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Effect of strength training on the slow component of V'O2 kinetics in young adults

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Abstract

Background: When performing exercise above gas exchange threshold, we attain oxygen uptake at steady state, with a delay due to the appearance of a slow component of $\dot{V}O_2$ kinetics that usually starts 150 – 200 s after exercise onset. The slow component is related to the loss of homeostasis and to the increased susceptibility to fatigue. Studies suggests that the progressive recruitment of type II fibers during heavy exercise is the main determinant due to high *ATP* cost and O_2 consumption. Strength training leads to a decrease in the numbers of motor units recruited at the same work-rate and may attenuate the slow component as a smaller number of less efficient type II fibers are recruited at the same work-rate. This paper investigated whether the slow component is determined by the progressive recruitment of type II fiber, or rather by the decay of efficiency of already recruited fibers.

Methods: 7 male subjects (age: 24 ± 3 years, height: 185 ± 3 cm, weight: 83 ± 14 kg) completed 6-weeks of one leg strength training on the *quadriceps*, while the other leg worked as a control. The subjects performed maximum one leg kicking test to determine 50 % and 80 % of WL_{max} before the training intervention, and then tested the same absolute work-rate pre- and post. Both $\dot{V}O_2$ and muscle activation were measured. Maximum isokinetic and isometric strength were also measured before and after the training intervention.

Results: The training increased both the isokinetic and isometric strength ($60°/s^{-1}$: 8 %, $P = 0.062$; $120°/s^{-1}$: 12 %, $P = 0.033$; 60°: 16 %, $P = 0.006$). The $\dot{V}O_2$ was significantly smaller at the 6th minute after the intervention in the training leg ($P = 0.009$), and the slow component was reduced from 19 % (291 $mL \cdot min^{-1}$) to 13 % (209 $mL \cdot min^{-1}$). The strength training also significantly decreased the *EMG* activity in *rectus femoris* ($P = 0.003$) and *vastus lateralis* ($P = 0.013$) at the 6th minute. At last, there was a significant less decay in the slope of regression in *MPF* after training in *vastus medialis* ($P = 0.027$) and *vastus lateralis* ($P = 0.001$). No difference in the results regarding *NIRS*.

Conclusion: The training of the *quadriceps* led to a significant increase in strength. The training also seemed to have decreased the neuromuscular fatigability of the trained muscle and lowered the slow component of $\dot{V}O_2$ kinetics.

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1. Introduction

After the start of a constant-work-rate exercise (*CWR*) below the lactate threshold (*LT*), or gas exchange threshold (*GET*), the pulmonary oxygen uptake ($\dot{V}O_2$) rises rapidly to attain a new steady state ($\dot{V}O_{2ss}$) within few minutes of the exercise onset (Jones et al., 2011). When exercise is performed at an intensity above the *LT*, there is a persistent lactic acidosis (Lucía, Hoyos, & Chicharro, 2000). This is concomitant with the occurrence of the slow component of the $\dot{V}O_2$ ($\dot{V}O_{2sc}$) (Poole & Jones, 2012), which is defined as the continued rise in $\dot{V}O_2$ beyond the third minute of exercise (Barstow, 1994; Poole et al., 1991).

Various factors, such as lactate, adrenaline and lower chemical-mechanical efficiency have been proposed as determinants of the $\dot{V}O_{2sc}$ during heavy exercise (Barstow, 1994; Poole et al., 1991). The increasing contribution to force production of muscle fibers higher in the recruitment hierarchy (type II muscle fibers), may contribute to the slower overall $\dot{V}O_2$ kinetic. The "higher-order" fibers are suggested to have a slower $\dot{V}O_2$ kinetic (and lower efficiency) relative to early-recruited fibers (type I muscle fibers) (Poole & Jones, 2012). However, there is not convincing evidence for a lower efficiency of type II muscle fibers in humans since the experiments on mechanical efficiency of human slow / fast twitch fibers is scanty and lead to ambiguous results (He, Bottinelli, Pellegrino, Ferenczi, & Reggiani, 2000; Reggiani et al., 1997).

The magnitude of the $\dot{V}O_{2sc}$ can exceed 1000 mL $O_2 \cdot min^{-1}$ and can account for > 25 % of the exercise induced increase in $\dot{V}O_2$. By definition, there is an increase in O_2 cost of work, a shortens of time to exhaustion, and an impaired power generation capability at $\dot{V}O_{2max}$ during the presence of the $\dot{V}O_{2sc}$ (Korzeniewski & Zoladz, 2015).

The $\dot{V}O_{2sc}$ is of great interest because it is closely related to the development of fatigue during exercise above LT (Jones, Poole, Grassi, & Christensen, 2010). It is also of interest because it can enhance or understanding about muscle energetics, metabolic control and the determinants of the efficiency of skeletal muscle contractions, since the slow component of $\dot{V}O_2$ kinetics is considered to be caused by a progressive recruitment of muscle fibers as exercise proceeds, and fatigue of those recruited fibers (Jones et al., 2011).

1.1 Research question

Strength training likely decreases the number of motor units (*MUs*) recruited at the same work-rate and may theoretically attenuate $\dot{V}O_{2sc}$ as a smaller number of less efficient type II fibers would be activated at the same work-rate.

We therefore proposed to investigate the effects of strength training on:

1) $\dot{V}O_{2sc}$ during heavy intensity exercise performed at the same absolute work-rate, before and after a 6-week strength training intervention
2) Muscular strength

1.2 Hypothesis

We hypothesized that strength training would reduce the amplitude of the $\dot{V}O_{2sc}$ because a smaller number of type II *MUs* will be recruited at the very same heavy exercise intensity after training than before.

2. Theory

2.1 Skeletal muscle energetics

To perform muscular contractions, skeletal muscles need to convert chemical energy into mechanical energy during the cross bridge interaction between actin and myosin (Whipp, 2006). Breakdown of adenosine triphosphate (*ATP*) is required for these interactions;

$$ATP \rightarrow ADP + [P_i] + \Delta G$$

where *ADP* are adenosine triphosphate, $[P_i]$ are inorganic phosphate and ΔG is the high free energy of hydrolysis. Despite the fact that *ATP* is only available in small concentrations in skeletal muscle (~ 5 mM/kg), the availability of *ATP* is maintained by aerobic and anaerobic metabolic reactions, unless exercise is performed at extremely high work-rates (Sahlin, Tonkonogi, & Söderlund, 1998; Whipp, 2006).

Oxidative phosphorylation is the aerobic process of resynthesize *ATP* and occurs in the electron transport chain of the mitochondria. The immediate anaerobic metabolic reactions for maintaining *ATP* supply is the breakdown of phosphocreatine ($[PCr]$) to creatine (Cr) and $[P_i]$ and contributes to the O_2 deficit.

$$[PCr] \rightarrow Cr + [P_i] + \Delta G$$

If O_2 is not delivered at appropriate rates to the electron transport chain, the *ATP* supply can be maintained anaerobic from lactate (La^-) production with one *ATP* per lactate molecule from glucose, or 1.5 *ATP* per lactate molecule from glycogen. At high work-rates, lactate production is therefore an important cellular process, despite the fact that the process occurs at a cost of both ventilatory control mechanisms and the contractile properties of the muscle (Whipp, 2006).

2.2 Pulmonary $\dot{V}O_2$ kinetics

The body's ability to transfer oxygen (O_2) to the skeletal muscle mitochondria at rates sufficient to meet the increased energy demand during physical tasks, such as exercise, is of paramount importance in order to sustain the oxidative metabolism of the muscle and to adapt its rate to the sudden changes of energy requirements imposed by exercise (Whipp, 2006). The kinetics of the pulmonary O_2 uptake ($\dot{V}O_2$) is dependent on the circulatory and muscular factors, as shown by the equation of Fick applied to O_2 uptake:

$$\dot{V}O_2 = \dot{Q} \; x \; (C_aO_2 - C_vO_2)$$

where $\dot{Q}$ is the cardiac output and $C_aO_2 - C_vO_2$ are the arterial and mixed venous blood concentrations of O_2 (Hill, Poole, & Smith, 2002). From the functional point of view, the more rapid the $\dot{V}O_2$ kinetics, the faster the $\dot{V}O_{2ss}$ can be achieved and a smaller O_2 deficit will consequently derive (Fig. 1). This would entail a smaller perturbation of the muscular milieu and diminished risk to incur in muscular fatigue (Whipp, 2006).

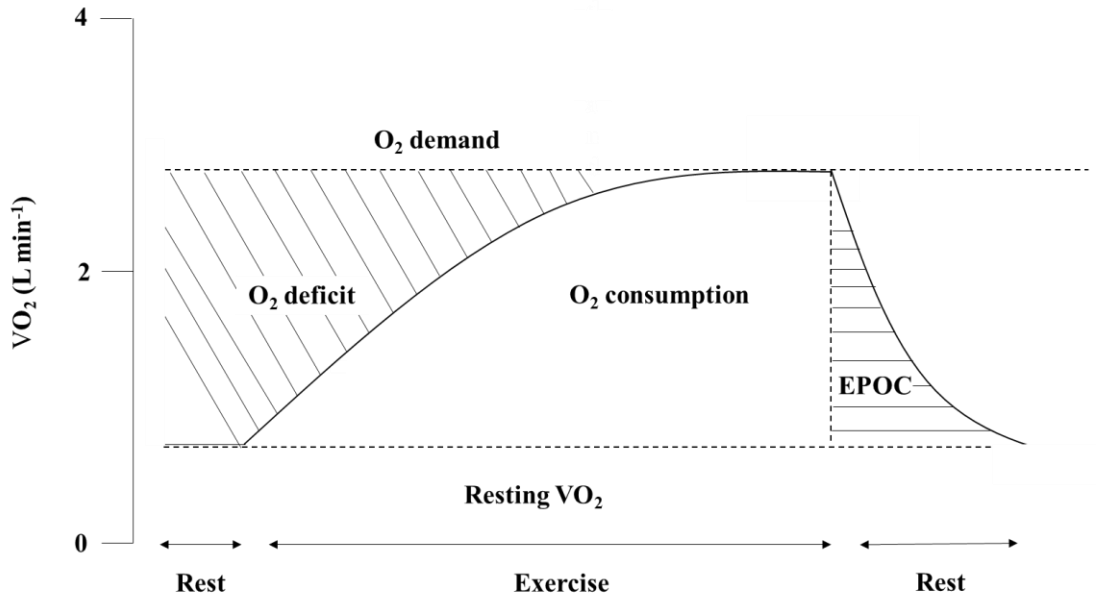

***Fig 1**. Schematic illustration of oxygen uptake during moderate intensity exercise. Oxygen uptake increases from transition of rest to exercise, and the O_2 deficit is covered by anaerobic processes until steady state is achieve (O_2 demand). At the end of the exercise, excess post exercise O_2 consumption (EPOC) restore the metabolic and cellular processes to resting state. Modified from Whipp and Wasserman (1972) and Poole and Jones (2012).*

2.2.1 Phases of $\dot{V}O_2$ kinetics

Following the onset of *CWR* exercise, the dynamic response of $\dot{V}O_2$ profile is determined by the intensity of the exercise. After the onset of *CWR* exercise performed in the moderate (*MOD*) intensity domain, i.e. below the *LT* or *GET*, $\dot{V}O_2$ rises rapidly to $\dot{V}O_{2ss}$ within 3 – 4 minutes (Jones et al., 2011; Krustrup, Söderlund, Mohr, & Bangsbo, 2004a) following a complex kinetics that is usually modeled as the sum of two mono-exponential components.

The first rapid component, called Phase I (or cardiodynamic phase), is driven by the increase in pulmonary blood flow in the absence of altered venous O_2 contents. The instantaneous increase in $\dot{Q}$ initiated by the mechanical pumping action of the contracting muscles is responsible for the rapid rise of the $\dot{V}O_2$ observed during Phase I. Phase I is followed by the second component – Phase II – until $\dot{V}O_{2ss}$ is attained. $\dot{V}O_2$ kinetics in Phase II, considered to be a reliable proxy of muscular O_2 uptake (Poole & Jones, 2012) is remarkably slower than in Phase I, as its time constant, τ_2, is about 20 s in young, healthy trained subjects.

During heavy intensity exercise (*HI*, metabolic rates between *LT* and critical power, *CP*) the attainment of $\dot{V}O_{2ss}$ is delayed due to the appearance of a slow component of $\dot{V}O_2$ kinetics ($\dot{V}O_{2sc}$, or Phase III) that usually starts about 150 – 200 s after exercise onset and, if modeled as an exponential increase, is characterized by a much longer τ than τ_2 (Jones et al., 2011). *CP* is the highest work-rate, or $\dot{V}O_2$, that can be sustained for a prolonged period. It is the highest submaximal $\dot{V}O_2$ that can be stabilized, and it also corresponds to highest metabolic rate still compatible with lactate steady state (maximal lactate steady state). It also constitutes the highest metabolic rate where [H^+] and [*PCr*] of the muscle can stabilize (Poole & Jones, 2012).

If the exercise is performed in the very heavy domain (*VH*), i.e. above *CP*, $\dot{V}O_{2ss}$ cannot even be attained, since $\dot{V}O_2$ keeps increasing up to $\dot{V}O_{2max}$ (Poole & Jones, 2012). At this intensity, also blood lactate ($[La^-]_b$) does not stabilize but keeps increasing until exhaustion. In the severe intensity domain (> $\dot{V}O_{2max}$), $\dot{V}O_2$ rises rapidly to $\dot{V}O_{2max}$ following a complex kinetics (Jones et al., 2011) leading to exhaustion: the work-rate is so high that it may lead to fatigue (approximately 140 – 180 s) or to the interruption of the exercise even before $\dot{V}O_{2max}$ is attained (Poole & Jones, 2012).

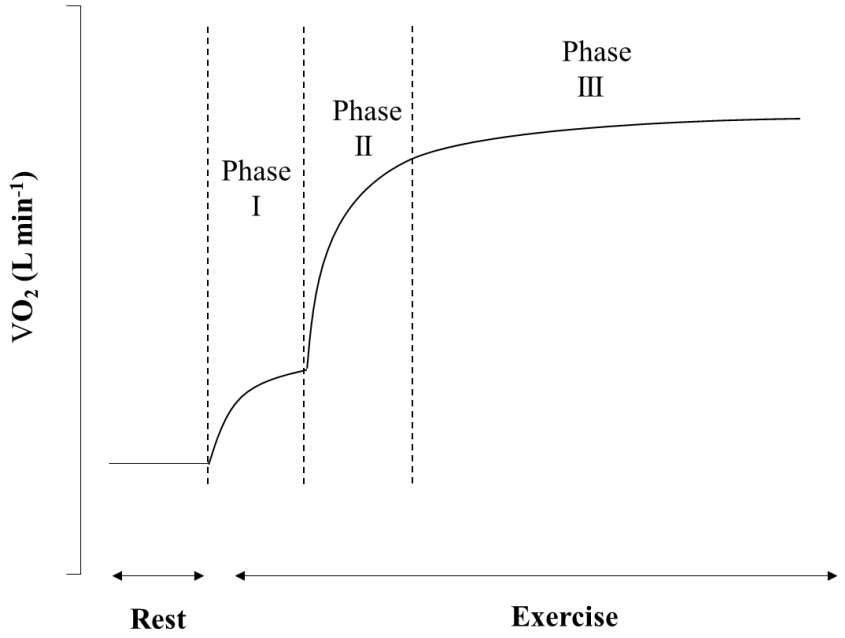

Fig 2. *Schematic illustration of the different phases of $\dot{V}O_2$ kinetics from rest to exercise. Modified from Poole and Jones (2012) and Fawkner and Armstrong (2003).*

2.2.2 Mathematical description of the $\dot{V}O_2$ kinetics

The different phases of the $\dot{V}O_2$ kinetics can be described in the time domain by a two- (*MOD*) or three- (*HI*) component exponential functions. Each exponential curve describes one phase; the first phase begins at the onset of exercise, whereas the other terms begin after independent time delays:

Phase I: $\dot{V}O_2\,(t) = \dot{V}O_2\,(b) + A_0 \times (1 - e^{-t/\tau 0})$

Phase II: $+ A_1 \times (1 - e^{-(t - TD1)/\tau 1})$

Phase III: $+ A_2 \times (1 - e^{-(t - TD2)/\tau 2})$

where $\dot{V}O_2\,(b)$ is the baseline $\dot{V}O_2$ measured in the onset of exercise; A_0, A_1 and A_2 are the asymptotic amplitude for the exponential curves; τ_0, τ_1 and τ_2 are the constants; and TD_1 and TD_2 are the time delays (Burnley, Jones, Carter, & Doust, 2000).

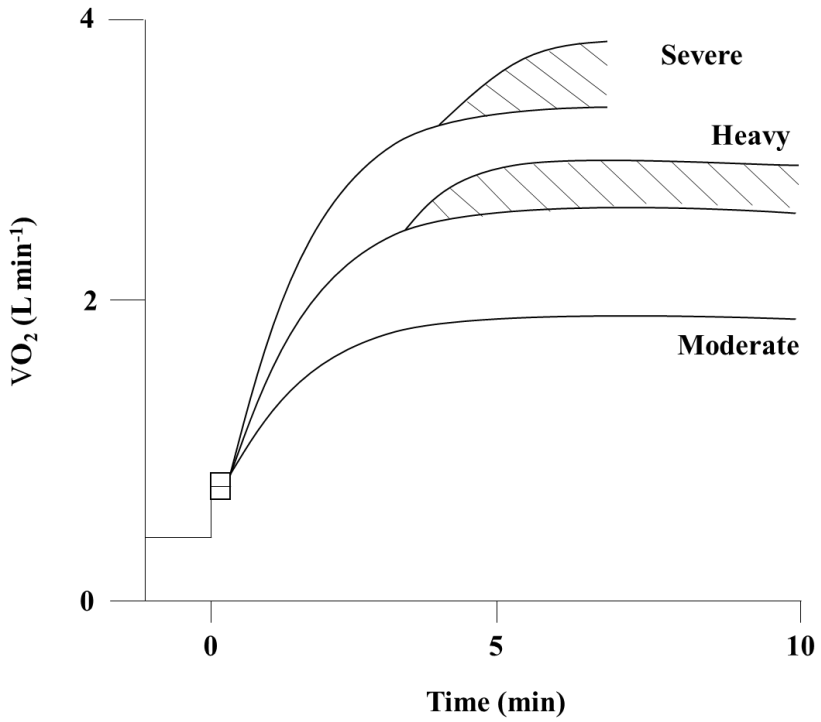

***Fig 3**. Schematic illustration of the $\dot{V}O_2$ response to CWR exercise in the moderate, heavy and severe domains. Hatched area represents the $\dot{V}O_{2sc}$. Modified from Poole and Jones (2012).*

The parameters that describe the three phases of $\dot{V}O_2$ kinetics are usually estimated by fitting the time series of the breath-by-breath $\dot{V}O_2$ data recorded from the onset of the exercise to the appropriate model that mainly depends on the intensity of the exercise. The fitting procedure is usually performed by using iterative, computerized procedures (De Roia, Pogliaghi, Adami, Papadopoulou, & Capelli, 2012) that implement algorithms developed for fitting non-linear equations (Marquardt, 1963).

A simplified version of the mathematical description of the $\dot{V}O_2$ kinetics has been proposed for both *MOD* and *HI*/*VH* domains. In these cases, only $\dot{V}O_2$ above the values at rest or above the baseline O_2 uptake during unloaded or very low workload (*WL*) pedaling is considered ($\Delta\dot{V}O_2(t)$). In addition, the breath-by-breath $\dot{V}O_2$ values recorded in the first 20 second after the onset of exercise are removed and phase I is disregarded, as our interest is focused on the analysis of phase II and phase III (Barstow & Molé, 1991).

In this case, the equation of *MOD* boils down to:

$$\Delta\dot{V}O_2(t) = A_1[1 - e^{-(t - TD1)/\tau 1}]$$

and during *HI*/*VH* to:

$$\Delta\dot{V}O_2(t) = A_1[1 - e^{-(t - TD1)/\tau 1}] + A_2[1 - e^{-(t - TD2)/\tau 2}]$$

2.3 The slow component of $\dot{V}O_2$ kinetics

We have already pointed out that after the onset of *CWR* exercise of *MOD* intensity, $\dot{V}O_2$ rises rapidly to attain the $\dot{V}O_{2ss}$ within few minutes (Jones et al., 2011; Krustrup et al., 2004a). Also, during *HI* exercise the attainment of $\dot{V}O_{2ss}$ is delayed due to the appearance of a slow component of $\dot{V}O_2$ kinetics ($\dot{V}O_{2sc}$ or phase III) that usually starts about 150 – 200 s after exercise onset and, if modeled as an exponential increase, is characterized by a much longer τ_3 than τ_2 (Jones et al., 2011). Furthermore, if the exercise is performed in the *VH* domain, i.e. above *CP*, $\dot{V}O_{2ss}$ cannot even be attained, since $\dot{V}O_2$ keeps increasing up to $\dot{V}O_{2max}$, a condition that portends the interruption of exercise because of exhaustion (Poole & Jones, 2012).

$\dot{V}O_{2sc}$ may amount to as much as 1000 – 1500 mL $O_2 \cdot min^{-1}$ and it can account for more than 25 % of the total $\dot{V}O_2$ during *HI*/*VH* exercise: in these cases, the end-exercise gain may increase considerably above 9 – 11 mL $O_2 \cdot min^{-1} \cdot W^{-1}$ (Jones et al., 2011). $\dot{V}O_{2sc}$ causes an increase in O_2 cost of work, anticipates the time to exhaustion, and it is related to increased susceptibility to fatigue: $\dot{V}O_{2sc}$ amplitude, e.g., is linearly related to the time to fatigue in obese adolescents (Salvadego et al., 2010).

$\dot{V}O_{2sc}$ somehow challenges our understanding of muscle energetics and the foundational tenets of exercise physiology, as f.i., the concept of steady state. In addition, it is closely related to the loss of muscle homeostasis and the development of fatigue during exercise above *LT* (Jones et al., 2011; Jones, Vanhatalo, Burnley, Morton, & Poole, 2010). It is also of interest because the exploration of its determinants may help us enhance our understanding about muscle energetics, metabolic control and the determinants of the efficiency of skeletal muscle contraction (Jones et al., 2011).

2.3.1 The possible mechanisms underpinning $\dot{V}O_{2sc}$

The possible causes of $\dot{V}O_{2sc}$ may be classified as central or peripheral factors. Peripheral factors may be the recruiting of lower-efficient muscle fiber and the accumulation of metabolites, which leads to decreased efficiency. Central factors may include larger O_2 uptake associated with increased ventilatory and cardiac work (Poole et al., 1991).

There is compelling evidence that muscular mechanisms are largely responsible for $\dot{V}O_{2sc}$. It is demonstrated that muscle oxygen consumption ($\dot{V}O_m$) is significantly associated with $\dot{V}O_{2sc}$ when the blood flow and arterio-venous O_2 (a-vO_2) content difference across an exercising limb (Poole et al., 1991) are measured. In the quoted study, the authors claimed that ~ 85 % of the $\dot{V}O_{2sc}$ arose in the exercising muscle. These findings suggest that processes outside the muscle, like ventilatory, cardiac and auxiliary muscle work play a marginal role in inducing the $\dot{V}O_{2sc}$ (Poole & Jones, 2012).

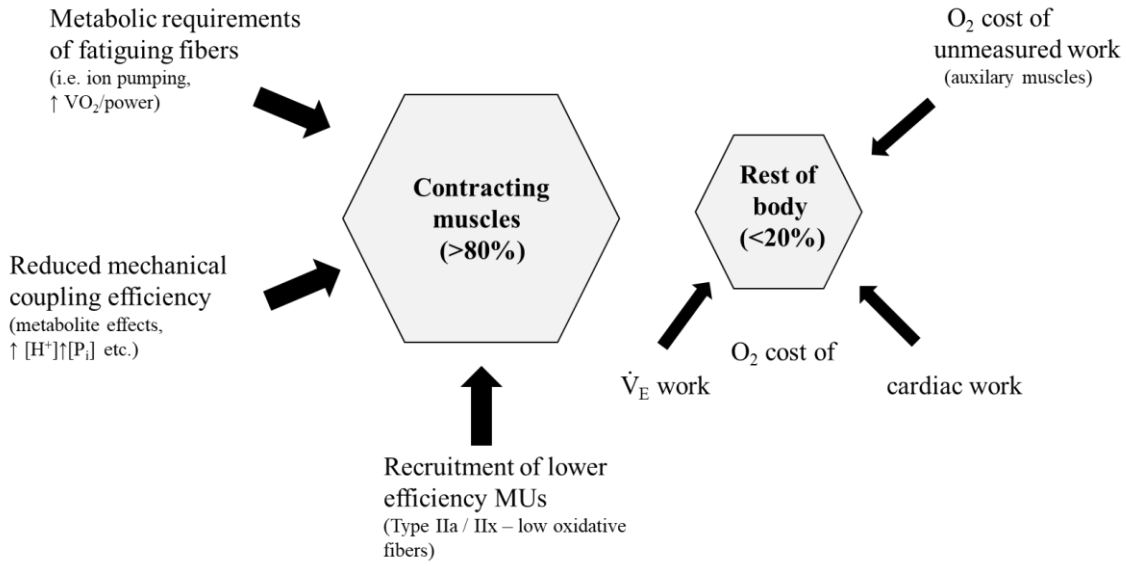

Fig 4. *Schematic illustration of putative mediators for the slow component of $\dot{V}O_2$ kinetics. Modified from Poole and Jones (2012)*

Several data support the notion that the progressive recruitment of type II muscle fibers during *HI*/*VH* exercise is the main determinant of $\dot{V}O_{2sc}$ (Poole & Jones, 2012) and, from the empirical point of view, it has been demonstrated that $\dot{V}O_{2sc}$ is more evident in humans with a higher percentage of type II fibers (Barstow, Jones, Nguyen, & Casaburi, 1996).

Type II muscle fibers have different metabolic characteristics in comparison with type I fibers, and these may well explain how and why they are implied in causing $\dot{V}O_2$. First, a lower "metabolic stability" characterizes them. Good metabolic stability during rest-to-work transition is associated with good exercise tolerance and results, for a given increase in $\dot{V}O_2$ in a less pronounced decrease in [*PCr*] and in the cytosolic phosphorylation potential, as well as in a less pronounced decrease in [P_i], [ADP_{free}], [AMP_{free}] and [IMP_{free}] (Jones et al., 2011). Compared to type II fibers, type I fibers would obtain the same $\dot{V}O_2$ for less [*PCr*] decrease and less enhanced anaerobic glycolysis (and therefor a lower muscle pH). Or conversely, for the same [*PCr*] decrease (and [ADP_{free}] increase), type I fibers would have a higher $\dot{V}O_2$ (Grassi, Porcelli, Salvadego, & Zoladz, 2011).

We know that the $\dot{V}O_{2sc}$ is associated with the slow decay of [*PCr*] and that endurance training, by improving the metabolic stability of the muscle, leads to a lower decrease in [*PCr*] and a diminished intramuscular acidosis during *HI* in connection with a less evident $\dot{V}O_{2sc}$ (Poole & Jones, 2012). Since low levels of intramuscular [*PCr*] and of pH characterize *HI*/*VH* exercise (Jones et al., 2011; Jones, Wilkerson, DiMenna, Fulford, & Poole, 2008), these results suggest again that $\dot{V}O_{2sc}$ is mechanistically linked to the slow decrease in [*PCr*] and increase of [H^+] occurring observed during *HI*/*VH* exercise (Jones et al., 2008).

Additional causes of $\dot{V}O_{2sc}$ may reside in higher *ATP* cost of force production and higher O_2 cost for *ATP* turnover of type II fibers (Poole & Jones, 2012). Type II muscle fibers has fewer mitochondria than type I muscle fibers, and both lower oxidative efficiency and slower kinetics (Rossiter et al., 2002). Type I muscle fibers have a higher amount of citric acid cycle and electron transport chain enzyme activities, and greater capillary density (Pringle et al., 2003). Therefore, type I muscle fibers are more efficient to convert chemical energy to mechanical work at slow velocity muscular contractions (Horowitz, Sidossis, & Coyle, 1994). The reason that type II muscle fibers have a greater *ATP* cost for contractile activity than type I muscle fiber can be related to different chemical-to-mechanical coupling efficiencies, faster calcium pump activity, and faster actomyosin turnover in type II versus type I fibers. Another factor is the FAD-linked α-glycerol phosphate shuttle activity, which is higher in type II fibers. This reduces the P-O ratio with ~ 18 % (Poole & Jones, 2012).

It is also claimed that type II fibers are characterized by a lower mechanical efficiency, leading to a higher O_2 consumption for the same mechanical power output in comparison with type I fibers. In this regard, we know that endurance trained athletes with a higher percentage of type I fibers in the *vastus lateralis* (73 vs. 48 %) were able to generate more power (9 %) during high-intensity endurance performance compared with well-trained athletes with a normal percentage of type I fibers, despite identical $\dot{V}O_{2max}$ (Horowitz et al., 1994). The authors claimed this was due to a superior efficiency in converting chemical energy from *ATP* hydrolysis during contraction into mechanical work, rather than a greater ability to re-synthesize *ATP* aerobically. It is worth underlying, however, that there is not convincing evidence for a lower efficiency of type II fibers in humans. The experiments on mechanical efficiency of human slow / fast twitch fibers are scanty, and lead to ambiguous results. Thermodynamic efficiency, f.i., seems to be rather remarkably similar in slow and fast fibers (He et al., 2000). This is consistent with the identical peak mechanical efficiency and net efficiency in mouse muscle (Barclay & Weber, 2004). On the other hand, thermodynamic efficiency in rat-skinned fibers seems to be larger in slow fibers than in fast fibers (Reggiani et al., 1997). Efficiency also depends on the velocity of the contraction, and therefore of the speed of the movements. The two types of fibers attain their peak efficiencies at different velocity of shortening. This may be the only cause of the different mechanical efficiencies observed at moderate and heavy exercise intensities (Rossiter, 2011).

Also, the increase of body temperature occurring during exercise has been called upon as a possible determinant of $\dot{V}O_{2sc}$ via the increase of the Q_{10} effect. However, in order to increase $\dot{V}O_2$ it may be necessary to elevate muscle temperature above 43°C, which is unlikely during exercise in humans (Poole & Jones, 2012). Since the muscle temperature increases in the range of 36 - 39°C (Krustrup et al., 2004a), the muscle temperature does not seem to influence muscle $\dot{V}O_{2sc}$. This conclusion seems to be strengthened by the findings that prior warming (average of 2.6°C) of the lower limbs in a hot bath (40 min of 42°C) did not alter the $\dot{V}O_2$ response to subsequent heavy exercise (Burnley, Doust, & Jones, 2002).

On the basis of the premises reported above, several experimental results support the notion that the progressive recruitment of type II muscle fibers during *HI*/*VH* exercise is the main determinant of $\dot{V}O_{2sc}$ (Poole & Jones, 2012). Type II muscle fibers are recruited in addition to type I fibers at heavy and severe work-rate, according to Henneman's size principle (Henneman, 1957). As already stated, they are characterized by a higher *ATP* cost of force production and by higher O_2 consumption for *ATP* synthesis than type I fibers. When they fatigue, they would no longer contribute to substantial force production, but continue to consume O_2 (Pringle et al., 2003; Vanhatalo, Poole, DiMenna, Bailey, & Jones, 2011). In addition, to maintain the same *WL* in presence of fatigued muscle fibers, additional type II fibers will be recruited and a higher O_2 uptake would result. In conclusion, the appearance of $\dot{V}O_{2sc}$ would be explained by an "extensive" process attributable to the progressive recruitment of type II *MU* in a fatiguing muscle.

Recent findings have however challenged the view that progressive recruitment of less efficient type II fibers is a prerequisite for inducing $\dot{V}O_{2sc}$, suggesting that it instead arises from metabolic processes inside already recruited fibers (Zoladz, Gladden, Hogan, Nieckarz, & Grassi, 2008). This may directly cause the progressive decay of the mechanical efficiency of contraction (Grassi et al., 2011). According to this view an "intensive" mechanism inside already recruited MUs would be the main determinant of $\dot{V}O_{2sc}$.

Finally, the magnitude of $\dot{V}O_{2sc}$ is sensitive also to perturbations that alter blood flow and O_2 delivery and increased arterial O_2 content. Reduced muscle blood flow induced by partial vascular occlusion impacts muscle fiber recruitment profiles, and profoundly impacts the $\dot{V}O_2$ response. It is suggested that either blood flow, or O_2 delivery conditions within the muscle can impact the mechanisms that is responsible for the $\dot{V}O_{2sc}$ (Poole & Jones, 2012). Therefore, decreased O_2 availability may affect $\dot{V}O_{2sc}$ of individuals in whom local O_2 delivery during exercise is impaired and a clear mismatch between O_2 delivery and consumption is present (Murias, Kowalchuk, & Paterson, 2010, 2011).

2.3.2 The relationship between EMG and $\dot{V}O_{2sc}$

In order to assess the progressive recruitment of *MUs* during *CWR* exercise of *HI*/*VH* intensities, surface electromyographic (*sEMG*) analysis, because of its low cost and non-invasive nature, is usually used to examine temporal changes in muscle fiber recruitment. The overall recruitment of *MUs* is indicated by the integrated *EMG* (*iEMG*), whose amplitude is proportional to the numbers of *MUs* implied in the motor task, but that does not provide any information about the specific recruitment pattern of type I and type II fibers. Greater muscle activation indicated by increases in *iEMG* signals is observed during cycling exercise (Burnley et al., 2002). However, the same protocol performed in a different study showed unchanged fiber recruitment (Scheuermann, Hoelting, Noble, & Barstow, 2001).

During *HI CWR* exercise, a significant correlation between the increase in *iEMG* and the increase in $\dot{V}O_2$ has been shown (Moritani, Sherman, Shibata, Matsumoto, & Shinohara, 1992). An indication of a progressive recruitment of new fibers was also shown in the study of Vanhatalo et al. (2011), where the muscle activation (measured with *iEMG*) progressively declined throughout the all-out cycle test (3 min all-out ramp test), whereas it increased during the *CWR* test. This may support the contention that the $\dot{V}O_{2sc}$ may be associated with changes in muscle recruitment patterns. However, the onset of the $\dot{V}O_{2sc}$ ~ 90 – 180 s after the onset of exercise, and the increase in *iEMG*, which is evident only after ~ 240 s, are somehow dissociated (Barstow & Molé, 1991; Scheuermann et al., 2001).

The specific recruitment pattern of type I and type II fibers may be inferred from the frequency content (i.e. power density spectrum) and, specifically, by the shift of the median or mean power frequencies (*MPF*) of the *EMG* signal (Scheuermann et al., 2001). *MPF* is dependent on the action potential velocity of the muscle fiber across the sarcolemma. This is influenced by the fiber diameter (i.e. the lager the diameter, the faster the velocity). As type I muscle fibers have slower velocity than type II muscle fibers, increased activation of type II fibers might be expected to increase *MPF* (Garland, Wang, & Ward, 2006; Kupa, Roy, Kandarian, & De Luca, 1995).

By coupling the *iEMG* and *MPF* analysis, f.i., it was possible to show that there was an increase in both *EMG* and *MPF* associated with the $\dot{V}O_{2sc}$ (Borrani et al., 2001; Burnley

et al., 2002) and these findings were considered to support the view that $\dot{V}O_{2sc}$ arises from shifts in recruitment patterns of *MUs* (Cannon, Kolkhorst, & Cipriani, 2007). We have to consider, though, that during intense dynamic exercise we may have difficulties in obtaining valid *sEMG* measurements due to changes in muscle temperature and noise in the signal due to movement (Konrad, 2005). Different protocols, and different muscles investigated may therefore explain the different results observed in different studies.

2.4 Training and the slow component of $\dot{V}O_2$ kinetics

Endurance training improves the so-called metabolic stability, leading to a lower decrease in [*PCr*] concentration and a diminished intramuscular acidosis during *HI* in connection with a less evident $\dot{V}O_{2sc}$ (Poole & Jones, 2012). Since low levels of intramuscular [*PCr*] and pH characterize *HI*/*VH* exercise (Jones et al., 2011; Jones et al., 2008), these results further strength the view suggesting that $\dot{V}O_{2sc}$ is mechanistically linked to the slow decrease in [*PCr*] and increase of [H^+] occurring during *HI*/*VH* (Jones et al., 2008). In addition, endurance training improves metabolic hyperemic response and optimize the matching between local O_2 delivery and utilization, especially in individuals with suboptimal vascular response, such as elderly subjects (Murias et al., 2010, 2011). Accordingly, the correlation between the indexes that describe amelioration of local peripheral perfusion and the attenuation of the amplitude of $\dot{V}O_{2sc}$ might suggest a potential mechanistic link between O_2 delivery and $\dot{V}O_{2sc}$. Also, strength training, by decreasing the number of *MUs* recruited at the same work-rate may theoretically attenuate $\dot{V}O_{2sc}$ as a smaller number of less economic type II fibers would be recruited at the same work-rate. However, this hypothesis has been somehow disproved in young adults in whom isometric strength training failed to abate the amplitude of $\dot{V}O_{2sc}$ (Zoladz et al., 2012).

2.5 Strength training

Strength training leads to functional and structural adaptions of the neuromuscular system (Ahtiainen, Pakarinen, Alen, Kraemer, & Häkkinen, 2003). The early training-induced increase in strength are mainly due to neural factors, with a gradually increasing contribution of muscular hypertrophy (Moritani, 1979).

2.5.1 Stimulus for muscle growth

The initial phase of strength training mobilizes the two fundamental adaptions necessary for muscle hypertrophy (i.e. increased protein synthesis and satellite cells proliferation) (Seynnes, de Boer, & Narici, 2007). Hypertrophy is mainly caused by an increased volume of each muscle fiber. Type II muscle fibers normally grows more than type I muscle fibers as a result of strength training, but for all fiber types, hypertrophy is a result of accumulation of protein inside each fiber (Ahtiainen et al., 2003). This accumulation is due to increased protein synthesis, decreased protein breakdown, or a combination of both. The growth of type II muscle fibers are however claimed to be primarily caused by the increased synthesis, and the growth of type I muscle fibers by decreased breakdown (Goldspink, 1998). Satellite cells cause the necessary increase of the number of nuclei in combination with the increase in muscle fiber volume, to maintain the so-called nuclear domain (each nucleus covers a certain volume in the muscle cell). However, it is possible to increase protein synthesis around each nucleus, to gain hypertrophy without increasing the number of nuclei (Kadi et al., 2005).

Mechanical loading / stress and metabolic stress are the two main factors leading to hypertrophy (Ahtiainen et al., 2003; de Freitas, Gerosa-Neto, Zanchi, Lira, & Rossi, 2017; Schoenfeld, 2013). Studies on muscle cells, and on animals have shown that mechanical loading leads to muscle growth by both an increase in cross-sectional area (*CSA*) and length (Goldberg, Etlinger, Goldspink, & Jablecki, 1975; Wernbom, Augustsson, & Thomeé, 2007). The mechanical loading activates mechanosensitive receptors inside each muscle cell, and in the connective tissue around the muscle cells. This mechanical loading leads to a release of growth factors (*HGF*) bound in the connective tissue between the muscle cells. In addition, passive stretch can lead to an increase in the amino acid transport into the muscle, which can sustain to an increase in protein synthesis (Goldberg et al., 1975).

Metabolic stress, in turn, activates many of the same pathways activated by the mechanical loading, via the osmotic swelling of the muscle cells as a result of metabolic accumulation (de Freitas et al., 2017). The accumulation of metabolites like La^-, $[P_i]$ and $[H^+]$ increases when hypoxia (i.e., lack of oxygen) is induced. Post-exercise hormones like insulin-like growth factor-1 (*IGF-1*), testosterone and *HGF* are some of the anabolic hormones released after training induced hypoxia (Schoenfeld, 2013)

2.5.2 Muscular adaptions

12 weeks of strength training could increase the *CSA* of the muscle by 3 – 25 % in untrained people (Wernbom et al., 2007). This is equivalent to approximately 0.1 – 0.5 % increase per session, but there are huge individual differences.

Strength training could also lead to an increase in muscle fiber *CSA* in all the three fiber types (i.e. I, IIa and IIx). Dynamic strength training in the range of 60 – 70 % of 1RM would not activate all *MUs* maximally unless performed to failure. This type of training would primarily lead to hypertrophy of the type I muscle fiber. However, if we train to failure with heavier load (80 – 90 % of 1RM), we would activate all the *MUs* maximally, and this often leads to greater hypertrophy in type II than in type I muscle fiber (Macdougall, 2003; Roman et al., 1993; Staron et al., 1991). Since it is unusual to see type I muscle fibers larger than 7000 μm^2, and type II muscle fibers lager than 9000 μm^2, there seems to be an upper limit for the fiber *CSA*. This may indicate that we start to produce more fibers (hyperplasia) when the fiber has reached a certain size. However, hyperplasia is primarily shown in animal studies, and yet to be demonstrated in humans (D'antona et al., 2006).

Generally, no changes in muscle length the first 5 weeks of strength training seem to occur (Blazevich, Gill, Deans, & Zhou, 2007), but Alegre, Jiménez, Gonzalo-Orden, Martín-Acero, and Aguado (2006) demonstrated an increase of 10 % in the fascicle length in the *vastus lateralis* after 13 weeks of strength training. Regarding changes in muscle mass, studies with *DXA* have shown an average increase of ~ 2 kg lean body mass after 14 weeks of strength training, which is equivalent to ~ 200 g per week, or ~ 60 g per session (Hanssen et al., 2013).

Morphological adaptions are also paralleled by substantial changes in muscle architecture. There is a general agreement on the fact that the pennation angle of the muscle fibers increases with hypertrophy (Folland & Williams, 2007; Kawakami, Abe, & Fukunaga, 1993). The increase of the pennation angle would allow a larger packing of fibers for the same anatomical *CSA*, and lead to the increase of the physiological cross-sectional area (*PSCA*), i.e. the area perpendicular to the line of application of the force produced by the fibers. However, an increase of the pennation angle would bring about a decrease of the force applied to the tendon because the angle between the fibers

and the line along which the force is projected decreases. Yet, it can be geometrically demonstrated that, if the pennation angle stays below 45°, its increase is compensated by the increase of *PCSA* so that an augmentation of force results after training (Alexander, 1990). Indeed, it has been shown that an increase of the pennation angle from 8.0 to 10.7 (+ 36 %) increased *PCSA* and force (+ 16 %) more than *CSA* (+ 10 %) (Aagaard et al., 2001).

If we consider the changes inside the muscle cells, the volume of sarcoplasmic reticulum (*SR*) and the amount of *SR*-related proteins increases in line with the *CSA*. This increase is important, so that the contractile properties of the fibers can be maintained with an increased *CSA* (MacDougall et al., 1998). Primarily, mitochondrial biogenesis is not an effect of heavy strength training, but strength training with higher range of repetitions (10 – 12 RM) and short breaks can maintain, or even increase, mitochondrial density (Wang, Hikida, Staron, & Simoneau, 1993). The concentration of nucleus is a result of an activation of satellite cells that activates and melts together with the growing fiber. The activation of satellite cells occurs during mechanical stress due to strength training. Each satellite cell can therefore contribute with one new nucleus to a growing fiber (Macdougall, 2003). The amount of capillaries per fiber is held constant or increases slightly as a result of strength training. However, the capillary density will be reduced due to a higher increase in fiber *CSA* than in the number of capillaries. Strength training with higher repetitions (10 – 20 RM) and shorter breaks can however increase the capillary density (Campos et al., 2002).

Fiber type composition can change due to strength training. It is well reported that strength training leads to a decrease in type IIx fibers, and an increase in type IIa. Activation of type IIx fibers can also lead to hybrid fibers (IIax) (Staron et al., 1991).

2.5.3 Neural adaptions

At the start of a strength training period, the increase measured as 1RM is ~ 1 % per session, and this increase is often higher than the increase in *CSA* of the trained muscle group (0.1 – 0.5 % per session). This difference is often contributed to the increased capability to activate the muscle (Moritani, 1979).

We know that *MUs* are arranged in a recruitment hierarchy according to the Henneman principle (Henneman, 1957). The amount of *MUs* recruited, and the firing rate, determines the power output of the muscle. The correlation between firing frequency and force output in each fiber is directly linked to the Ca^{2+} concentration in the cytosol. The higher the concentration of Ca^{2+}, the more cross-bridges can engage the binding site of the actin (Alegre et al., 2006).

Strength training leads to the increase of the recruitment and to a better synchronization of the *MUs* of the agonist muscles (Folland & Williams, 2007). This improvement can in turn lead to the increase of the rate of force development (*RFD*), shorter time in developing the same amount of force, with a longer relaxation time in a given cycle, and therefore better blood flow and improved endurance (Sale, 1988). Finally, early effects of strength training also include the decrease of the activation of the antagonist muscle (Folland & Williams, 2007).

When performing a training intervention with one arm and / or leg as control, there is often an increase in strength parameters from pre- to posttest, even though the limb has not been exercising. This increase is often contributed to the so-called crossover effect. A meta-analysis has shown an 8 % increase on the side that did not trained, but when tested in a simple isokinetic or isometric exercise, the crossover effect is often absence (Munn, Herbert, & Gandevia, 2004). One of the reasons proposed is changes at the spinal level, which can lead to an easier excitation of the α-motoneuron on the equivalent muscles on the other side, or changes in motor cortex which can affect the activation. The crossover effect can also be a result of habituation. Little habituation in the exercise before a pre-test would therefore possibly lead to a greater improvement posttest (2004).

2.6 Summary

The $\dot{V}O2sc$ is the continuous rise in $\dot{V}O_2$ after ~ 3rd minute of exercise above *LT* or *GET* and is associated with a progressive recruitment of additional type II fibers during *HI*. The low efficiency of these fibers might contribute to the increased O_2 cost of exercise. However, fibers that are fatigued may also require a greater O_2 consumption per unit of *ATP* turnover, and / or greater *ATP* turnover per unit of power output due to their loss of efficiency. Since the $\dot{V}O_{2sc}$ represents a progressive loss of muscle efficiency, and is associated with development of fatigue, further studies on the $\dot{V}O_{2sc}$ is of great importance due to designing interventions that reduces the $\dot{V}O_{2sc}$. Strength training which leads to a decrease in the numbers of *MUs* recruited at the same work-rate may theoretically attenuate $\dot{V}O_{2sc}$ as a smaller number of less efficient type II fibers would be recruited at the same work-rate. The parallel assessment of $\dot{V}O_2$ and *EMG* can help us understand whether the $\dot{V}O_{2sc}$ is determined by the progressive recruitment of type II-fibers, or rather by the decay of efficiency of the already recruited fibers.

3. Methods

3.1 Subjects

Seven healthy, moderate endurance trained young men was recruited to the study (Table 1). All subjects signed an informed consent (appendix I) and were medically screened (appendix II). The assessment of blood pressure at rest and $\dot{V}O_{2max}$ determination during a maximal test on cycle-ergometer were also included in the medical screening. Inclusion criteria were: men between 20 and 30 years old, with moderate experience with strength training (< 2 sessions per week for the last year), and no cardiovascular diseases. The project was approved by the local ethical board (October 9th, 2017). During the first visit to the laboratory, we randomly assigned the legs of the volunteers to the training leg (T_{leg}: $N = 7$) and the control leg (C_{leg}: $N = 7$) leg groups.

Table 1. *Characteristics of the subjects (N = 7)*

Age (year)	Height (cm)	Weight pre (kg)	Weight post (kg)	$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)
24 ± 3	185 ± 3	83.4 ± 14.3	84.4 ± 15.0	51.3 ± 7.6

3.2 Study design

The subjects referred to the laboratory of the Department of Physical Performance at the Norwegian School of Sports Sciences to undergo 3 sessions of familiarization (see description below), during which a simplified version of the experimental sessions was carried out. Briefly, the subjects performed approximately 10 minutes of submaximal 1-KE per leg per familiarization session, and one submaximal session to the isokinetic-isometric dynamometer. Then, pre-training tests took place in 3 different days (separated by 24 – 48 hours). After the pre-training tests, the subjects entered a 6-week strength training intervention. At the end of the intervention, post-training tests were completed in two days.

$\dot{V}O_{2max}$ test (ramp test during cycling) and 1-KE maximal test were performed only during the pre-training test to characterize the fitness level of the subjects and to assess maximal workload (WL_{max}) achieved during the 1-KE test. During the pre-training and

post-training tests, muscle strength of the limb extensors of T_{leg} and C_{leg} and two single 1-KE tests at 50 % and 80 % of WL_{max} were carried out. The absolute *WL* during 1-KE tests was identical both before and after training. In the paragraphs below, a schematic summary of the measurements carried out in the two pre- and post-training sessions is presented.

Pre-training tests:

- Day 1 – Anthropometrics and $\dot{V}O_{2max}$ test (ramp test)
- Day 2 – Strength test and 1-KE maximal test on training and control leg
- Day 3 – 1-KE test at 50 % and 80 % of WL_{max} on training and control leg

Post-training tests:

- Day 1 – Strength tests
- Day 2 – 1-KE at 50 % and 80 % of WL_{max} on training and control leg

3.3 Experimental protocols and Methods

3.3.1 Anthropometrics

Height was measured with SECA 213 portable stadiometer and weight was measured with SECA 877 flat scale (SECA, Hamburg, Germany) before and after training. Subcutaneous fat of the *vastus lateralis* was measured with ultrasound (Phillips HD11 XE, Netherlands) on both the T_{leg} and C_{leg} at pre- and post-training test.

The thigh volume (*V*) was calculated by the following formula:

$$V = L \cdot (12\pi)^{-1} \cdot (O_1^2 + O_2^2 + O_3^2)$$

$$- (S - 0.4) \cdot 2^{-1} \cdot L \cdot (O_1 + O_2 + O_3) \cdot 3^{-1}$$

where *L* is the estimated muscle length; O_1, O_2 and O_3 are the circumferences 10 cm above, at the middle and 10 cm below the middle of the segment of the muscle length. S is the correction for subcutaneous fat measured with skinfold caliper (Holtain LTD, Crosswell, U.K.) at each circumference.

The muscle mass of the *quadriceps* (M_{qf}) was calculated according to:

$M_{qf} = 0.307 \cdot V$ (l) + 0.353 kg (Andersen & Saltin, 1985)

and adjusted to:

$0.792 \cdot M_{qf} - 0.382$ kg (Raadegran, Blomstrand, & Saltin, 1999).

3.3.2 Maximal oxygen uptake ($\dot{V}O_{2max}$)

The test was performed on an ergometer cycle (Lode Excalibur sport), separated by a 5-min break. $\dot{V}O_2$ and heart rate (*HR*) were continuously measured during exercise and capillary blood from fingertip was taken 1 min after the ending of the ramp test and analyzed for lactate concentration. The entire procedure was divided in two phases (Fig. 5).

1. Steady state test

First, the subjects pedaled for 6 minutes at a *WL* of 50, 100 and 150 W. Then, we extrapolated the linear relationship between *WL* and *HR* to the estimated HR_{max} of the subject (Tanaka, Monahan, & Seals, 2001) to obtain the maximal mechanical aerobic power (WL_{max}). Afterwards, we subtracted from WL_{max} the *WL* corresponding to warm up, set equal to 100 W, to obtain a ΔWL, which one by 10 minute yielded the increment in watt per minute (W/min) of the subsequent ramp test.

2. Ramp test

5 minutes after the steady state test, the subjects initiated the ramp test to measure $\dot{V}O_{2max.}$ After 3 minutes of active warm up pedaling at 100 W, the *WL* was progressively increased according to the preset W/min until exhaustion. The test was terminated either voluntarily by the subject, i.e. when the self-selected *RPM* (usually between 70 – 90) could not be maintained despite strong verbal encouragement, and / or when the established test criteria of test termination was met (Edvardsen, Hem, & Anderssen, 2014):

- $\dot{V}O_2$ reaches a plateau
- *RER* > 1.1
- + - 10 beats/min of estimated HR_{max}
- $[La^-]_b \geq 8.0$ mmol·L^{-1}

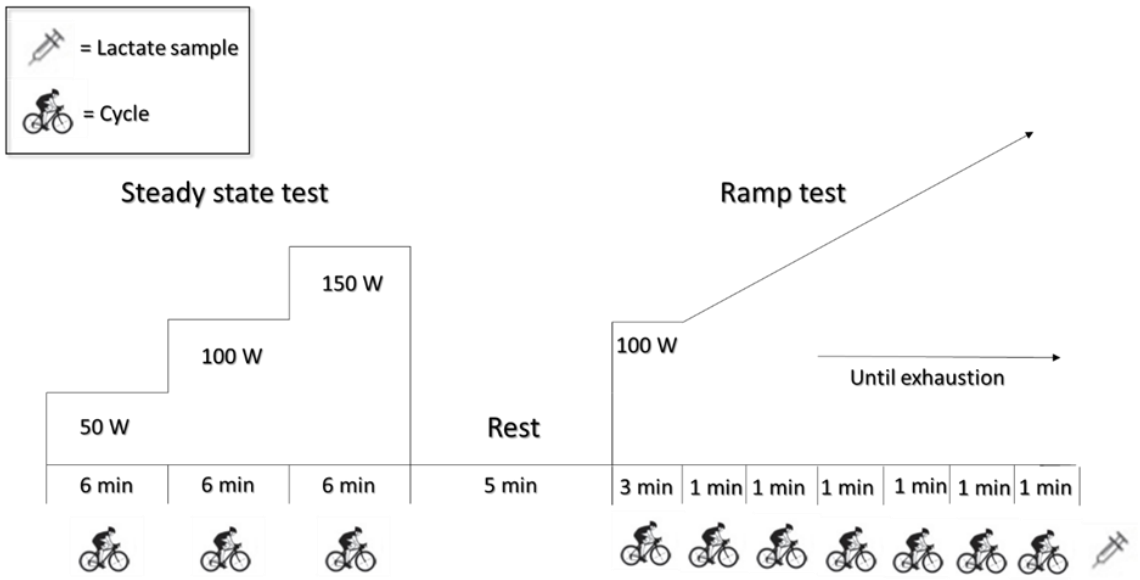

Fig 5*. Schematic illustration of the experimental protocol for the steady state test and the ramp test on ergometer cycle*

3.3.3 One leg knee extension (1-KE) maximal test

The 1-KE was performed on a custom built electromagnetically braked knee extensor ergometer, previously described by Andersen and Saltin (1985), and modified by Hallen, Saltin, and Sejersted (1996). $\dot{V}O_2$ and *HR* were measured throughout the test and capillary blood from fingertips was taken 1 minute after the end of the test and analyzed for lactate concentration. During the extension, only the *quadriceps* was used, and a four-point safety belt was used for minimizing upper body movement. The leg that was not exercising was placed extended on a chair to minimize movements that could influence the test. Both the T_{leg} and C_{leg} was tested in randomly order, separated by a 30-minute break. The test started at 10 W, with a stepwise *WL* increments of 5 W per minute until exhaustion (Fig. 6). The subjects terminated the test voluntarily, or when the subject was not able to maintain a frequency of 60 *RPM* despite strong verbal encouragement. During the test, there was a PC monitor which gave the subject visual feedback to control their frequency. In addition, a mirror was set up for visual control of

the kicking frequency. This test allowed us to select the *WL* corresponding to 50 % and 80 % of WL_{max} for single leg exercise.

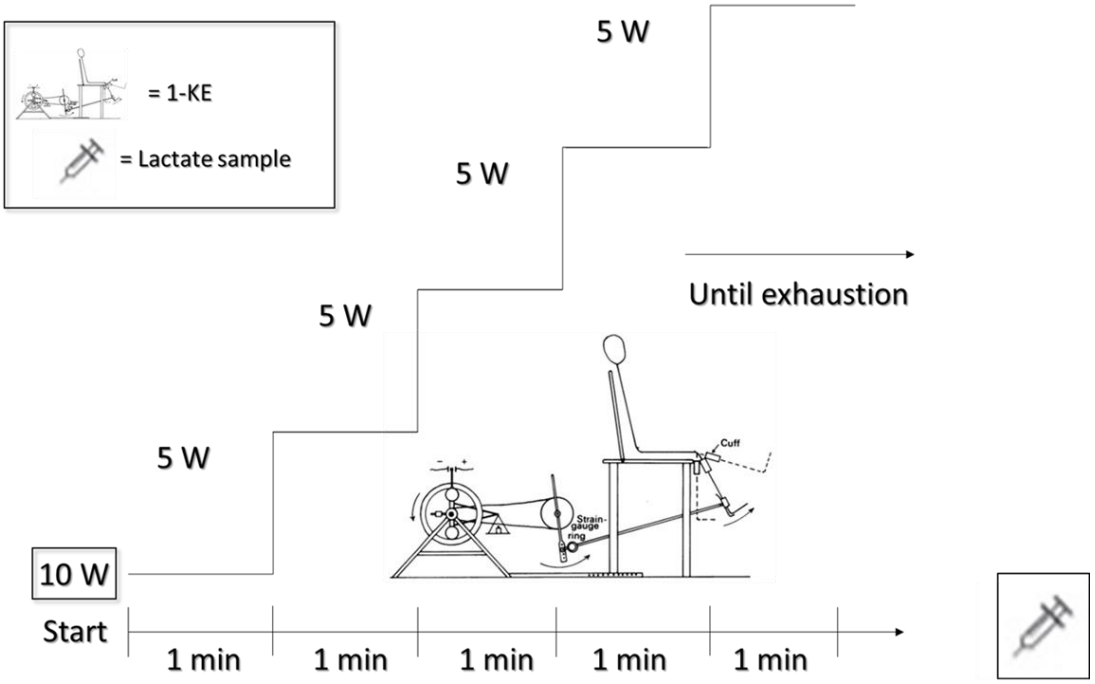

Fig 6. *Schematic illustration of the experimental protocol for* WL_{max} *in 1-KE*

3.3.4 1-KE: 50 % and 80 % of WL_{max}

After testing 1-KE to max, we selected the *WL* corresponding to 50 % and 80 % of WL_{max}. On order to evaluate if the strength training was able to modify the slow component of $\dot{V}O_2$ kinetics because of a less pronounced recruitment of *MU* at the very same *WL*, we performed the test at the same absolute *WL* before and after the strength training intervention. This test was performed on the same electromagnetically braked knee extensor ergometer as described above, with the same standardized design. Both legs were tested in randomized order. The recovery time between the tests were 30 minutes per leg, and the recovery time between the test on the two legs were 30 minutes (Fig. 7). Also, in this case, $\dot{V}O_2$ and *HR* was measured throughout the test and capillary blood from fingertip was taken before and after the two tests and analyzed for lactate concentration. Both the test at 50 % and 80 % lasted for 7 minutes, and the presence of the slow component was evaluated by computing the difference between $\dot{V}O_2$ at 6th and 3rd minute of the exercise. During the test, we also recorded *sEMG* to measure the

muscle activation on *vastus lateralis*, *vastus medialis* and *rectus femoris*. Finally, the oxygenation of the exercising *vastus lateralis* was evaluated by measuring the concentration of deoxygenated hemoglobin by using a wireless device for near-infrared spectroscopy (*NIRS*).

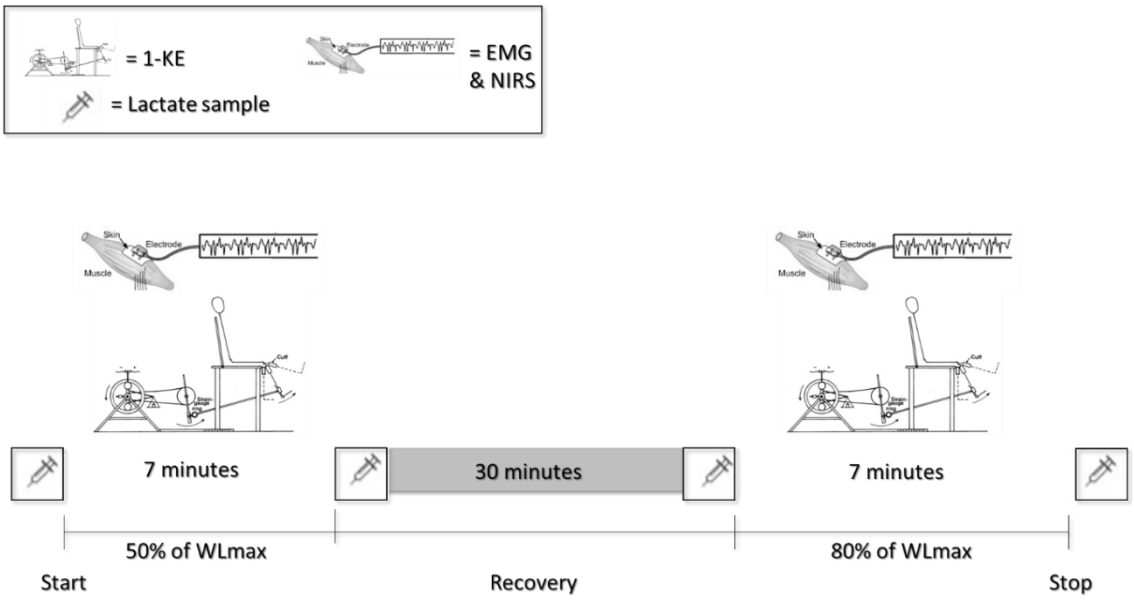

Fig 7*. Schematic illustration of the experimental protocol for the 1-KE at 50 % and 80 % of* WL_{max}

3.3.5 Strength tests – quadriceps

Maximal isometric (T_{MVC}) and isokinetic concentric (T_C) torques produced by the knee extensors of the C_{leg} and T_{leg} were evaluated with an isometric-isokinetic dynamometer at 60° of knee angle during maximal voluntary contractions during T_{MVC} and at angular velocities of 60° s^{-1} and 120° s^{-1} T_C (Fig. 8). The subjects were seated in the chair with their arms crossed over the chest and fastened with a four-point safety belt to minimize extraneous body movement during the extension.

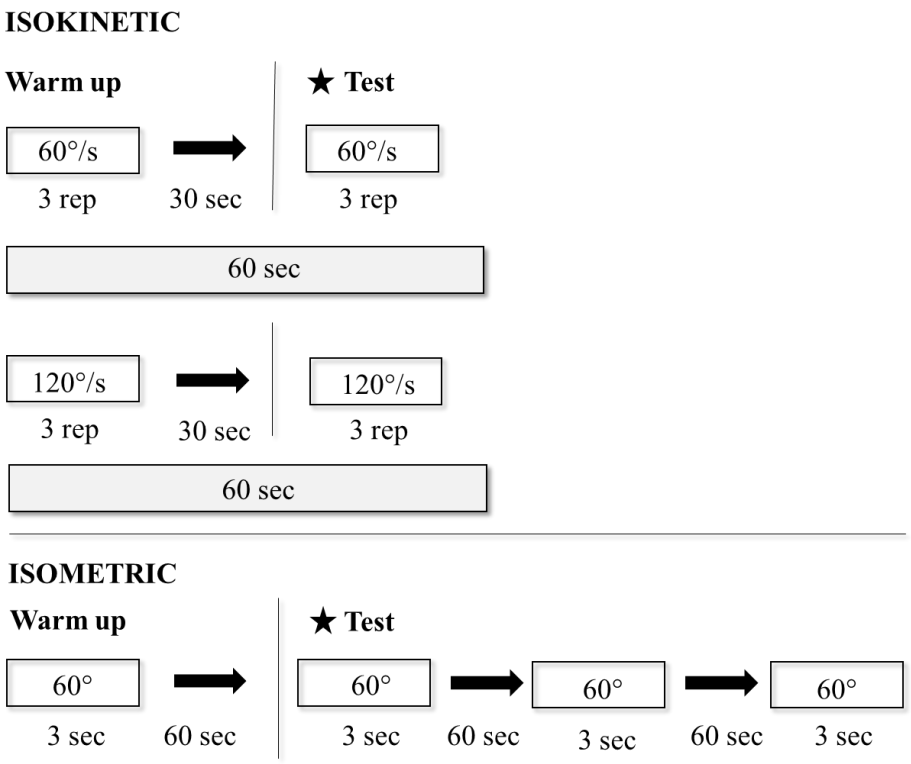

***Fig 8**. Schematic illustration of the experimental protocol for T_C and T_{MVC} in Humac dynamometer.*

3.4 Strength training intervention

The subjects underwent a 6-week strength training intervention on the randomized T_{leg}, on the leg extension apparatus (Technogym, Cesena, Italy). To establish the individual load during training, we preliminary tested the maximal strength (1RM) of the *quadriceps* with the same apparatus, of both the T_{leg} and the C_{leg} separately.

The protocol consisted in:

- General warm up: 10 minutes on ergometric cycle (100 W at 60 – 90 *RPM*)
- Specific warm up: 10 – 6 – 3 repetitions on light load, with increasing weight
- 3 attempts at 1RM (Fully stretched knee)

The C_{leg} was not trained during the intervention, but the subjects were free to do other types of activity during the period, except from single leg exercises. All subjects had to attain a minimum of 90 % of the training sessions, or else the data / subject was removed from the study. All subjects attained the minimum, and no subjects were removed. The training intervention started 2 – 3 days after the final pre-test, and the first

two weeks contained 2 sessions per week (approximately 20 minutes per session). The last four weeks of the training intervention contained 3 sessions per week. All sessions were supervised, and the load were adjusted within the percent given in Table 2 according to how the subject felt during the different sessions (i.e. the weight was adjusted to fit the nRM of repetition). In Table 2, the progression of the load and the number of repetitions applied during training is schematically reported.

***Table 2**. Strength training protocol*

Week	Set (No.)	Repetition (No.)	Load (% 1RM)	Rest (min)
1	3	10 – 15	50 – 60	2
2	5 – 6	15 – 20	60 – 70	2
3	6	15 – 20	60 – 70	2
4	4 – 5	10 – 12	70 – 80	1
5	3 – 4	4 – 6	80 – 90	1
6	3 – 4	4 – 6	80 – 90	1

3.5 Materials

Blood pressure

The clinic blood pressure was measured using standard methodology with an oscillometric device (Spot Vita Signs LXi, Welch Allyn, Skaneateles Falls, NY, USA), as the average of 3 attempts.

Oxygen uptake

$\dot{V}O_2$, carbon dioxide output ($\dot{V}CO_2$) and pulmonary ventilation ($\dot{V}_E$) were measured every 15 seconds by using a mixing box system (Oxycon Pro, Jaeger, Hochberg, Germany). The equipment was calibrated prior to test using gas mixtures with known concentrations of O_2 and CO_2 (14.93 % O_2 and 5.99 % CO_2) and ambient air

(approximately 20.95 % O_2 and 0.039 % CO_2). Volume was calibrated manually using a 3-liter pump (Calibration Syringe, Series 5530, Hans Rudolph Inc., MO, USA). During testing, the subjects breathed through a mouthpiece connected to a two-way low resistance valve (Hans Rudolph, Shawnee, KS 66227 USA) with the nostrils sealed with a nose clip.

EMG

We used surface *sEMG* to measure changes in muscle activity, and to measure changes in activity before and after the strength training intervention at 50 % and 80 % of WL_{max}. The skin was shaved, prepared with scrubbing gel and cleaned with alcohol in the zone explored by the electrodes. Transparent sheet marked with pen marks were used for each subject to enable reproduction / placement of the electrodes from pre- to post-test.

- *Rectus femoris*
  Location: 50 % of the line from the anterior *spina iliaca* superior to the superior part of *patella*
- *Vasus medialis*
  Location: 80 % on the line between anterior *spina iliaca* superior, and the joint space in front of the anterior border of medial ligament
- *Vastus lateralis*
  Location: 2 / 3 on the line from the anterior *spina iliaca* superior to the lateral side of *patella*

The *EMG* signals was recorded at a sampling rate of 1000 Hz (National instruments, software Labview – custom made) by using an amplifier, and band pass filtered (high-pass cut-off was 20 Hz, and low-pass cut-off was 500 Hz). The signals were stored on a PC for off-line analysis. *EMG* signals were then processed both in the time-domain and in frequency domain by computing Root Mean Square (*RMS*) and mean- and median frequency of the spectrum. This allowed us to gain information of the recruitment pattern of the involved muscles, the number of the recruited *MUs*, and the indexes of peripheral fatigue. Mean power frequency (*MPF*) was plotted as a function of time (Ng, Richardson, Kippers, Parnianpour, & Bui, 1996).

The slope of the regression line describes the fatigue rate of the muscle. It is usually negative as median or mean frequency decrease during repeated contractions. If the muscle shows no signs of fatigue, the slope has a value close or not significantly different from zero. *RMS* was taken as an expression of *MU* recruitment (Coburn, Housh, Cramer, & Weir, 2005). To normalize the *iEMG*, the peak value ($iEMG_{max}$) recorded during the entire 1-KE test was set, for each individual and test, equal to 100 %. The integrated *sEMG* was expressed relative to the value (Ringelberg, 1985):

$$\%EMG = \frac{iEMG}{iEMGmax} * 100\%$$

NIRS

A wireless device for near-infrared spectroscopy (*NIRS*, PortaMon, Artinis Medical Systems, Elst, Netherlands) was placed longitudinally on the belly of the *vastus lateralis* of the working leg to measure muscle deoxyhemoglobin ([*HHb]*) and oxy-hemoglobin ([HbO_2*]*) concentrations. Data were recorded at 10 Hz and the PortaMon was wrapped in plastic to protect it from sweat. The values were analyzed in Oxysoft 3.0.53 (Artinis Medical Systems, Elst, Netherlands). To calculate the [*HHb*] and [HbO_2] we used Δ-values of the 3rd and 6th minutes from the baseline, which was defined as the average of 1 minute where the subject sat still. The ratio between the net increase of $\dot{V}O_2$ and [*HHb*] at the 3rd and 6th minutes of 1-KE were calculated as an index of change in fractional muscle O_2 extraction required to elicit a given $\dot{V}O_2$.

HR monitor

HR was measured using an *HR* monitor from Polar RS 800 (Kempele, Finland).

Lactate

Lactate of capillary blood ($[La^-]_b$) obtained from fingertip was measured by using an automated analyzer (Biosen C-line Analyser, EFK Diagnostics, Barleben, Germany). The analyzer was calibrated before each test following the indications of the producer.

Muscle strength

T_{MVC} and T_C torques were measured on both legs by using a dynamometer (Humac NORM, CSMi, Stoughton, MA, USA). 1RM of the *quadriceps* was evaluated with a leg extension apparatus (Technogym, Cesena, Italy) of both legs separately.

3.6 Data analysis

All data was checked for normality distribution with a Shapiro-Wilk test. Data are presented as mean values ± standard deviation (SD). Difference between C_{leg} and T_{leg} where examined with unpaired student's *t*-test, while the difference from pre- to post in one leg where examined with paired student's *t*-test. 2way ANOVA for repeated measurement where used to examine the difference between each minute pre- and post for each leg. The significance threshold (*P*-value) was set to 0.05, and when differences were found to be significantly different from 0, the 95 % CI of the difference were always reported. Regression lines were calculated by using the least – square method and the slopes and intercepts of two regression lines were analyzed according to Zar (1984). Analysis was performed using GraphPad Prism version 7.00 (GraphPad Software, La Jolla California, USA, www.graphpad.com)

4. Results

4.1 Training

In Figure 9 the progression of the training load of the seven subjects is presented. The training load applied during the sessions increased from 50 % to about 80 % of the maximal repetition load at the end of the six weeks of training. For both control and trained legs, the load significantly increased from the beginning to the end of training (Table 3). The 1RM increased significantly in both legs, respectively 21 % pre- to post in the T_{leg} ($P < 0.01$; 95 % CI of the difference -19.6/-13.3 kg) and 5 % in the C_{leg} ($P = 0.03$; 95 % CI of the difference -6.7/-0.5 kg).

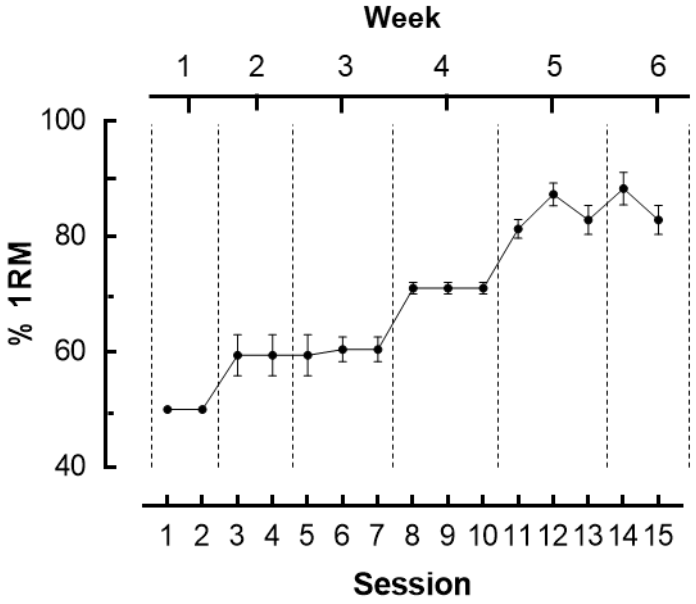

Fig 9. *Average increase in loading (% of 1RM) of each session, and each week*

Table 3. *Dynamic strength results pre- and post-training for C_{leg} and T_{leg}*

	Cleg		**Tleg**	
	Pre	Post	Pre	Post
1RM (kg)	54 ± 13	57 ± 14 *	58 ± 13	74 ± 13 **

* Significant difference between pre- and post-training ($P < 0.05$) ** ($P < 0.01$)

4.2 Anthropometrics

In Table 4, the results of the subcutaneous fat measured on the surface of the *vastus lateralis* are presented before and after training for the T_{leg} and the C_{leg}. Results showed that there was no difference in subcutaneous fat pre- to post in C_{leg}. There was a 5.3 % decrease in subcutaneous fat in the T_{leg}, but no significant difference.

Table 4. *Difference in subcutaneous fat on vastus lateralis between C_{leg} and T_{leg} pre- and post-training*

	C_{leg}		T_{leg}	
	Pre	Post	Pre	Post
Subcutaneous fat (mm)	4.9 ± 1.2	4.9 ± 0.8	4.9 ± 1.0	4.6 ± 1.0

In Figure 10, the estimated M_{qf} in the C_{leg} (Fig. 10a) and the T_{leg} (Fig. 10b) are reported. No difference between the legs at pre-training was present. Estimated M_{qf} in the C_{leg} did not change from pre- to post-training; conversely, estimated M_{qf} increased 0.14 kg (5 %) in the T_{leg} pre- to post-training, but not significantly so (P = 0.085).

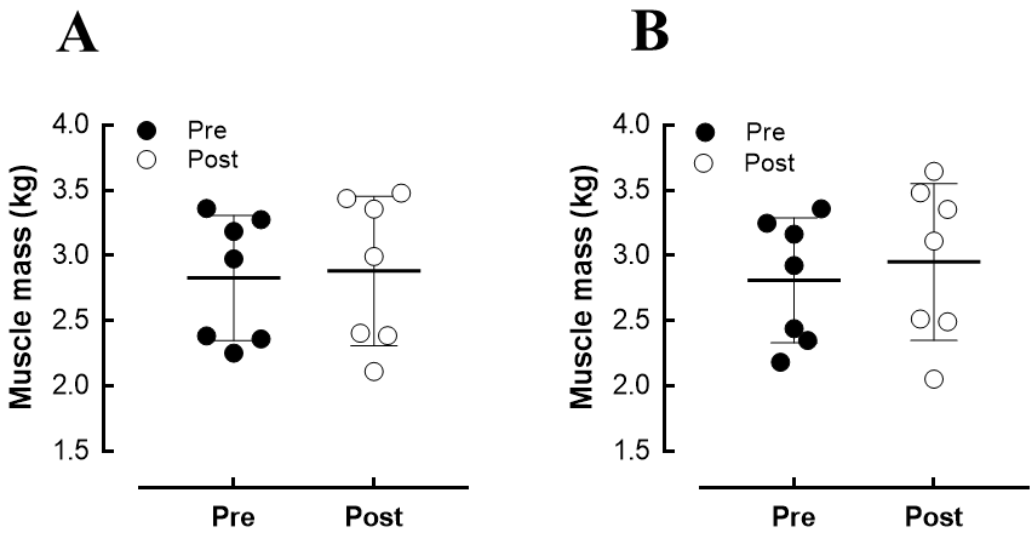

Fig 10. *Changes in estimated M_{qf} for C_{leg} (A) and T_{leg} (B)*

4.3 Strength

In Table 5, the results of the T_C and T_{MVC} strength tests are presented before and after the training intervention. There were no significant differences in any parameters between the C_{leg} and T_{leg} at pre-training.

T_C increased by 8 % in the T_{leg} at 60°/s^{-1} (P = 0.062; 95 % CI of the difference -1.5/46.3 Nm), while there was no difference in C_{leg}. At this angular speed, the T_C of T_{leg} was significantly larger than the one of C_{leg} (P = 0.024; 95 % CI of the difference 27.8/81.0 Nm) after the training intervention. At 120°/s^{-1} there was a 12 % increase in T_C from pre- to post-training in T_{leg} (P = 0.033; 95 % CI of the difference 3.0/50.1 Nm), but no difference in C_{leg}. After the training intervention, the T_C of T_{leg} was on the average 12 % larger than the one of C_{leg}, but not significantly so (P = 0.153).

T_{MVC} torque of T_{leg} turned out to be significantly larger than before the training intervention (P = 0.007; 95 % CI of the difference 17.9/73.8 Nm). Likewise, the T_{MVC} torque of C_{leg} before the training intervention was significantly larger than the one assessed after training (P = 0.019; 95 % CI of the difference -37.1/-4.9 Nm). After training, the T_{MVC} of T_{leg} was larger than the one of C_{leg} (P = 0.006; 95 % CI of the difference 72.4/21.3 Nm).

Table 5. *T_C and T_{MVC} results pre- and post-training for the C_{leg} and T_{leg}*

	C_{leg}		T_{leg}	
	Pre	Post	Pre	Post
60°/s^{-1}	229 ± 38	219 ± 33	251 ± 43	274 ± 26 #
120°/s^{-1}	198 ± 26	202 ± 27	201 ± 31	228 ± 37 *
60°	290 ± 45	269 ± 47 *	295 ± 28	341 ± 32 # **

* Significant difference between pre- and post-training (P < 0.05) **(P < 0.01)

Significant difference between C_{leg} and T_{leg} (P < 0.05)

4.4 Oxygen uptake

In Table 6, 7 and 8 are the gas exchange data together with the data of *HR* and $[La^-]_b$ concentration assessed during ramp cycling exercise and 1-KE tests presented. As far as the parameters collected during the maximal 1-KE, no parameter showed significant difference between C_{leg} and T_{leg}.

Figure 11 shows the $\dot{V}O_2$ measured every minute from the onset of the 1-KE exercise at 50 % of WL_{max} and at 80 % of WL_{max}. For the tests at 50 % of WL_{max}, a steady state is attained after approximately 3 minutes of exercise both in the C_{leg} (Fig. 11a) and T_{leg} (Fig. 11b), and after training $\dot{V}O_2$ assessed at the end of the test performed at 50 % of WL_{max} was not significantly different compared to the one assessed before training both in the C_{leg} and T_{leg}. By the same token, also the other parameters seemed not to be affected by training. At 80 % of WL_{max} there is a continuous rise of $\dot{V}O_2$ both before and after the training intervention in C_{leg} (Fig. 11c) and T_{leg} (Fig. 11d).

At 80 % of WL_{max} there was a 9 %, not significant, decrease of both absolute $\dot{V}O_2$, mL min^{-1} ($P = 0.144$) and relative $\dot{V}O_2$, mL kg^{-1} min^{-1} ($P = 0.153$) in T_{leg} after training intervention at the end of exercise in comparison with what observed before the training intervention (Table 8). By the same token also $\dot{V}_E$ decreased by 19 %, but not significantly so in the same leg ($P = 0.147$). Conversely, there was a significant increase of both absolute $\dot{V}O_2$, mL min^{-1} ($P = 0.036$; 95 % CI of the difference 14.0/289.7 mL min^{-1}) and relative $\dot{V}O_2$, mL kg^{-1} min^{-1} ($P = 0.048$; 95 % CI of the difference 0.0/3.1 mL kg^{-1} min^{-1}) in C_{leg} measured at the end of the exercise after the training intervention (Table 8)

Table 6. *Gas exchange data from $\dot{V}O_{2max}$ test on ergometric cycle (Ramp test) and WL_{max} test (1-KE_{max})*

	Ramp test	**1-KE_{max}**	
		C_{leg}	T_{leg}
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	51.3 ± 7.6	17.9 ± 5.1	19.1 ± 4.4
$\dot{V}O_{2max}$ (L·min^{-1})	4.3 ± 0.7	1.5 ± 0.5	1.6 ± 0.5
$\dot{V}_E$ (L·min^{-1})	170.4 ± 26.2	61.2 ± 21.6	69.4 ± 24.1
RER	1.18 ± 0.07	1.14 ± 0.11	1.18 ± 0.12
HR_{max} (BPM)	181 ± 10	132 ± 17	128 ± 8
WL_{max} (W)	360 ± 87	48 ± 8	48 ± 9
$[La^-]_b$ (mmol·L^{-1})	11.4 ± 2.4	4.4 ± 0.7	4.5 ± 0.7

$\dot{V}O_2$ oxygen uptake, $\dot{V}_E$ respiratory minute volume, *RER* respiratory exchange ratio, *HR* heart rate, *WL* workload, $[La^-]_b$ capillary blood lactate

Table 7. *Gas exchange data from 50 % of WL_{max} (1-KE_{submax})*

	1-KE_{submax} 50 %			
	C_{leg}		T_{leg}	
	Pre	Post	Pre	Post
$\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1})	10.6 ± 0.8	11.0 ± 0.9	10.8 ± 1.1	10.6 ± 1.0
$\dot{V}O_{2peak}$ (L·min^{-1})	0.9 ± 0.2	1.0 ± 0.2	0.9 ± 0.2	0.9 ± 0.2
$\dot{V}_E$ (L·min^{-1})	29.4 ± 7.0	28.0 ± 6.9	29.3 ± 7.7	28.5 ± 5.5
RER	0.95 ± 0.05	0.91 ± 0.05	0.94 ± 0.05	0.90 ± 0.05
HR_{peak} (BPM)	98 ± 16	97 ± 6	97 ± 14	99 ± 9
WL (W)	24 ± 4	24 ± 4	24 ± 5	24 ± 5
$[La^-]_b$ start (mmol·L^{-1})	2.0 ± 0.5	1.5 ± 0.6	1.8 ± 0.7	1.7 ± 0.5
$[La^-]_b$ stop (mmol·L^{-1})	2.5 ± 0.6	1.9 ± 0.5	2.2 ± 0.6	2.0 ± 0.5

$\dot{V}O_2$ oxygen uptake, $\dot{V}_E$ respiratory minute volume, *RER* respiratory exchange ratio, *HR* heart rate, *WL* workload, $[La^-]_b$ capillary blood lactate

***Table 8**. Gas exchange data from 80 % of WL_{max} (1-KE_{submax})*

	1-KE_{submax} 80 %			
	C_{leg}		T_{leg}	
	Pre	Post	Pre	Post
$\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1})	16.1 ± 2.2	17.6 ± 3.1 *	18.1 ± 4.0	16.6 ± 4.6
$\dot{V}O_{2peak}$ (L·min^{-1})	1.3 ± 0.3	1.5 ± 0.4 *	1.5 ± 0.4	1.4 ± 0.4
$\dot{V}_E$ (L·min^{-1})	53.4 ± 18.4	55.5 ± 18.8	62.6 ± 22.3	52.5 ± 20.8
RER	1.07 ± 0.08	1.04 ± 0.08	1.09 ± 0.13	1.04 ± 0.08
HR_{peak} (BPM)	125 ± 26	124 ± 20	127 ± 26	122 ± 22
WL (W)	38 ± 6	38 ± 6	38 ± 7	38 ± 7
$[La^-]_b$ start (mmol·L^{-1})	1.7 ± 0.5	1.6 ± 0.4	1.4 ± 0.3	1.6 ± 0.4
$[La^-]_b$ stop (mmol·L^{-1})	4.3 ± 1.0	4.3 ± 1.2	4.7 ± 1.0	4.6 ± 1.0

$\dot{V}O_2$ oxygen uptake, $\dot{V}_E$ respiratory minute volume, *RER* respiratory exchange ratio, *HR* heart rate, *WL* workload, $[La^-]_b$ capillary blood lactate

* Significant difference between pre- and post-training ($P < 0.05$)

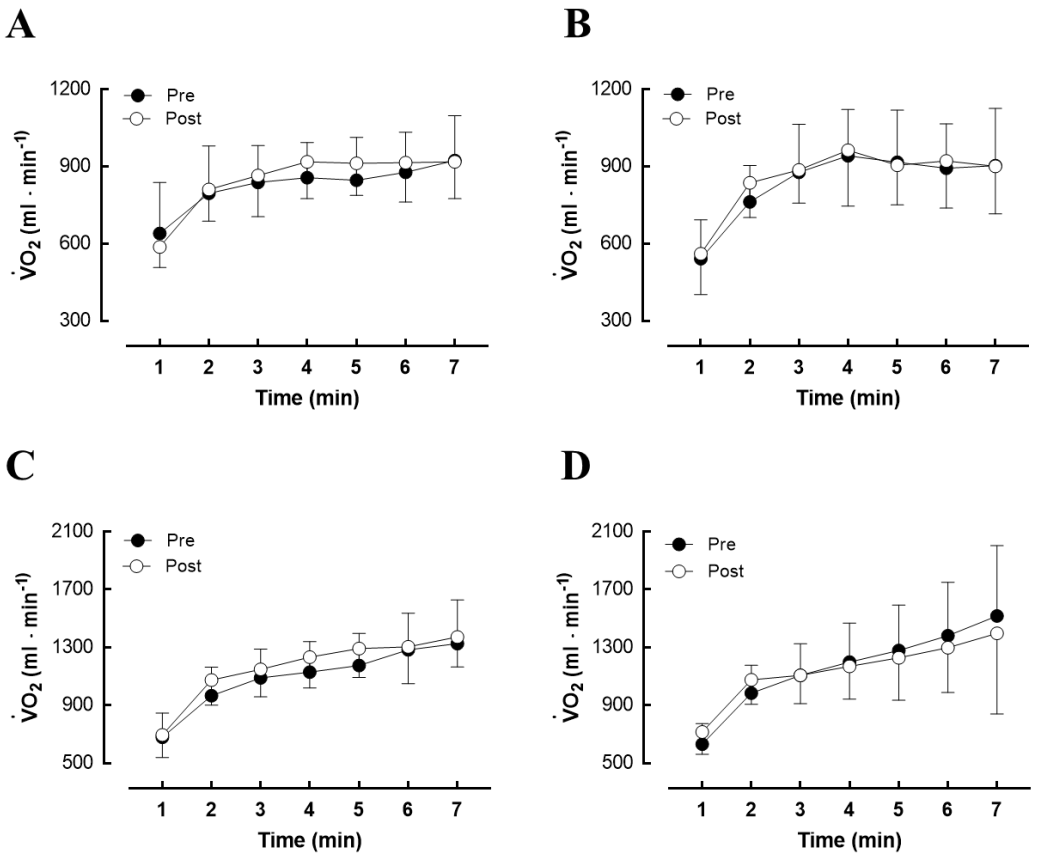

Fig 11. *Changes in $\dot{V}O_2$ as average of each minute pre- and post-training during 1-KE at 50 % of WL_{max} for C_{leg} (A) and Tl_{eg} (B) and 80 % of WL_{max} for C_{leg} (C) and T_{leg} (D)*

As explained in the Methods, the entity and the amplitude of the slow component of $\dot{V}O_2$ kinetics was evaluated by computing and comparing the mean values of $\dot{V}O_2$ measured at the 3rd and 6th minutes (Fig. 12a-d).

At 50 % of WL_{max}, there was no difference between the 3rd and 6th minute, or between pre- and post-training for C_{leg} (Fig. 12a) or T_{leg} (Fig. 12b). At 80 % of WL_{max} in the C_{leg}, $\dot{V}O_2$ at the 6th minute was significantly larger than at the 3rd minute before (P = 0.019; 95 % CI of the difference -298.1/-34.8), but not after training (P = 0.057; 95 % CI of the difference -259.6/4.4) (Fig. 12c). Before training, the $\dot{V}O_2$ increased from 1098 ± 230 mL min^{-1} at the 3rd minute to 1265 ± 282 mL min^{-1} at the 6th minute, resulting in an increase of 166 mL min^{-1} (15 %). After training intervention, $\dot{V}O_2$ increased from 1187 ± 213 mL min^{-1} at the 3rd minute to 1315 ± 283 mL min^{-1} at the 6th minute, which corresponded to a net increase of 128 mL min^{-1} (11 %). At 80 % of WL_{max} in the T_{leg}, $\dot{V}O_2$ at the 6th minute was significantly larger than at the 3rd minute before (P = 0.0001;

95 % CI of the difference 364.8/216.4 mL min^{-1}) and after training intervention (P = 0.003; 95 % CI of the difference 282.8/134.4 mL min^{-1}). However, after training, $\dot{V}O_2$ at the 6th minute was significantly smaller than before (P = 0.0098; 95 % CI of the difference 181.4/32.9 mL min^{-1}) (Fig. 12d). Before training, the $\dot{V}O_2$ raised from 1145 ± 258 mL min^{-1} at the 3rd minute to 1436 ± 443 mL min^{-1} at the 6th minute, with a net increase of 291 mL min^{-1} (19 %). After training, there was an increase from 1120 ± 275 mL min^{-1} at the 3rd minute to 1329 ± 386 mL min^{-1} at the 6th minute, corresponding to a difference of 209 mL min^{-1} (13 %).

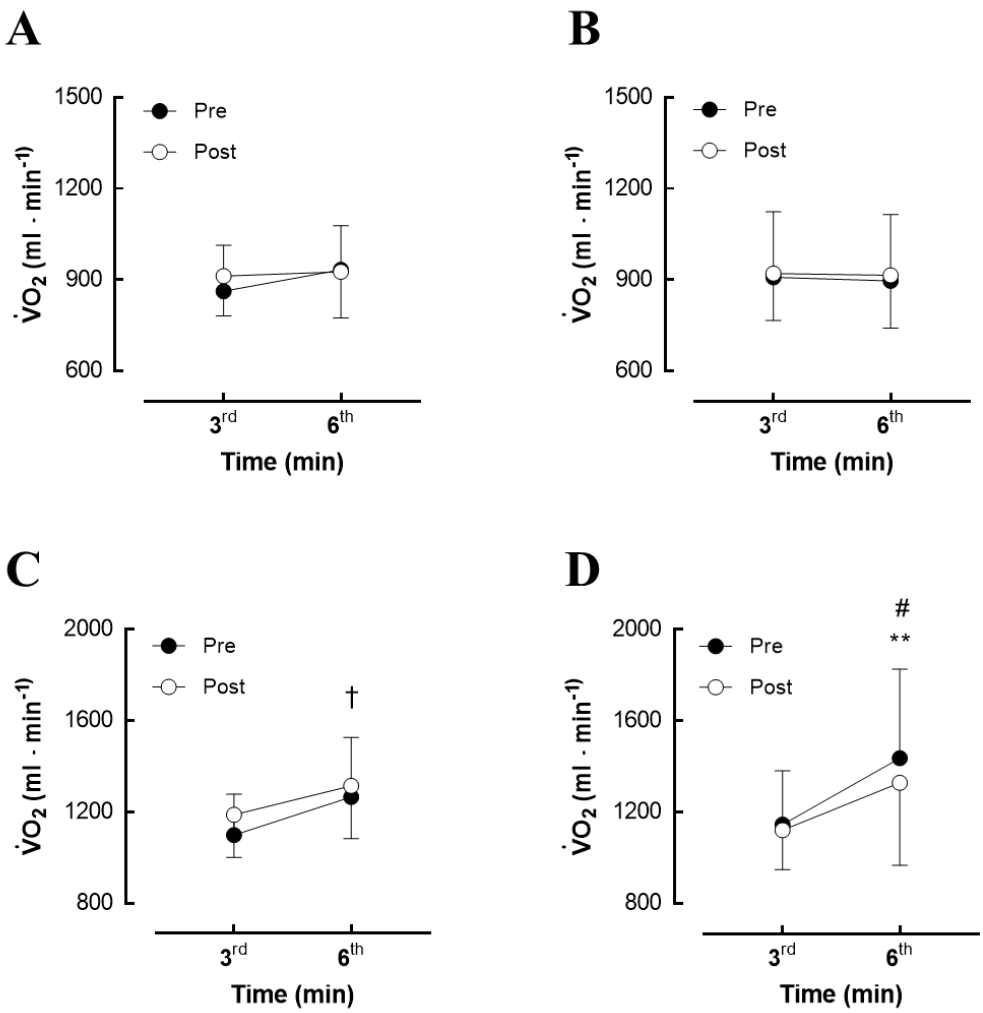

Fig 12. *Changes in the slow component of $\dot{V}O_2$ kinetics from 3rd to 6th minute of 1-KE during 50 % of WL_{max} for C_{leg} (A) and T_{leg} (B), and during 80 % of WL_{max} for C_{leg} (C) and T_{leg} (D).*
*# Significant difference 3rd to 6th minute pre- and post-training ($P < 0.05$) † Significant difference 3rd to 6th pre-training ($P < 0.05$) **Significant difference pre- to post-training ($P < 0.01$).*

4.5 iEMG

In Figure 13, the difference in *iEMG* during 50 % of WL_{max} for C_{leg} and T_{leg} are presented before and after training. For the tests at 50 % of WL_{max}, there was a steady state from the 1st to the 6th minute of exercise. There was no difference between pre- and post-training in either C_{leg} or T_{leg}, except for in *vastus lateralis* C_{leg} (Fig. 13c), where the 3rd minute was significantly higher during the post-training (P = 0.02; 95 % CI of the difference 11.9/0.7 %).

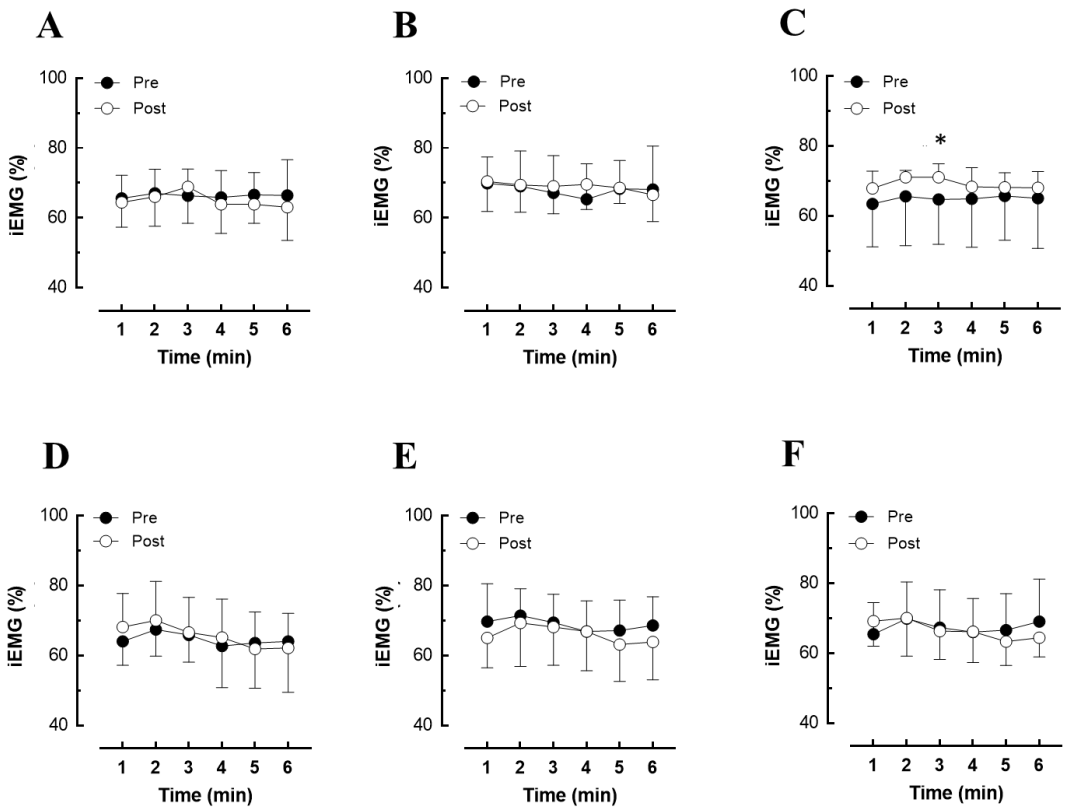

Fig 13. *Change in iEMG activity in percent during 1-KE at 50 % of WL_{max} for vastus medialis C_{leg} (A) and T_{leg} (D), rectus femoris C_{leg} (B) and T_{leg} (E) and vastus lateralis C_{leg} (C) and T_{leg} (F).*
**Significant difference between pre- and post-training ($P < 0.05$)*

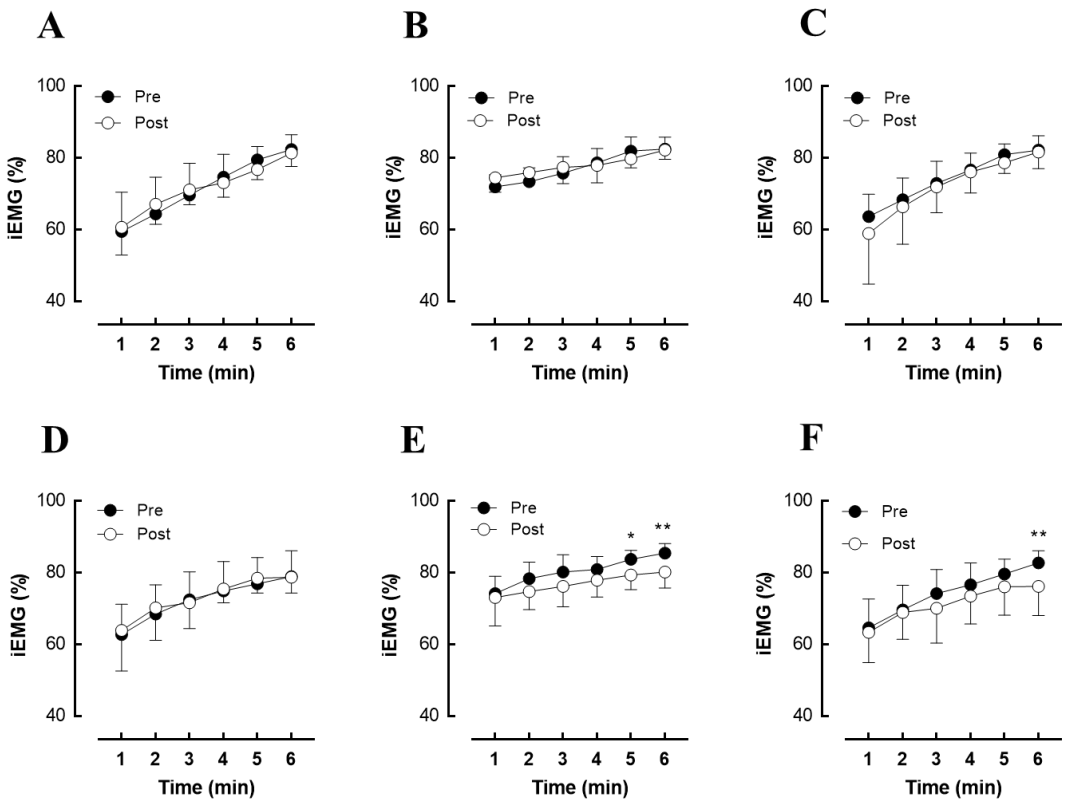

Fig 14. *Change in iEMG activity in percent during 1-KE at 80 % of WL_{max} for vastus medialis C_{leg} (A) and T_{leg} (D), rectus femoris C_{leg} (B) and T_{leg} (E) and vastus lateralis C_{leg} (C) and T_{leg} (F).*
** Significant difference between pre- and post-training (P < 0.05) **(P < 0.01)*

In Figure 14, the difference in *iEMG* during 80 % of WL_{max} before and after training are presented, for both C_{leg} and T_{leg}. There was no difference in *iEMG* activity between pre- and post-training intervention for C_{leg} in either *vastus medialis* (Fig. 14a), *rectus femoris* (Fig. 14b) or *vastus lateralis* (Fig. 14c). There was no difference pre- to post-training in *vastus medialis* for the T_{leg} (Fig. 14d), but for the *rectus femoris*, there was a significant decrease during the 5th minute (P = 0.035; 95 % CI of the difference 0.2/8.7) and 6th minute (P = 0.006; 95 % CI of the difference 1.1/9.5) (Fig. 14e). In *vastus lateralis*, there was a significant decrease in the 6th minute between pre- and post-training (P = 0.005; 95 % CI of the difference 1.3/11.8) (Fig. 14f).

In Figure 15, the change in *iEMG* from the 3rd to the 6th minute before and after training at 80 % of WL_{max} is presented. For the C_{leg}, the 6th minute was significantly higher than the 3rd minute both pre- (P = 0.017; 95 % CI of the difference -22.8/-2.6) and post-training (P = 0.046; 95 % CI of the difference -20.4/-0.2) in *vastus medialis* (Fig. 15a), but no difference before and after training. The value at 6th minute was significantly higher than the one at 3rd minute pre- (P = 0.006; 95 % CI of the difference -10.9/-2.6) and post-training (P = 0.026; 95 % CI of the difference -9.0/-0.7) in *rectus femoris* (Fig. 15b), but no difference before and after training. There was also a significant difference between the 6th and the 3rd minute both pre- (P = 0.026; 95 % CI of the difference -17.2/-1.4) and post-training (P = 0.021; 95 % CI of the difference -17.7/-1.8) in *vastus lateralis* (Fig. 15c), but no difference before and after training.

Regarding the *vastus medialis* in the T_{leg}, there was no difference between pre- and post-training (Fig. 15d). Before training, the difference between the values of *iEMG* at the 6th and the 3rd minute was not significant (P = 0.06; 95% CI of the difference -13.2/0.3). After training, the difference between the two values was significant (P = 0.04; 95 % CI of the difference -13.9/-0.4). There was a significant decrease at the 3rd minute of exercise from pre- to post-training in *rectus femoris* (Fig. 15e) (P = 0.011; 95 % CI of the difference -1.2/-6.9) and in the 6th minute (P = 0.003; 95 % CI of the difference 2.4/8.2). The *iEMG* at the 6th minute was significantly larger than at the 3rd minute before training (P = 0.003; 95 % CI of the difference -8.2/-2.4); after the training intervention, there was still a significant difference between the two values (P = 0.011; 95 % CI of the difference 2.4/8.2). There was also a decrease of *iEMG* in the 3rd minute in *vastus lateralis*, but not significantly so (P = 0.089; 95 % CI of the difference -0.7/8.9) (Fig. 15f). However, at the 6th minute, the decrease was significant (P = 0.013; 95 % CI of the difference -1.7/-11.4). The *iEMG* at the 6th minute was significantly larger than the one at the 3rd minute before training (P = 0.004; 95 % CI of the difference -13.4/-3.8) and after training (P = 0.018; 95 % CI of the difference -10.9/-1.3).

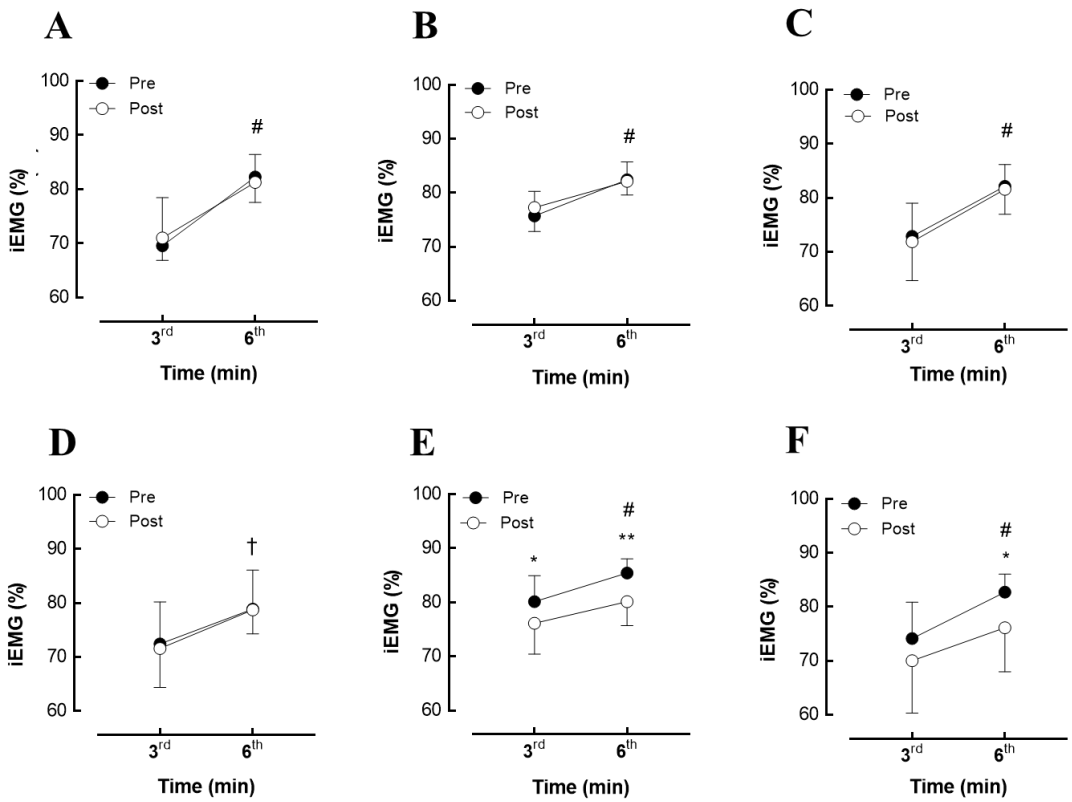

Fig 15. *Change in iEMG activity in percent from the 3rd to the 6th minute in vastus medialis C_{leg} (A) and T_{leg} (D), rectus femoris C_{leg} (B) and T_{leg} (E) and vastus lateralis C_{leg} (C) and T_{leg} (F).*
 # Significant difference between 3rd and 6th minute pre- and post-training ($P < 0.05$).
† Significant difference between 3rd and 6th minute only post-training ($P < 0.05$).
** Significant difference between pre- and post-training ($P < 0.05$) ** ($P < 0.01$)*

4.6 MPF

In Figure 16 and 17, the normalized values ± SD of *MPF* averaged every minute from the first to the seventh minute of exercise in C_{leg} (Fig. 16) and in T_{leg} (Fig. 17) are plotted as a function of time calculated before (filled dots) and after training (open dots) from the *sEMG* recorded on *vastus medialis*, *rectus femoris* and *vastus lateralis* at 80 % of WL_{max}. The values were normalized according to what explained in the Methods, and a decaying *MPF* is usually considered an index of peripheral neuromuscular fatigue and an indication of the progressive recruitment of type II *MUs*.

MPF recorded on *vastus medialis* of C_{leg} significantly decreased with time after training ($P = 0.041$; $F = 7.53$; $r^2 = 0.60$). However, the slopes of linear regressions observed before and after the training intervention were not significantly different. Also, in the *rectus femoris* of C_{leg} we observed a significant decrease with the time of exercise of

MPF only after training ($P = 0.031$; $F = 8.85$; $r^2 = 0.64$); likewise, the slopes of the linear regressions calculated before and after training were not significantly different. Finally, the *MPF* calculated from *sEMG* recorded from the *vastus lateralis* of C_{leg} significantly decreased with time only before training ($P = 0.032$; $F = 8.68$; $r^2 = 0.64$). Also, in this case, however, the slopes of the two linear regression lines obtained before and after training were not significantly different.

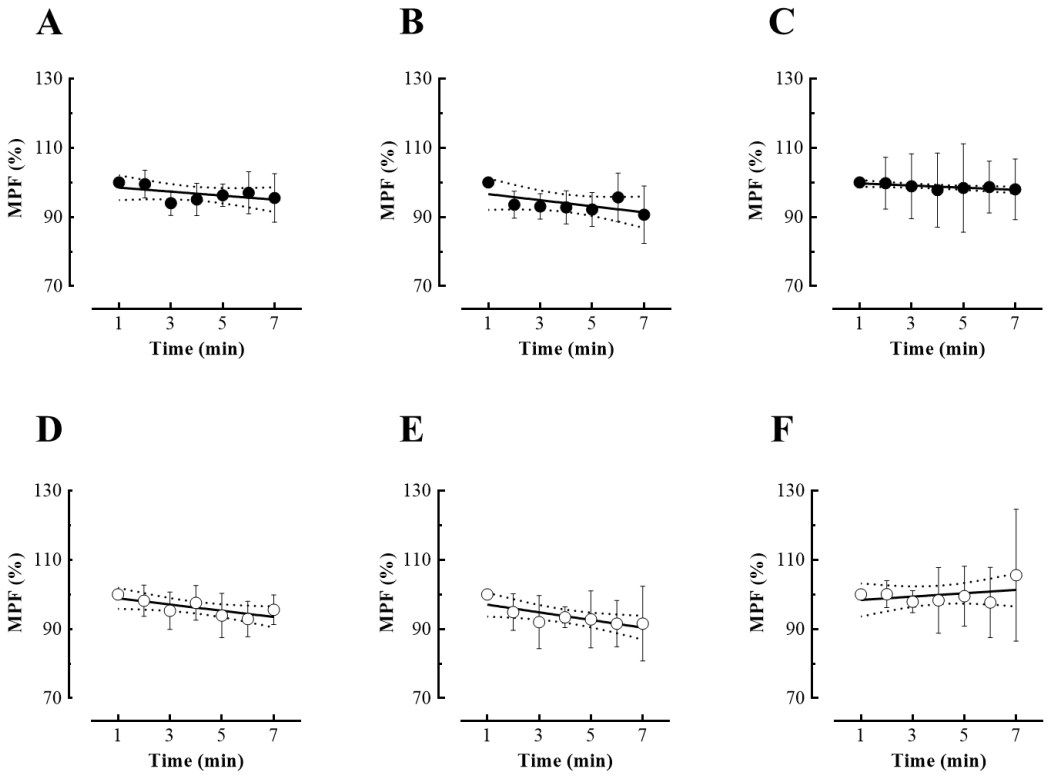

Fig 16. *Change in MPF during 80 % of WL_{max} in the C_{leg} for the vastus medialis pre- (A) and post-training (D), rectus femoris pre- (B) and post-training (E) and vastus lateralis pre- (C) and post-training (F).*

MPF recorded on the *vastus medialis* of T_{leg} significantly decreased with time only before training ($P = 0.003$; $F = 29.5$; $r^2 = 0.85$), and the slopes of the linear regressions observed before and after the training intervention were significantly different ($P = 0.027$; $F = 6.7$). In the *rectus femoris* of T_{leg} we observed a significant decrease with time of exercise of *MPF* both before ($P = 0.020$; $F = 11.4$; $r^2 = 0.69$). and after the training intervention ($P = 0.014$; $F = 13.7$; $r^2 = 0.73$), and the slopes of the linear regressions calculated before and after the training were not significantly different. Finally, the *MPF* calculated from *sEMG* recorded from the *vastus lateralis* of T_{leg}

significantly decreased with time only before training (P = 0.007; F = 19.2; r^2 = 0.79). Also, in this case the slopes of the two linear regression lines obtained before and after training were significantly different (P = 0.001; F = 21.5).

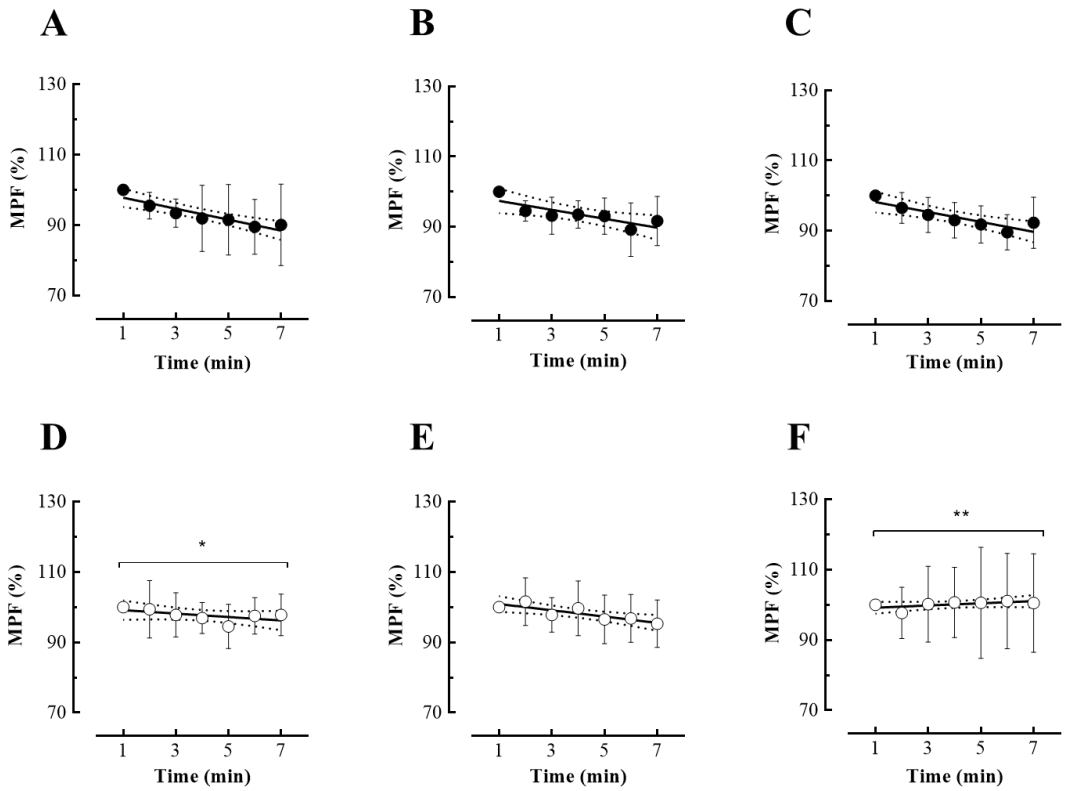

***Fig 17**. Change in MPF during 80 % of WL_{max} in the T_{leg} for the vastus medialis pre- (A) and post-training (D), rectus femoris pre- (B) and post-training (E) and vastus lateralis pre- (C) and post-training (F).*
** Significant difference pre- to post-training ($P < 0.05$) ** ($P < 0.01$)*

4.7 NIRS

In Figure 18, the average values of [*HHb*], expressed as net variations from the baseline values (see Methods for further details) and measured at the 3rd and 6th minute of exercise during 50 % and 80 % of WL_{max} in both C_{leg} and T_{leg} are presented. No difference between the 3rd and 6th minute from pre- to post-training at 50 % of WL_{max} were detected in C_{leg} (Fig. 18a) and T_{leg} (Fig. 18b). By the same token, no significant difference between the 3rd and 6th minute at 80 % of WL_{max} in C_{leg} (Fig. 18c) and T_{leg} (Fig. 18d) were observed.

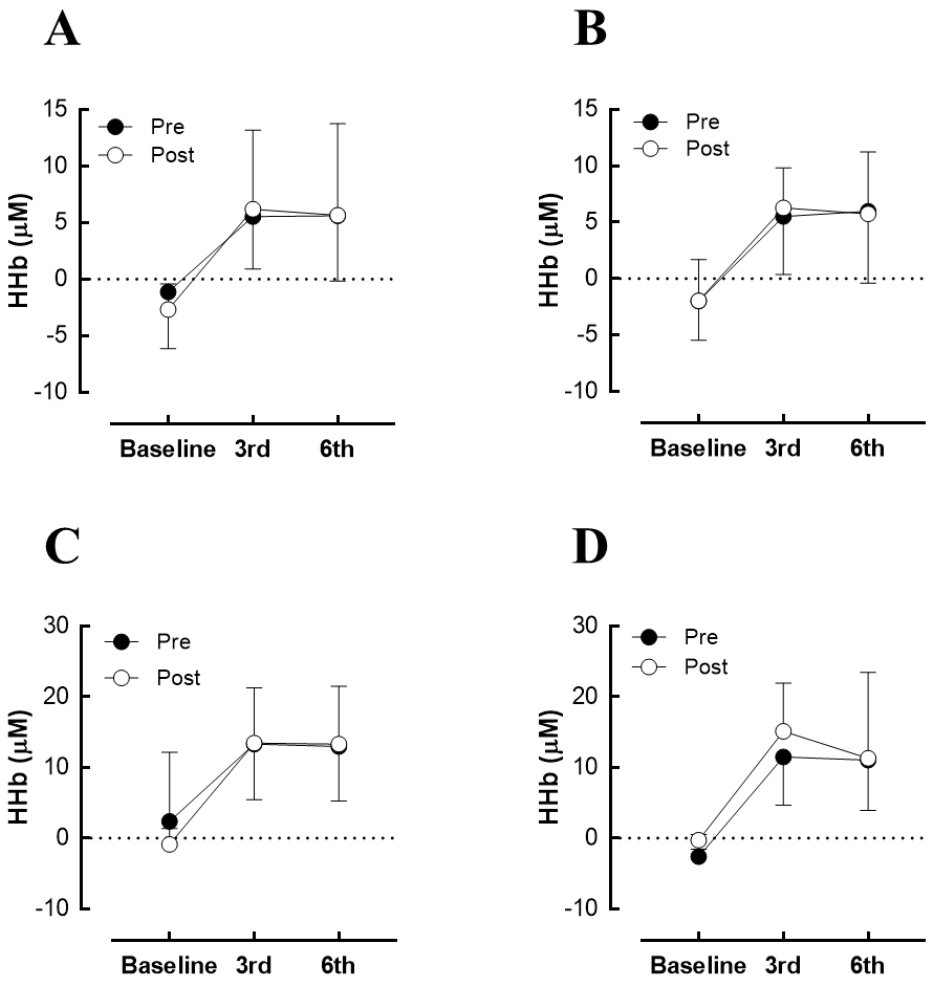

Fig 18. *Change in [HHb] from the 3rd to 6th minute of exercise during 50 % of WL_{max} in Cl_{eg} (A) and T_{leg} (B), and during 80 % of WL_{max} in C_{leg} (C) and T_{leg} (D).*

The ratio between the net increase of $\dot{V}O_2$ and [*HHb*] at the 3rd minute of exercise (19.4 L min^{-1} / µM ± 11.8) in T_{leg} was significantly higher than the value prevailing at the 6th minute (13.5 L min^{-1} / µM ± 9.9) only before training (P = 0.02; 95 % CI of the difference 0.84/6.2 L min^{-1} / µM).

5. Discussion

The main aim of this study was to investigate the effect of 6 weeks of strength training on the $\dot{V}O_{2sc}$. The main results showed that strength training of the *quadriceps* brought about an increase of muscular strength and a decrease in the amplitude of the $\dot{V}O_{2sc}$ of the trained leg in connection with a smaller recruitment of *MUs* and a less pronounced peripheral fatigue during 1-KE at 80 % of the WL_{max} in the T_{leg}.

5.1 Effect of strength training on the slow component

Several studies have shown that endurance training could reduce the amplitude of the $\dot{V}O_{2sc}$ (Carter et al., 2000; Murias et al., 2010; Womack et al., 1995). However, to our knowledge there are few studies that have looked at the effect of strength training on the $\dot{V}O_2$ kinetics. Millet, Jaouen, Borrani, and Candau (2002) found no effect on the $\dot{V}O_2$ kinetics after 14 weeks of combined strength and endurance training in well-trained subjects when testing running, despite a 2.7 % increase in $\dot{V}O_{2max}$ and 25 % increase in strength (measured as half squat). The only study, as far as we know, who have tested how strength training affects the slow component, demonstrated no change in the $\dot{V}O_2$ kinetics after 8 weeks (3 sessions per week) of full body strength training (Womack, Flohr, Weltman, & Gaesser, 2000). The authors found a significant increase in leg strength (~ 27 %) but no influence on the $\dot{V}O_{2sc}$ during a 10-minute constant velocity running test (2000).

5.1.1 Anthropometrics

Our training intervention was modified from the one proposed by Golik-Peric, Drapsin, Obradovic, and Drid (2011) who performed 4 weeks of strength training on the extensors. The periodization of the strength training intervention took into consideration the basic strength training principles with increased load and progression. We therefore used a typical linear periodization (Fig. 9) starting with lighter load and a higher number of repetitions, before we increased the load and decreased the number of repetitions (Willoughby, 1993).

The significant increase of 21 % (+ 16 kg) in 1RM for the T_{leg} (Table 3) are in accordance with the literature, which as stated earlier is ~ 1 % per session (Kraemer et al., 2002; Moritani, 1979). There was also an increase in estimated M_{qf} of 5 % (+ 0.14

kg) in the T_{leg} (Fig. 10b) and a 5.3 % (- 0.3 mm) decrease, albeit not significant, in subcutaneous fat in *vastus lateralis* (Table 4). The muscular adaptions regarding *CSA* in untrained subjects are as stated earlier ~ 0.1 – 0.5 % per session (Wernbom et al., 2007).

The neural adaptions are usually considered to be the main causes of the increase in strength during the first period of a strength training program in untrained subjects, since there is an delay in the onset of muscle hypertrophy (Seynnes et al., 2007). However, the same authors were the first to demonstrate a significant *quadriceps* muscle hypertrophy after only 20 days of strength training (3.5 – 5.2 %). The training, however, consisted of 3 sessions per week with flywheel ergometer that imposes a greater eccentric load to the muscle, an efficient stimulus for hypertrophy. The increased *CSA* of the *quadriceps* were also measured using *DXA*. It is important to notice that the muscle volume and *CSA* in our study were estimated by using skinfold, measured with caliper, and circumferences with tape according to Raadegran et al. (1999). These authors demonstrated that muscle volume of the knee extensors was markedly overestimated by using the indirect approach. Therefore, the validity and reliability of our estimation may not be as accurate as in studies where *DXA* or imaging *MRI* were utilized. For instance, a study by Krustrup, Söderlund, Mohr, González-Alonso, and Bangsbo (2004b) found that muscle mass of the *quadriceps* was 22 % lower when they used *MRI* compared to skinfold measurement according to Raadegran et al. (1999). Also, muscle and region specificity of hypertrophic response is of great importance, due to different increase in *CSA* in distal and proximal regions of the muscle (Folland & Williams, 2007).

The significant increase of 1RM found in the C_{leg} (+ 5 %, + 3 kg) (Table 3) was likely the effect of familiarization, learning effect and crossover effect. The adjustment in the increase in the dynamic strength apparatus used in training were minimum 2.5 kg, and since the estimated M_{qf} in the C_{leg} did not change from pre- to post (Fig. 10a) and no difference in subcutaneous fat (Table 4) were found, we may indeed attribute the increase in 1RM in the C_{leg} to neural factors.

5.1.2 Muscular strength

Strength training of the knee extensors led to a significant increase in both T_C and T_{MVC} of the T_{leg}, whereas there was no significant difference in the C_{leg} (Table 5). The 8 % (+ 23 Nm) increase in the T_{leg} at 60°/s^{-1} however, was not significant. This result was somehow unexpected, since both the increase at 120°/s^{-1} (+12 %, + 27 Nm) and 60° were statistical significant (+ 16 %, + 46 Nm).

When we perform tests that prescribe different modalities of muscular contraction in respect with those applied during training, it is not unusual to obtain trivial changes in strength between pre- to post measurement sessions, even evaluating the trained muscle group (f.i., isokinetic vs. 1RM). For instance, it has been shown that by using isometric test and *MVC*, the result can be as much as 50 % lower than the ones corresponding to the 1RM test after training (Rønnestad, Egeland, Kvamme, & Refsnes, 2007).

12 weeks of strength training on one leg with the other leg as control demonstrated a 15 % increase in T_{MVC} strength of the trained leg, whereas there was no change in the control leg (Jones & Rutherford, 1987). We need however to take into consideration that the intervention in this study was twice as long as ours. A 6-week study, which compared dynamic, eccentric and isometric strength training found ~ 32 % increase in T_C in both the dynamic and eccentric group (Scanlon et al., 2014). However, in this study the entire chain of the extensor muscles of the lower limb was trained with leg press. Then, strength was evaluated by using isotonic contraction modality, and it was performed on older men and woman. A short period of 4 weeks (5 sessions per week) of isokinetic strength training was followed by a ~ 10 % increase of strength of the right leg, and by a ~ 16 % increase in the left leg in a group of athletes (Golik-Peric et al., 2011). The control group performed isotonic training, increased the strength ~ 8 % in both the right and left leg. In both groups, strength was tested during isokinetic, low angular speed contractions (60°/s^{-1}). We can therefore conclude that the gains in isometric and isokinetic strength obtained in our investigation closely matched the ones found in other studies where subject of similar characteristics were studied.

Although there was an increase in strength in the C_{leg}, as assessed during the dynamic test (Table 3), there was no increase during T_C or T_{MVC} (Table 4). Torque values at 120°/s^{-1} were unchanged pre- to post; and the ones at 60°/s^{-1} and 60° slightly decreased.

This can be attributed to either learning effect, or the so-called crossover effect from familiarization to pre-training. However, a meta-analysis by Munn et al. (2004) reported that the crossover effect, which can amount to as much as 8 %, is often not found in isometric and isokinetic exercises. In addition, there may be a marginal learning effect when testing isometrically, as our results may also suggest.

5.1.3 Oxygen uptake and $\dot{V}O_2$ slow component

Studies performed on untrained subjects showed little to non-effect of strength training on $\dot{V}O_{2max}$ (Loveless, Weber, Haseler, & Schneider, 2005; McCarthy, Agre, Graf, Pozniak, & Vailas, 1995). To increase the $\dot{V}O_{2max}$, exercise intensity must be at least maintained at a level corresponding to minimum 50 % of $\dot{V}O_{2max}$ for extended periods of time (Laursen & Jenkins, 2002). Therefore, the metabolic stimulation prevailing during strength training is not sufficient to elicit the cardiovascular modifications that may be beneficial for the increase of $\dot{V}O_{2max}$. In addition, the peripheral muscular adaptions induced by strength training, with a possible and likely shift from type I to type II muscle fibers, and no beneficial changes of the metabolic vascular dilatation in response to exercise, would not contribute to the increase of $\dot{V}O_{2max}$.

After the training intervention, there was a significantly higher $\dot{V}O_{2peak}$ assessed during one-leg kicking post-training for the C_{leg}, both in $mL \cdot kg^{-1} \cdot min^{-1}$ and $L \cdot min^{-1}$, while it decreased in the T_{leg}, albeit not significantly so (Table 8). Although strength training does not elicit beneficial modifications of the maximal aerobic power, we must however acknowledge that it may however improve endurance performances. Strength training, by reducing type II muscle fiber recruitment at a given absolute *WL* may lead to a lower metabolic demand and to improvement in work economy during prolonged, submaximal exercise (Morgan et al., 1995). As a matter of fact, strength training results in an improved $\Delta\ \eta$ (Bastiaans, Diemen, Veneberg, & Jeukendrup, 2001) and work η (Sunde et al., 2010) in cycling.

Fig. 11 illustrated the $\dot{V}O_2$ kinetics during both *MOD* and *HI* exercise. During *MOD* exercise at 50 % of WL_{max}, O_2 uptake increased during the first minutes and attained a steady state after approximately 3 minutes in accordance with the literature (Poole & Jones, 2012) in both the C_{leg} (Fig. 11a) and the T_{leg} (Fig. 11b) pre- and post-training. During one-leg kicking at 80 % of WL_{max}, the O_2 uptake continued to rise after the 3rd

minute both in the C_{leg} (Fig 11c) and the T_{leg} (Fig. 11d). The progressive increase of $\dot{V}O_2$ after ~ 3 minutes from the onset of the exercise is described as the slow component of the $\dot{V}O_2$ kinetics (Jones et al., 2011).

There were no significant differences before and after the training intervention for either the C_{leg} (Fig. 11c) or T_{leg} (Fig. 11d). However, the values are slightly higher in post-training condition from the 2nd minute onward for the C_{leg} until the 6th minute. For the T_{leg}, after the 3rd minute the linear rise was lower at post-training than at pre- until the end of the exercise. There are however huge individual differences, as witnessed by the pretty large SD.

As expected, during *MOD* exercise (50 % of WL_{max}), no slow component was present in either the C_{leg} (Fig. 12a) or the T_{leg} (Fig.12b). Krustrup et al. (2004a) showed, in accordance with our findings, that there was no slow component during *MOD* intensity exercise from the 3rd to the 6th minute. Therefore, we can conclude that the subjects, when performing one-leg kicking at 50 % of WL_{max}, were performing *MOD* exercise, a condition where the O_2 uptake attains a steady state after approximately 3 minutes (Jones et al., 2011).

At 80 % of WL_{max}, defined as *HI* exercise, there are no significant changes in the slow component from pre- to post-training (15 vs. 11 %) for the C_{leg} (Fig. 12c). However, the increase from the 3rd to the 6th minute of exercise was significant only at pre-training. Regarding the T_{leg} (Fig. 12d), the increase in slow component was reduced from 19 % pre-training to 13 % post-training (97 to 70 $mL \cdot min^{-1}$) and the O_2 uptake at the 6th minute was not significantly larger than the one prevailing at the 3rd minute at post-training. Therefore, despite the fact that the slow component in $mL \cdot min^{-1}$ are higher for the T_{leg} than the C_{leg}, we noticed that strength training was followed by the reduction of the amplitude of the slow component.

Several studies have documented a huge range of the amplitude of the slow component of $\dot{V}O_2$ kinetics. Studies on well-trained cyclists have demonstrated an increase of 8 $mL \cdot min^{-1}$ (Lucía et al., 2000) and 33 $mL \cdot min^{-1}$ (Billat, Richard, Binsse, Koralsztein, & Haouzi, 1998), and even a range from 40 to 60 mL min^{-1} has been shown in subjects with varying fitness level (Jacobsen, Coast, & Donnelly, 1998). Our data showed an

increase of 55 mL·min^{-1} pre-, and 43 mL·min^{-1} post-training for the C_{leg}, and 97 mL·min^{-1} pre-, and 70 mL·min^{-1} post-training for the T_{leg}. These average values, however, are characterized by marked interindividual variability. In addition, the reported studies have performed the testing on cycling at 80 % of $\dot{V}O_{2max}$, whereas our study performed one-leg kicking at 80 % of WL_{max}.

5.1.4 EMG recruitment and $\dot{V}O_2$ slow component

Except from the 3rd minute in the *vastus lateralis* (Fig. 13c), there were no changes in *iEMG* activity during 50 % of WL_{max} (Fig.13a-f). The post-training values for the *vastus lateralis* were slightly higher from the 1st to the 6th minute, with a single significant difference at the 3rd minute. Our data would therefore confirm that peripheral muscular fatigue was not occurring in the *MOD* exercise and that the pool of the recruited *MUs* remained likely constant. In accordance with these data, it has been shown that during 10 minutes of knee extension at moderate intensity, *iEMG* maintained a fairly clear steady state (Garland et al., 2006).

At 80 % of WL_{max}, there was a linear increase in *iEMG* from the 1st to the 6th minute in all tests (Fig. 14a-f) and this evidence supports the hypothesis that a progressive recruitment of less efficient fiber types occurs in parallel with the establishment of the slow component. The decrease of *iEMG* activity elicited by training seemed to be different in the different bellies of the *quadriceps*. We did not find any changes pre- to post in the C_{leg} for either *vastus medialis* (Fig. 14a), *rectus femoris* (Fig. 14b) or *vastus lateralis* (Fig. 14c). We also noticed that for the T_{leg} pre- to post, there was no difference in the *vastus medialis* (Fig. 14d), but *iEMG* increased less in both *rectus femoris* (Fig. 14e) and *vastus lateralis* (Fig. 14f). This behavior may be tentatively explained by the different magnitude of their activation due to the load imposed by the mechanic gain of each single muscle (Folland & Williams, 2007).

There was a significant increase in *iEMG* activity from the 3rd to the 6th minute of exercise (Fig. 15a-f), which again indicated a progressive recruitment as the exercise proceeded. There were no differences from pre- to post-training in the C_{leg} (Fig. 15a-c); for the *vastus medialis* in the T_{leg}, the only significant difference was post-training (Fig. 15d). For both *rectus femoris* and *vastus lateralis* in the T_{leg}, the *iEMG* activity was lower after the training intervention. In *rectus femoris* (Fig. 15e) the activity was

significant lower at both the 3rd ant the 6th minute. *Vastus lateralis* was however only significant lower at the 6th minute (Fig. 15f). These findings show that there was an increased activity in the *quadriceps* muscles with the increase in $\dot{V}O_2$ (Fig. 12a-d).

In accordance with our findings, Moritani et al. (1992) found a significant correlation between the increase of $\dot{V}O_2$ and *iEMG*. However, the increase in *iEMG* demonstrated in their study appeared after ~ 240 s, while the $\dot{V}O_{2sc}$ typically appear at ~ 90 – 180 s (Barstow & Molé, 1991). Our results show that both the *iEMG* (Fig. 15a-f) and $\dot{V}O_2$ (Fig. 12a-d) increases significantly from the 3rd to the 6th minute. It is however wort underlying that *iEMG* alone does not give any information about the types of muscle fibers recruited (Kupa et al., 1995). Krustrup et al. (2004b) demonstrated, in accordance with our findings, a progressive recruitment in the *vastus lateralis* as the exercise proceeded during *CWR* at ~ 80 % of $\dot{V}O_{2max}$, leading to exhaustion in approximately 4 minutes. The same authors also found that both type I and type II muscle fibers were recruited at the onset of the knee extension exercise, and that most of the fibers was already activated at the 3rd minute. Other studies demonstrated in agreement with our findings that additional type I and type II muscle fibers were recruited with time during *HI* cycling (~ 80 % of $\dot{V}O_{2max}$) in association with a significant $\dot{V}O_{2sc}$ (Krustrup et al., 2004a) and increased *iEMG* that was evident from the 2nd to the 6th minute (Burnley et al., 2002). Finally, increased *EMG* activity in the *vastus lateralis* during a cycling *CWR* exercise was found in parallel with the occurrence of slow component (Vanhatalo et al., 2011). During a 3-minute all-out test however, the authors demonstrated a greater $\dot{V}O_{2sc}$, but no progressive fiber recruitment. It was suggested that other putative mediators such as slow $\dot{V}O_2$ kinetic, reduced contractile efficiency and metabolic cost of recovery process in already recruited fibers were the main reasons for the slow component.

Yet, other studies are inconsistent with our findings. Based on the absence of experimental evidence of the progressive recruitment during *HI* exercise, someone suggested that the slow component originates from intrinsic factors to the already recruited fibers (Cannon et al., 2007; Garland et al., 2006; Lucía et al., 2000; Scheuermann et al., 2001). However, Garland et al. (2006) investigated a *WL* well below *CP*, whereas Cannon et al. (2007) imposed a *WL* at or above the *CP*. There were also differences in the protocols. While Garland et al. (2006) used a knee extension apparatus that implied the use of the isolated *quadriceps* alone, Cannon et al. (2007),

Lucía et al. (2000) and Scheuermann et al. (2001) performed cycling tests on ergometer. As stated earlier, during whole-body movements, like f.i. cycling, the contribution of other muscle groups is higher than during dynamic knee extension.

The slope of the decay of *MPF* during *CWR* exercise describes the fatigue rate of the muscle: It is negative as median or mean frequency decrease during repeated contractions and if the muscle shows no signs of peripheral muscular fatigue, the slope has a value close or not significantly different from zero (Ng et al., 1996). The shift of *MPF*, moreover, suggests the progressive recruitment of type II *MUs*. Results showed that there was no change in the slope from pre- to post-training for the C_{leg} in either the *vastus medialis* (Fig. 16a, d), *rectus femoris* (Fig. 16b, e) or *vastus lateralis* (Fig. 16c, f). This indicates that although the C_{leg} had increased in dynamic strength measured as 1RM (Table 3), it has not become less prone to develop fatigue. This is also consistent with no change in $\dot{V}O_2$ data (Fig. 11a, b; Fig. 12a, b) an *iEMG* (Fig. 14a, b, c; Fig. 15a, b, c).

Regarding the T_{leg}, the slope of the decay was significantly lower after the training intervention in both *vastus medialis* (Fig. 17a, d) and *vastus lateralis* (Fig. 17c, f). A decrease in the *MPF* is as mentioned earlier a sign of peripheral muscular fatigue (Ng et al., 1996), and our results indicates that the training have increased the muscles capability to resist fatigue, likely because of a diminished recruitment of the highly fatigable type II *MUs*. The only slightly atypical results are the ones concerning the *rectus femoris* (Fig. 17b, e), where the slope after the training intervention is lower, but not significantly so. However, regarding the activity in the *quadriceps* muscles, it has been demonstrated by using *MRI* during isometric knee extension to fatigue that *rectus femoris* is the most active, and *vastus lateralis* the least active of the *quadriceps* muscles. The main reason is thought to be that *rectus femoris* is the only biarticular *quadriceps* muscle (i.e., both a hip flexor and a knee extensor) (Krustrup et al., 2004b). Also, as a result of varying anatomical, biomechanical and morphological characteristics the *quadriceps* muscle component may generate different contribution to knee extensor torque. Lower *EMG* values are observed for *vastus medialis* compared to *rectus femoris*, and the greater proportion of type I muscle fibers in the *vastus medialis* may account for a greater degree of fatigue resistance than both the *rectus femoris* and *vastus lateralis* (Pincivero, Gandhi, Timmons, & Coelho, 2006).

In general, the *MPF* and *EMG* data from the present study would confirm the efficacy of the training and suggests that a smaller number of *MUs* were recruited at 80 % of WL_{max} after the training of the extensors. However, our data must be interpreted with a pinch of salt. The hypothesis that we aimed to challenge was the "extensive" mechanism of the slow component in contrast with the alternative "intensive" mechanism. According to the former, the progressive appearance of the slow component would be the direct consequence of a progressive addition of type II *MUs* to the pool of recruited *MUs* so that the given and fixed *WL* can be maintained in presence of peripheral muscular fatigue. According to the second mechanism, we would not need to admit the extension of the pool of *MUs* for justifying the presence of the slow component if we hypothesize that fatigue may lead to the decrease of muscle efficiency of the already recruited *MUs* for some not identified mechanism(s).

In this regard, the results presented in the current literature are somehow controversial. Scheuermann et al. (2001) found no change in *MU* recruitment when comparing the $\dot{V}O_2$ response with *iEMG* and *MPF* in the *vastus lateralis* during both *MOD* and *HI* cycling. Our study found that *vastus lateralis* had the most significant change in slope of the decay of the *quadriceps* muscles, but it is important to notice that Scheuermann and colleagues investigated cycling subjects, and we know that cycling activates the *vastus lateralis* in a different manner compared with knee extension (da Silva et al., 2016).

The increased O_2 cost (i.e. $\dot{V}O_{2sc}$) represents a progressive loss of muscle efficiency. Vanhatalo et al. (2011) related this to a higher *ATP* cost of force production, rather than to a greater O_2 cost of oxidative *ATP* production. Scheuermann et al. (2001) associated the increased O_2 cost with a progressive *ATP* requirement of already recruited *MUs*, rather than change in the recruitment pattern. Furthermore, the unchanged *MPF* demonstrated in their study suggested that any *MUs* that dropped out due to fatigue was replaced by *MUs* with similar *EMG* characteristics. However, *HI* exercise with a significant $\dot{V}O_{2sc}$ showed a significant decrease in glycogen content in both type I and type IIa fibers, but only in type I fibers during *MOD* exercise (Krustrup et al., 2004a). Further, they also found a significant decrease in [*PCr*] content in both type I and type II fibers after 3 and 6 minute of exercise, with no further change from 6 – 20 minutes.

Good metabolic stability during exercise is associated with a smaller decrease in [*PCr*] and smaller increase in [P_i], [ADP_{free}], [AMP_{free}] and [IMP_{free}] (Zoladz, Korzeniewski, & Grassi, 2006) which is directly linked to muscle fatigue (Fitts, 2008). This due to the fact that endurance trained skeletal muscle undergoes smaller [*ADP*] and [P_i] increases and [*PCr*] decrease at any given work-rate compared with untrained skeletal muscles (Grassi et al., 2011). During *HI* exercise, disturbance in metabolic stability may be responsible for the decrease in both muscle efficiency and also the slow component of $\dot{V}O_2$ kinetics as well (Zoladz & Korzeniewski, 2001) as the slow component of O_2 uptake has been phenomenologically linked to the progressive drop of [*PCr*] and to a more marked perturbation of the muscular milieu (Poole & Jones, 2012).

Unchanged *EMG* and *MPF* data may however be a result of fatigue of type II muscle fibers, which in turn would be replaced by more type I muscle fibers. Type I muscle fibers are less efficient at high power output (i.e., generate less force) and the net result would therefore be unchanged *EMG* signals (Garland et al., 2006; He et al., 2000). Muscular contraction may also lead to loss of [K^+] and may blunt the propagation of action potentials across the cell membrane, which would result in reduced *MPF* (Gamet, Duchene, Garapon-Bar, & Goubel, 1993).

5.1.5 Muscular NIRS

Changes in deoxy-hemoglobin ([*HHb*]) from the 3rd to the 6th minute was measured on the *vastus lateralis* both pre- and post-training. Our results found no difference at either 50 % or 80 % of WL_{max} for either the C_{leg} (Fig. 18a, c) or the T_{leg} (Fig. 18b, d). This finding indicates that there was no change in relative peripheral O_2 extraction during $\dot{V}O_{2sc}$. Likewise, Tew, Ruddock, and Saxton (2010) did not find any increase in muscle O_2 saturation in the *vastus lateralis* during dynamic knee extension exercise. However, the intensity and duration of their exercise was much lower – shorter than the ones applied in the present study (i.e. 4 x 4 minutes with ~ 20 % of *MVC*). One study observed an increase in [*HHb*] after strength training intervention during cycling at ~ 80 % of $\dot{V}O_{2max}$ (Turner et al., 2013), a finding that suggest a better O_2 muscular extraction.

The unchanged [*HHb*] values found in our study may however suggest that at least in young, active subjects, the decreased availability of O_2 may not be one of the major determinants of $\dot{V}O_{2sc}$. An unchanged [*HHb*], and index of relative peripheral O_2

extraction, would strongly suggest that the ratio between local O_2 delivery and extraction was hardly affected after training in presence, however, of a modified amplitude of the $\dot{V}O_{2sc}$ in the trained leg.

The changes in oxygenation detected by *NIRS* mainly reflects capillary ([*HHb*] related) and intracellular (myoglobin related) changes in O_2 levels (Grassi & Quaresima, 2016). Furthermore, the muscle area investigated with the *NIRS* probe may not represent a reliable picture of the overall relative O_2 extraction in the muscle, as it has been shown a high inhomogeneous distribution of this parameter.

5.2 Methodological considerations

5.2.1 $\dot{V}O_2$ measurements

Most of the studies who have looked into the $\dot{V}O_{2sc}$ have used breath-by-breath measurement. It is also worth mentioning the muscle to time delay for $\dot{V}O_2$ which is ~ 15 s (Krustrup et al., 2004a). Based on calculations / methods of measuring O_2 uptake, this time delay might have an impact on the different results. In addition, we also used Oxycon Pro Mixing Chamber to measure $\dot{V}O_2$, which on average measure 0.8 % lower $\dot{V}O_2$ values than the Douglas Bag method (Foss & Hallen, 2005).

5.2.2 EMG

One of the most important things regarding *EMG* measurements is to normalize the data obtained. As described in the Method chapter, we normalized the *iEMG* signal according to Ringelberg (1985), where the peak value ($iEMG_{max}$) was set equal to 100 %, and the integrated *sEMG* was expressed relative to the value. However, other studies often normalize *EMG* according to *MVC*, as this is a commonly used amplitude analysis technique. By normalizing to peak value, we reduced the variability between individuals, like strength differences (Halaki & Ginn, 2012). Noise, changes in distance between signal origin and detection site and cross-talk (i.e. *EMG* from neighboring muscles) are factors who could influence the *EMG* signal, especially during dynamic movements (De Luca, 1997; Konrad, 2005). This is important to notice when comparing our results with other studies performing f.i. cycling.

5.2.3 NIRS

The calibration procedure we selected for standardizing the [*HHb*] signal obtained with the *NIRS* probe may have affected the results. The *NIRS* technology applied in this investigation does not provide absolute values of [*HHb*]. Instead, it measures relative values expressed in arbitrary units that, therefore, need an internal reference. In the majority of the studies, the measured relative values are expressed as percentage of the [*HHb*] measured after 4 – 5 minutes of arterial occlusion of the explored vascular bed. The [*HHb*] measured at this plateau is then taken as the reference value equal to 100 %. It must be however underlined, that the present calibration procedure was adopted in other investigations (Zandonai et al., 2016).

5.3 Perspectives

As far as we know, the present study is one of the first that have investigated the effect of strength training on the slow component of $\dot{V}O_{2sc}$. Since we did not perform muscle biopsies in our study, it is difficult to conclude which adaptions the strength training has led to (f.i. increased capillary density, shift of fiber types).

The results presented and discussed in this thesis suggest further experiments that should aim to clarify the physiological mechanisms underpinning the slow component, namely to understand whether the progressive: i) decrease of mechanical efficiency and / or; ii) increase of *ATP* cost of force production of the recruited *MUs* are the main phenomena underpinning $\dot{V}O_{2sc}$.

5.4 Practical application

The slow component of $\dot{V}O_2$ kinetics is important from a performance standpoint, since it is closely related to the development of fatigue during exercise at high intensities. Also, since $\dot{V}O_{2sc}$ is considered to be caused by a progressive recruitment of muscle fibers as exercise proceeds, it is of great interest since it can enhance our understanding about muscle energetics, metabolic control and the determinants of the efficiency of skeletal muscle contractions.

The present study strengthens the theory that $\dot{V}O_{2sc}$ is related to a progressive recruitment of less efficient muscle fibers. In addition, it brings further evidence to the table that strength training may decrease the slow component, which is of great interest

for both athletes and subjects who have different restrictions related to low or limited O_2 transport capacity. Further studies are warranted to gather more and stronger evidence about the topic.

6. Conclusion

This paper presents the effect of 6 weeks strength training on the slow component of $\dot{V}O_2$ kinetics in young, male adults. Our results showed that one-leg strength training of the extensors increased muscle strength and decreased the amplitude of the $\dot{V}O_{2sc}$. In addition, a smaller recruitment of *MUs* were demonstrated after the training intervention at high intensity exercise at 80 % of WL_{max}.

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Nomenclature

$[ADP_{free}]$	Adenosine diphosphate concentration
$[AMP_{free}]$	Adenosine monophosphate concentration
ATP	Adenosine triphosphate
$a\text{-}vO_2$	Arterio venous difference of O_2 content
BPM	Beats per minute
Ca^{2+}	Calcium
C_aO_2	Arterial blood concentrations of O_2
C_{leg}	Control leg
CO_2	Carbon dioxide
CP	Critical power
Cr	Creatine
CSA	Cross-sectional area
C_vO_2	Mixed venous blood concentrations of O_2
CWR	Constant-work-rate exercise
ΔG	Free energy
DXA	Dual-energy X-ray absorptiometry
EMG	Electromyography

GET	Gas exchange threshold
H^+	Hydrogen ion
$[H^+]$	Hydrogen ion concentration
$[HbO_2]$	Oxy-hemoglobin
HGF	Hepatocyte growth factors
[*HHb*]	Deoxyhemoglobin
HI	Heavy intensity exercise
HR	Heart rate
iEMG	Integrated electromyography
$iEMG_{max}$	Maximal value of integrated electromyography
$[IMP_{free}]$	Inosine monophosphate concentration
La^-	Lactate
$[La^-]_b$	Capillary blood lactate concentration
LT	Lactate threshold
MOD	Moderate intensity exercise
MPF	Mean power frequency
M_{qf}	Muscle mass of the quadriceps
MRI	Magnetic resonance imaging

MU	Motor unit
MVC	Maximal isometric voluntary contraction
NIRS	Near-infrared spectroscopy
O_2	Oxygen
PCr	Phosphocreatine
[*PCr*]	Phosphocreatine concentration
PCSA	Physiological cross-sectional area
P_i	Inorganic phosphate
[P_i]	Inorganic phosphate concentration
P-O ratio	ATP – oxygen ratio
$\dot{Q}$	Cardiac output
Q_{10}	Temperature coefficient
RER	Respiratory exchange ratio
RFD	Rate of force development
RPM	Revolutions per minute
sEMG	Surface electromyography
SR	Sarcoplasmic reticulum
T_C	Maximal isokinetic concentric torque

T_{leg}	Training leg
T_{MVC}	Maximal isometric torque
$\dot{V}CO_2$	Carbon dioxide output
$\dot{V}_E$	Ventilation
VH	Very heavy domain
$\dot{V}O_2$	Pulmonary oxygen uptake
$\dot{V}O_{2max}$	Maximal oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake
$\dot{V}O_{2sc}$	Slow component of $\dot{V}O_2$
$\dot{V}O_{2ss}$	Steady state of oxygen uptake
$\dot{V}O_m$	Muscle oxygen consumption
W	Watt
WL	Workload
WL_{max}	Maximal workload
η	Efficiency
τ	Time constant
1-KE	One leg knee extension
1RM	One repetition maximum

Appendix

Appendix I

Effekten av styrketrening på «slow component» av V'O_2 kinetikk

Bakgrunn og hensikt

Dette er en forespørsel til deg om å delta i en forskningsstudie som skal se på effekten av 6-ukers styrketrening på «slow component» av V'O_2 kinetikk.

Slow component av V'O_2 kinetikk defineres som økningen i V'O_2 etter det tredje minuttet av et arbeid, og forekommer kun ved arbeid over anaerob terskel. Slow component kan utgjøre opptil 1000 – 1500 mL O_2/min, og utfordrer vår forståelse av muskulær energiomsetning, og de grunnleggende prinsippene for treningsfysiologi. Dette er av stor interesse, da slow component er nært relatert til utviklingen av trøtthet under arbeid over anaerob terskel. Styrketrening som fører til en nedgang i antall rekrutterte motoriske enheter på samme absolutte arbeidsbelastning, kan teoretisk minke slow component, siden et mindre antall type II-fibre vil rekrutteres på samme belastning. Dette er av interesse for å kunne utvikle bedre treningsintervensjoner for å minske slow component, både for toppidrettsutøvere, men også for pasienter / eldre som har dagligdagse restriksjoner i form av begrenset O_2 kapasitet.

Hva innebærer studien?

Som forsøksperson må du møte på Norges idrettshøgskole for å gjennomføre tester og trening. Her vil det bli randomisert hvilket bein som vil være treningsbein, mens motsatt bein vil fungere som kontrollbein. Det vil være 3 økter tilvenning i sparkeergometer (1-KE) på ca. 10 min per bein, for å bli kjent med hvordan testen utføres. I tillegg vil forsøkspersonen fylle ut et spørreskjema for medisinsk screening. Deretter vil det gjennomføres 3 pre-tester. Treningsintervensjonen varer i 6 uker, og innebærer at du som forsøksperson møter 2 ganger per uke de 2 første ukene, og 3 ganger de 4 siste ukene. Under treningsperioden vil du kun trene styrketrening på ett-beins kneekstensjon. Etter treningsperioden vil det gjennomføres en post-test, som krever 1 oppmøte.

<u>Tilvenning:</u>

3 økter i sparkeergometer (1-KE) – 10 min per bein

Styrketest – isometrisk og isokinetisk kneekstensjon

<u>Pre-testene:</u>

Dag 1: Antropometriske målinger, og VO_{2maks} test på ergometersykkel

Dag 2: Styrketest – Isometrisk og isokinetisk kneekstensjon, og VO_{2maks} test i kneekstensjon (1-KE)

Dag 3: 1-KE på 50 % og 80 % av maksimal watt (fra dag 2).

Treningsperiode:

Uke 1 – 2: 2 oppmøter per uke

Uke 3 – 6: 3 oppmøter per uke

Hvert oppmøte vil vare mellom 15 – 30 min, og innebære oppvarming, og ett-beins kneekstensjon på treningsbeinet.

Post-testen:

Dag 1: Styrketest – Isometrisk og isokinetisk kneekstensjon, og 1-KE på 50 % og 80 % av maksimal watt (fra pre-test, dag 2).

Mulige ulemper og risiko

Deltakelse i prosjektet vil kreve litt tid og oppmerksomhet i form av de obligatoriske oppmøtene. Under VO_{2maks} testene vil man kunne oppleve ubehag i form av at man må presse seg til tilnærmet utmattelse, men dette innebærer ingen helserisiko for friske personer. Ettersom styrketreningen foregår i et kneekstensjonsapparat, vil det kunne medføre blant annet muskelstølhet etter testing / trening. Det kan også innebære en liten, men like fullt en risiko for skader i kneet.

Hva skjer med prøvene og informasjonen om deg?

Informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Du har rett til innsyn i hvilke opplysninger som er registrert om deg og rett til å få korrigert eventuelle feil i de opplysningene som er registrert.

Alle opplysningene vil bli behandlet konfidensielt og uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger gjennom en navneliste. Dette betyr at denne informasjonen er avidentifisert. Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Blodprøvene som tas av deg blir destruert umiddelbart etter analyse.

Prosjektleder har ansvar for den daglige driften av forskningsprosjektet og at opplysninger om deg blir behandlet på en sikker måte. Avidentifiserte opplysninger lagres på en passordbeskyttet datamaskin med kryptert harddisk. Koblingsnøkkelen oppbevares innlåst og adskilt fra resterende opplysninger. Informasjon om deg vil bli oppbevart i 5 år etter prosjektslutt for etterprøvbarhet og kontroll, og slettes/ destrueres deretter.

Frivillig deltakelse

Hvis du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Du kan når som helst, uten å oppgi grunn, trekke ditt samtykke til å delta i studien. Hvis du ønsker å trekke deg, eller har spørsmål til studien, vennligst ta kontakt med Thomas Lerhol Warvik (tlf. 93884159 / epost: thomasle@student.nih.no)

Samtykke til deltakelse i studien

Jeg er innforstått med hensikten med studien, mine rettigheter som forsøksperson, og er herved villig til å delta i studien:

(signer av forsøksperson, sted/dato)

Jeg bekrefter å ha gitt informasjon om studien:

(signert av testleder, sted/dato)

Appendix II

Egenerklæring for forsøkspersoner

Etternavn:	Fornavn:
Fødselsdato:	
E-post:	
Tlf.:	
FP nr.	
Idrettsbakgrunn (angi omtrent hvor mange timer du trener per uke):	

Takk for at du vurderer å delta som forsøksperson ved Norges idrettshøgskole! Før du kan delta, må vi imidlertid kartlegge om din deltakelse kan medføre noen form for helserisiko. Vær snill å lese gjennom alle spørsmålene nøye og svar ærlig ved å krysse av for JA eller NEI. Hvis du er i tvil, bør du be om å få snakke med legen som er ansvarlig for forsøket.

Hvis du krysser av for JA på ett eller flere av disse spørsmålene, må du gjennomgå en legeundersøkelse før forsøksstart.

Spørsmål	JA	NEI
1. Kjenner du til at du har en hjertesjukdom?		
2. Hender det du får brystsmerter i hvile eller i forbindelse med fysisk aktivitet?		
3. Kjenner du til at du har høyt blodtrykk?		
4. Bruker du for tiden medisiner for høyt blodtrykk eller hjertesjukdom? (f.eks. vanndrivende midler)?		
5. Har noen av dine foreldre, søsken eller barn fått hjerteinfarkt eller dødd plutselig (før fylte 55 år for menn og 65 år for kvinner)?		
6. Røyker du?		
7. Har du besvimt i løpet av de siste seks månedene?		
8. Hender det du mister balansen på grunn av svimmelhet?		
9. Har du sukkersjuke (diabetes)?		
10. Får du allergiske eller hypersensitive reaksjoner av bedøvelse?		
11. Kjenner du til noen annen grunn til at din deltakelse i prosjektet kan medføre helse- eller skaderisiko?		

Gi beskjed straks dersom din helsesituasjon forandrer seg fra nå og til undersøkelsen er ferdig, f.eks. ved at du blir forkjølet eller får feber.

_____

Sted – dato

_____

Underskrift