933.106.3.689.
27. **McCully KK, Iotti S, Kendrick K, Wang Z, Posner JD, Leigh J Jr, Chance B.** Simultaneous in vivo measurements of HbO_2 saturation and PCr kinetics after exercise in normal humans. *J Appl Physiol (1985)* 77: 5–10, 1994. doi:10.1152/jappl.1994.77.1.5.
28. **Meyer RA.** A linear model of muscle respiration explains monoexponential phosphocreatine changes. *Am J Physiol Cell Physiol* 254: C548–C553, 1988. doi:10.1152/ajpcell.1988.254.4.C548.
29. **Özyener F, Rossiter HB, Ward SA, Whipp BJ.** Influence of exercise intensity on the on- and off-transient kinetics of pulmonary oxygen uptake in humans. *J Physiol* 533: 891–902, 2001. doi:10.1111/j.1469-7793.2001.t01-1-00891.x.
30. **Poole DC, Jones AM.** Oxygen uptake kinetics. *Compr Physiol* 2: 933–996, 2012.
31. **Rossiter HB, Ward SA, Kowalchuk JM, Howe FA, Griffiths JR, Whipp BJ.** Dynamic asymmetry of phosphocreatine concentration and $O_{(2)}$ uptake between the on- and off-transients of moderate- and high-intensity exercise in humans. *J Physiol* 541: 991–1002, 2002. doi:10.1113/jphysiol.2001.012910.
32. **Rossiter HB.** Exercise: kinetic considerations for gas exchange. *Compr Physiol* 1: 203–244, 2011.
33. **Ryan TE, Brophy P, Lin CT, Hickner RC, Neufer PD.** Assessment of in vivo skeletal muscle mitochondrial respiratory capacity in humans by near-infrared spectroscopy: a comparison with in situ measurements. *J Physiol* 592: 3231–3241, 2014. doi:10.1113/jphysiol.2014.274456.
34. **Ryan TE, Erickson ML, Brizendine JT, Young HJ, McCully KK.** Noninvasive evaluation of skeletal muscle mitochondrial capacity with near-infrared spectroscopy: correcting for blood volume changes. *J Appl Physiol (1985)* 113: 175–183, 2012. doi:10.1152/japplphysiol.00319.2012.
35. **Ryan TE, Southern WM, Reynolds MA, McCully KK.** A cross-validation of near-infrared spectroscopy measurements of skeletal muscle oxidative capacity with phosphorus magnetic resonance spectroscopy. *J Appl Physiol (1985)* 115: 1757–1766, 2013. doi:10.1152/japplphysiol.00835.2013.
36. **Sanders JS, Mark AL, Ferguson DW.** Evidence for cholinergically mediated vasodilation at the beginning of isometric exercise in humans. *Circulation* 79: 815–824, 1989. doi:10.1161/01.CIR.79.4.815.
37. **Shoemaker JK, Badrov MB, Al-Khazraji BK, Jackson DN.** Neural control of vascular function in skeletal muscle. *Compr Physiol* 6: 303–329, 2015. doi:10.1002/cphy.c150004.
38. **Southern WM, Ryan TE, Kepple K, Murrow JR, Nilsson KR, McCully KK.** Reduced skeletal muscle oxidative capacity and impaired training adaptations in heart failure. *Physiol Rep* 3: e12353, 2015. doi:10.14814/phy2.12353.
39. **Tanaka H, Monahan KD, Seals DR.** Age-predicted maximal heart rate revisited. *J Am Coll Cardiol* 37: 153–156, 2001. doi:10.1016/S0735-1097(00)01054-8.
40. **Van Beekvelt MC, Colier WN, Wevers RA, Van Engelen BG.** Performance of near-infrared spectroscopy in measuring local O_2 consumption and blood flow in skeletal muscle. *J Appl Physiol (1985)* 90: 511–519, 2001. doi:10.1152/jappl.2001.90.2.511.

41. **Whipp BJ, Davis JA, Torres F, Wasserman K.** A test to determine parameters of aerobic function during exercise. *J Appl Physiol* 50: 217–221, 1981. doi:10.1152/jappl.1981.50.1.217.
42. **Whipp BJ, Rossiter HB, Ward SA.** Exertional oxygen uptake kinetics: a stamen of stamina? *Biochem Soc Trans* 30: 237–247, 2002. doi:10.1042/bst0300237.
43. **Wüst RC, van der Laarse WJ, Rossiter HB.** On-off asymmetries in oxygen consumption kinetics of single Xenopus laevis skeletal muscle fibres suggest higher-order control. *J Physiol* 591: 731–744, 2013. doi:10.1113/jphysiol.2012.241992.
44. **Zamani P, Rawat D, Shiva-Kumar P, Geraci S, Bhuva R, Konda P, Doulias PT, Ischiropoulos H, Townsend RR, Margulies KB, Cappola TP, Poole DC, Chirinos JA.** Effect of inorganic nitrate on exercise capacity in heart failure with preserved ejection fraction. *Circulation* 131: 371–380, 2015. doi:10.1161/CIRCULATIONAHA.114.012957.
45. **Zuccarelli L, Porcelli S, Rasica L, Marzorati M, Grassi B.** Comparison between slow components of HR and $\dot{V}O_2$ kinetics: functional significance. *Med Sci Sports Exerc* 50: 1649–1657, 2018. doi:10.1249/MSS.0000000000001612.

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