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The significance of the skeletal muscular oxidative capacity during whole body exercise

DISSERTATION FROM THE NORWEGIAN SCHOOL OF SPORT SCIENCES • 2011

ISBN nr 978-82-502-0472-0

Acknowledgements

The present work was accomplished at the Norwegian School of Sport Sciences, Department of Physical Performance, at Glittreklinikken and at Copenhagen Muscle Research Centre, Department of Anaesthesia, Rigshospitalet, University of Copenhagen, Denmark.

Special thanks to my supervising professor Jostein Hallén, for being my inspiring mentor and friend thorough the challenging and time consuming pathway.

I'm grateful for the tremendous work all the participating subjects have done, without you; nothing.

Thanks to all the personal staff at Glittreklinikken for making me feel so welcome when working there. Special thanks to my co-authors and co-workers in study I: Carl Christian Christensen, Anne Edvardsen, Turid Hagelund, Morten Skrede Ryg, Siri Skumlien and Marika Vargtun.

Thanks to my co-authors in study III: Øivind Foss, Peter Krustrup and Niels H. Secher. Also thanks to Peter and Niels for allowing us to borrow your equipped lab in Copenhagen during the invasive experiment and especially to Niels: thanks for taking your time to get to Norway during the invasive experiments in study IV. To Øivind: thanks for all the cooperation and fun times during study III and the Master and PhD period in general.

Thanks to the co-authors in study IV; Johnny Nilsson, Niels H. Secher and Gerald Smith.

Special thanks to the master students for cooperation. This includes Paul Johan Myklebust (study II), Åsmund Ekroll (III) and Lars-Erik Strand (IV). You all did a tremendous work in regard to subject recruitment, testing, the training period in study III and in analysis of data.

Thanks to Erlend Hem, Vidar Jakobsen, Svein Leirstein, Mone Eriksen Nastad, Johnny Nilsson, Tom Ringstad, Lars-Erik Strand and Ingrid Ugelstad for assistance and engineering work.

Thanks to Erik Bjørnstad for inspiring lessons in physiology, Truls Raastad for inspiring cooperation and discussions during my Master and to Leif Knivestøen who introduced me to scientific thinking and work during the Gyrodactylus salaris experiments on salmon, and who gave me the advice to follow my heart when choosing higher education.

My final thanks go to my family: Hilde, Sindre and Vegard who makes me grateful each day.

Table of Contents

1 Summary

The present thesis consists of four studies with the main objective to examine the significance of the skeletal muscular metabolic capacity during whole body exercise. Exercise capacity was studied in chronic obstructive lung disease (COPD) patients and a control group in study I, and in endurance-trained subjects and normal physical active subjects in study II. In both studies, muscular metabolic capacity was quantified during exercise with a small muscle mass (1.7-2.8 kg) to avoid the issue of muscular oxygen uptake being limited by oxygen supply. Thereafter, we tested how much the muscular metabolic capacity could be exploited during whole body exercise (bicycling). Exploitation less than 100% indicated a muscular metabolic reserve probably due to oxygen supply limitations. The muscular metabolic capacity ranged from 264 to 570 ml·kg^{-1}·min^{-1} for the four groups. However, there was less variation in the relative exploitation of the muscular metabolic capacity during whole body exercise for the healthy groups (31%-37%), while this percentage was smaller (17%) for the COPD patients. Based on these results, we concluded that the exploitation of the muscular metabolic capacity was relatively equal for the healthy groups despite large differences in age and maximal oxygen uptake (VO_2max). The COPD patients were least capable of exploiting the muscular metabolic capacity during bicycling and consequently, they had a relatively higher muscular metabolic reserve during whole body exercise compared with healthy subjects.

In study III, we added one-legged endurance training four times per week for 7 weeks to the regular training of the endurance-trained subjects. By using endurance-trained subjects we also avoided central adaptations which could have increased the cardiac output and hence the oxygen supply capacity. One-legged training resulted in higher activity of the oxidative enzymes, higher oxygen uptake and superior performance during maximal one-legged cycling with the trained leg, when compared with the control leg. During high intensity bicycling with the two legs exercising with exactly the same power output, we observed a 3.5% higher leg oxygen extraction and a 16% higher leg blood flow ($p=0.06$) for the trained compared with the control leg, resulting in 21% higher oxygen uptake in the trained leg. The fact that blood flow and oxygen extraction were higher in the trained leg supports the literature with respect to that hemoglobin deoxygenation plays a significant role for blood flow distribution. The absence of an increase in bicycling VO_2max supports the notion that oxygen supply limits VO_2max during whole body exercise. We concluded that oxygen extraction is important for muscular oxygen uptake, but not necessarily for whole body VO_2max.

In study IV we examined oxygen extraction, blood flow, oxygen uptake and the lactate balance during combined arm and leg exercise at low and moderate workload. The exercise mode used was double poling, which was performed by cross country skiers on a custom built double poling ergometer. During the low workload, blood flow and oxygen uptake were relatively equal between arms and legs, while oxygen extraction was 8% lower in the arms. When workload increased, oxygen uptake increased 40% more in legs than in the arms and oxygen extraction increased only in the legs (8%). Only the arms had a net release of lactate that increased from low to moderate workload. This indicated that the arm muscles were working close to their maximal oxidative

capacity. The blood flow, and hence the oxygen delivery, was relatively high in the arms indicating that the arms are limited by their muscular oxidative capacity, even during whole body exercise.

2 List of papers

I Rud B, Christensen CC, Ryg M, Edvardsen A, Skumlien S, & Hallen J (2008). Higher skeletal muscular metabolic reserve capacity in COPD patients than healthy subjects. *Scand J Med Sci Sports*.

II Rud B & Hallen J (2009). Is the balance between skeletal muscular metabolic capacity and oxygen supply capacity the same in endurance trained and untrained subjects? *Eur J Appl Physiol* **105**, 679-685.

III B. Rud, Ø. Foss, P. Krustrup, N.H. Secher and J. Hallén. One-legged endurance training: leg blood flow and oxygen extraction during cycling exercise (Paper in revision).

IV B. Rud, N. H. Secher, J. Nilsson, G. Smith, and J. Hallén. Metabolic balance between the arms and the legs during simulated skiing (Paper in preparation).

3 Abbreviations and acronyms

O_2	Oxygen
VO_2	Oxygen uptake
VO_2max	Maximal oxygen uptake
VE	Ventilation
Mass-specific VO_2	VO_2 per kg active muscle mass
a-v O_2 diff	Arterial blood - venous blood oxygen difference
SaO_2	The saturation level of oxygen in haemoglobin
SpO_2	The saturation level of oxygen in haemoglobin
PO_2	Oxygen partial pressure
HbO_2	Oxyhaemoglobin
La^-	Blood lactate concentration
HR	Heart rate
W	Watt
Yr	Years
$L \cdot min^{-1}$	Liters per min
$ml \cdot kg^{-1} \cdot min^{-1}$	Milliliters per kg per min
$mmol \cdot L^{-1}$	Millimoles per liter
SD	Standard deviation
SE	Standard error
COPD	Chronic obstructive lung disease

4 Definitions

Muscular metabolic capacity: Muscular metabolic capacity is in this thesis used synonymous with muscular aerobic metabolic capacity.

Muscular mass specific metabolic capacity: Muscular mass specific capacity means the oxygen uptake per kg estimated active muscle mass. For pulmonary measurements oxygen uptake at rest is subtracted from the total oxygen uptake, before dividing VO_2 on the active muscle mass.

5 Introduction

In 1924 Hill et al. described the relationship between running speed and oxygen intake, ventilation and the respiratory quotient. As running speed increased from 10.3 km/h, oxygen intake increased in a proportional manner up to a maximum of 4 $L \cdot min^{-1}$ at a speed of 16 km/h (Fig. 1). Regarding higher speeds, Hill wrote; "The oxygen intake failed to exceed this value…" and "At the higher speeds the requirement of the body for oxygen is far higher, but cannot be satisfied, and the oxygen dept continuously increases" (Hill *et al.*, 1924;Hill & Lupton, 1923). Later, the maximal oxygen uptake (VO_2max) has been defined as "A measure of the maximal energy output by aerobic processes" and is quantified as the body's volume of oxygen consumed per minute (Åstrand *et al.*, 2003). VO_2max differs significantly among mammal species and may range from 25 to >250 $ml \cdot kg^{-1} \cdot min^{-1}$ (Fig. 2), with untrained humans at the lower end of the range. However, there are huge inter-individual variations among humans due to genetic variation, gender, age and training status (Åstrand, 1960;Saltin & Astrand, 1967). The highest VO_2max of 80-85 $ml \cdot kg^{-1} \cdot min^{-1}$ is reported in highly endurance-trained athletes like cross country skiers and long distance runners (Saltin & Astrand, 1967), and a VO_2max higher than 90 $ml \cdot kg^{-1} \cdot min^{-1}$ is occasionally measured in our lab in highly trained cross country skiers (unpublished data), twice that normally found in untrained healthy subjects. At the lower end we may find severely sedentary subjects with a VO_2max less than 15 $ml \cdot kg^{-1} \cdot min^{-1}$ (Richardson *et al.*, 2004). For those subjects, even moderate walk can be strenuous. The close relationship

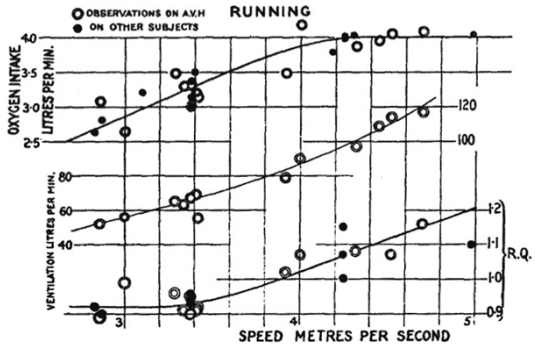

Figure 1. Hill's relation between speed of running, oxygen intake, lung ventilation and respirator quotient. From (Hill et al., 1924).

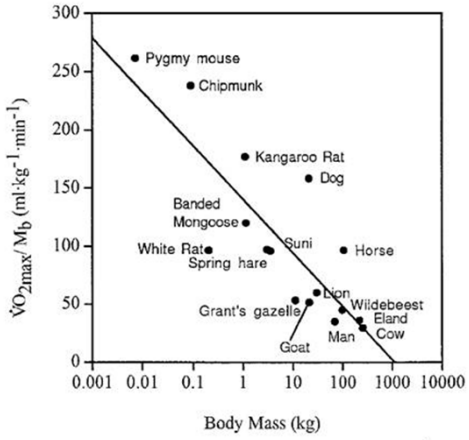

Figure 2. Maximal oxygen uptake of various species. From Bassett, Jr & Howley, (2000).

between high VO_2max and success in endurance sports (Saltin & Astrand, 1967) has led to t he assumption that VO_2max is the main predictor of endurance performance. Although such a statement has some limitations, it is easy to understand why limitations for VO_2max receive so much attention in exercise physiology literature. For example, the paper "Maximal perfusion of skeletal muscle in man" by Andersen and Saltin (1985), termed a "milestone in human physiology" by Greenhaff (2003), is currently the 8th-most cited paper published in the *Journal of Physiology*. In this study the authors reported much higher values of muscular blood flow and VO_2 capacity than previously measured. This was the first study to prove that during whole body exercise VO_2max is not limited by the aerobic muscular capacity, but rather by the heart's pumping capacity. This implies the existence of a muscular metabolic reserve during whole body exercise. This finding supported Ekblom et al. (1972a), who demonstrated that manipulation of red blood cells had a large impact on VO_2max, which by itself was evidence of oxygen supply dependency at the muscular level. In the wake of Andersen & Saltin (1985) many papers have been published on the topic, and a few years ago the issue was enthusiastically discussed in several letters in a "Point Counterpoint" debate in the Journal of Applied Physiology (Saltin & Calbet, 2006).

Despite the debate regarding central and peripheral limitations, it is generally agreed that the heart's pumping capacity is a significant limiting factor for VO_2max. However, Roca et al. (1989) found indications of an O_2 diffusion limitation of VO_2max at the capillary - muscle level in endurance-trained subjects (VO_2max 61.5 $ml \cdot kg^{-1} \cdot min^{-1}$) and later they reported that the diffusion capacity increased more (34%) than blood flow (19%) after 9 weeks of endurance training (Roca *et al.*, 1992). These studies were in line with Saltin et al. (1976), where a 16% higher VO_2 was achieved when exercising with the trained compared to the untrained leg, indicating increased muscular metabolic capacity after training. This was supported by findings of an increase in the oxidative enzyme succinate dehydrogenase only in the trained leg. Later, Wagner (2000a) hypothesized that training results in a switch from muscular metabolic limitation to O_2 supply limitation of VO_2max. This hypothesis supported the training studies by both Saltin et al. (1976) and Roca et al. (1992). Furthermore, the hypothesis was not necessarily in conflict with the concept of a muscular metabolic reserve during whole body exercise, because, with one exception, Andersen & Saltin (1985) used trained subjects (VO_2max; >60 $ml \cdot kg^{-1} \cdot min^{-1}$). Those subjects may have passed the switch point, meaning that -training had rendered their muscles oxygen-supply limited before the experiment started. A greater challenge for the Wagner hypothesis is that later studies indicate a muscular metabolic reserve during whole body exercise also in severely sedentary subjects (VO_2max: 15 $ml \cdot kg^{-1} \cdot min^{-1}$) (Richardson *et al.*, 2004), suggesting that the muscles are oxygen-supply limited regardless of training status. Even with subjects suffering from chronic heart failure and chronic lung disease, the muscles seem to

be oxygen-supply limited during whole body exercise, despite the severely untrained status associated with these patient groups (Magnusson *et al.*, 1997;Richardson *et al.*, 2004;Slettalokken *et al.*, 2010).

It seems obvious that the skeletal muscles have potential for significant adaptations to endurance training, but paradoxically, the muscles are oxygen-supply limited both in sedentary and trained subjects. Therefore, the meaning of the term "metabolic reserve capacity" may be confusing when higher a VO_2max in a trained leg can be caused by an increased metabolic capacity (Saltin *et al.*, 1976), or when diffusing capacity increases more than blood flow after training (Roca *et al.*, 1992). The question becomes: why does significant improvement of the muscular metabolic capacity occur if the metabolic capacity is in excess from the beginning? One could argue that one-legged training studies favor metabolic adaptations over systemic, but muscular metabolic adaptations occur also after whole body training (Roca *et al.*, 1992). A possible explanation to this paradox could be that the significance of the size of the muscular metabolic capacity is not completely understood, and therefore the metabolic capacity should not be interpreted as a reserve, or to be in excess, even during whole body exercise (Gollnick & Saltin, 1982). The size of the metabolic capacity could rather be built and maintained for essential purposes, as well as for all links in the O_2 pathway as described and discussed by Hoppeler & Weibel (2000), Weibel (1991), Weibel (1981) and di Prampero (1985) in their "symmorphosis" hypothesis. The main aim of the present work was therefore to study the significance of the skeletal muscular metabolic capacity for VO_2max. The next section will focus on the steps in the O_2 pathway from the lungs to the mitochondria in order to enlighten the theoretical background for the topic.

6 The oxygen pathways and potential VO_2 limitations

6.1 Central and peripheral limitations

As indicated in the introduction, it is appropriate to distinguish between central and peripheral factors in the O_2 pathway. The lungs' diffusion capacity, the heart's maximal cardiac output and the oxygen carrying capacity of the blood constitute the central factors, while the skeletal muscle characteristics including diffusion capacity, mitochondrial enzyme levels and capillary density constitute the peripheral factors (Bassett, Jr. & Howley, 2000). The central factors can thus be defined as the oxygen supply and the peripheral as the metabolic capacity. According to the Fick Principle VO_2 can be expressed as:

$VO_2 = Q \times (CaO_2 - CvO_2)$

where Q is the cardiac output and CaO_2 and CvO_2 are the arterial and venous content of O_2, respectively. Q is mainly determined by the heart's pumping capacity. However, this capacity is also dependent on the venous return which again is influenced by blood volume and the muscle pump. CaO_2 is determined by the haemoglobin oxygenation capacity of the lungs and the O_2 carrying capacity of the blood (Bassett, Jr. & Howley, 2000). The CvO_2 is determined by the muscular O_2 sink capacity, which itself is dependent of the capillarity (Roca *et al.*, 1989) and the mitochondrial oxidative capacity (McAllister & Terjung, 1990).

6.2 Lungs

The first potential barrier to O_2 being an electron acceptor to form water in the mitochondria is localized in the lungs. Hills hypothesized that no significant drop in blood O_2 saturation occurred (>25%), even during strenuous exercise, based on the fact that none of his subjects had shown signs of cyanosis. However, he was aware of the challenge a high cardiac output would put on O_2 saturation of the blood (Hill & Lupton, 1923;Hill *et al.*, 1924). Still, this is very much the picture today, although an O_2 arterial saturation of 75% would be considered significantly low by today's physiologists. Saltin (1986) concluded that for untrained and most endurance-trained individuals the maximal capacity of the lungs is far from taxed during exhaustive exercise eliciting maximal oxygen uptake. However, Saltin (1986) also referred to Dempsey et al. (1984) where some endurance-trained subjects had shown desaturation even at sea level. Later, Dempsey & Wagner (1999) wrote in a review that exercise-induced arterial hypoxemia (EIAH) at or near sea level occurs in a significant number of healthy subjects, and referred to Powers et al. (1988) who had found EIAH (SaO_2 <91%) in 52% of highly trained endurance athletes (VO_2max: 72 $ml \cdot kg^{-1} \cdot min^{-1}$) during strenuous exercise, but

not in lightly trained and untrained subjects (VO_2max: 46 and 55 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively). Because PO_2 drops with thinner air, altitude-induced O_2 desaturation occurs at a lower absolute running speed or power output compared to sea level, and will cause diminished oxygen uptake and performance (Wehrlin & Hallen, 2006;Mollard *et al.*, 2007). Altitude and EIAH diminish VO_2 in a similar manner by decreasing the O_2 supply to the muscles, but altitude will more or less affect all individuals because it reduces the O_2 content of the inspired air, and is not necessarily linked to the individual's lung function. Dempsey & Wagner (1999) describe the mechanism behind EIAH as ventilation-perfusion ratio maldistribution and diffusion limitation that cause excessive alveolar-to-arterial PO_2 difference. In turn, expiratory flow limitation presents a mechanical constraint to exercise hyperpnea. Diffusion limitation and absence of hyperventilation contribute equally to EIAH. Breathing air with an elevated fraction of O_2 can increase O_2-saturation and increase VO_2max, supporting the significance of EIAH. Dempsey & Wagner (1999) also stress the point that a rightward shift of the HbO_2-dissociation curve, which by itself causes desaturation, is usually present together with an EIAH-induced desaturation. Furthermore, they conclude by referring mainly from their own studies that for each percent desaturation below 95%, a 1-2% reduction in VO_2max will occur. This is supported by Wehrlin & Hallén (2006) and Mollard et al. (2007) who found 0.9% (VO_2max: 66 $ml \cdot kg^{-1} \cdot min^{-1}$) and 0.6-1.6% ($VO_2$max: 65.5 $ml \cdot kg^{-1} \cdot min^{-1}$) decrease in VO_2max per percent desaturation in endurance-trained subjects, respectively. In support of Powers et al. (1988), Mollard et al. (2007) found more pronounced desaturation and fall in VO_2max (11.5%) in endurance-trained than untrained subjects. However, Wehrlin & Hallén (2006) found significant inter-individual variation in the relationship between desaturation and VO_2max in endurance-trained subjects; therefore they failed to find a correlation between desaturation and VO_2max when evaluating only one altitude at a time, although they did find a correlation when looking at the mean values for multiple altitudes (personal communication). Wagner (2005) asks: "Why doesn't exercise grow the lungs when other factors do?" He points to the fact that of the four main systems that enable O_2 transport (lungs, heart, blood and muscle, as described by Weibel (1984)) all respond to endurance training with increased O_2 conductance except the lungs (Fig. 3).

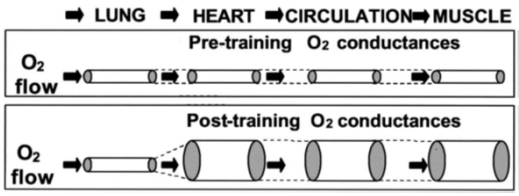

Figure 3. Wagner's "Conceptual diagram to show oxygen pumped from the lungs by the heart through the circulation to the muscle. This is an in series system (arrows) in which each component has a characteristic conductance for O_2 indicated by the diameter of the tube in each case". From Wagner (2005).

In summary, it seems that the lungs potentially limit O_2 supply to the muscles in healthy subjects due to incomplete O_2 saturation of the haemoglobin molecule, and that this limitation is individual in nature and more pronounced in endurance-trained than untrained subjects. With this in mind, one could sum up by quoting Wagner (2005): "It remains an enigma that despite the obvious gains in exercise that follow better developed lungs, exercise seems not to provide stimuli that lead to lung growth".

6.3 Heart and blood

By applying Fick's equation, Hill & Lupton, (1923) estimated cardiac output to be 28 $L \cdot min^{-1}$ at an oxygen intake of 4.2 $L \cdot min^{-1}$, but they were fairly certain that it could have been 30-40 $L \cdot min^{-1}$ because of uncertainties regarding saturation values of the arterial and venous blood. They concluded that it was obviously impossible to be a runner without possessing a powerful heart (Hill determined the O_2 extraction assumption by referring to the work by Lindhard, H. in 1915 who had found a mean O_2 extraction of 67%).

O_2 delivery to exercising muscles can be altered by changing Q and/or CaO_2. If no significant adaptation of O_2 saturation capacity occurs at the lung level following endurance training, oxygen delivery is solely dependent on the blood's O_2 carrying capacity multiplied by the cardiac output. Ekblom & Hermansen (1968) intended to study how large volumes of oxygen were transported from air to tissue. They studied the relationship between cardiac output, stroke volume, heart rate, the blood's concentration of haemoglobin and arterial and venous O_2 content during strenuous and maximal exercise. They divided their subjects in two groups; the first with a mean VO_2max of 74.6 $ml \cdot kg^{-1} \cdot min^{-1}$ and the second with a VO_2max of 66 $ml \cdot kg^{-1} \cdot min^{-1}$. When adding the finding from this study to their previous work they were able to conclude that the high VO_2max in top athletes was usually accompanied by high cardiac output and that the high cardiac output was entirely due to high stroke volumes, since maximal heart rate was similar to, or lower than in non-athletes (Fig. 4). Later, Saltin and Strange (1992) presented a figure from cross-sectional studies and longitudinal observations that supported the close

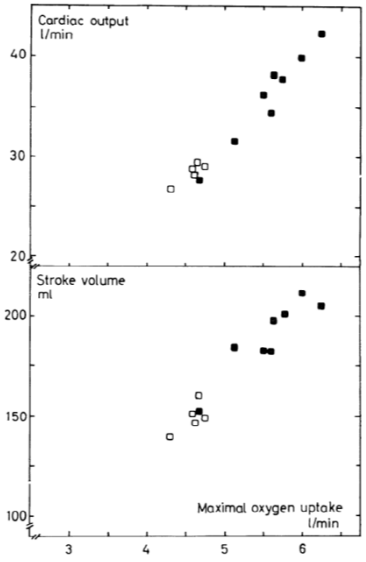

Figure 4. Cardiac output and stroke volume during maximal exercise in relation to maximal oxygen uptake. From Ekblom & Hermansen (1968).

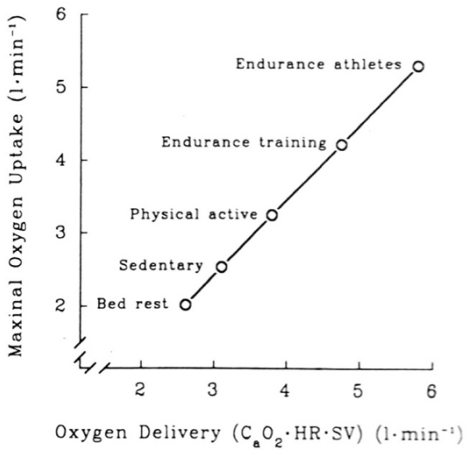

Figure 5. Saltin & Strange (1992) presented data on the relationship between oxygen delivery and maximal oxygen uptake. Data was obtained from cross-sectional and longitudinal observations.

relationship between oxygen delivery and VO_2max in subjects with different training status (Fig. 5). They stated that "The variation in oxygen delivery is solely a function of the size of the stroke volume as maximal heart rate and arterial oxygen content both are unaffected by training". Performance enhancements include increased contractile properties of the myocardium, increased inotropic response and increased ventricular filling (Saltin & Strange, 1992). The significance of oxygen delivery for VO_2max is also supported by studies that manipulated blood volume, haemoglobin concentration and heart rate. Ekblom et al. (1972a) administrated acute blood loss (800 ml) and thereafter reinfusion of the packed red blood cells 1 month later. The blood loss immediately induced a drop in VO_2max and performance. The reinfusion increased both VO_2max and performance by 9% and 23%, respectively. More recently, Birkeland et al. (2000) found a 7% increase in VO_2max (from 63.6 to 68.1 $ml \cdot kg^{-1} \cdot min^{-1}$) and an 8.5% increase in performance after they had increased the hematocrit 8% by administration of recombinant erythropoietin. By combined parasympatic and beta adrenoceptor blockade, Ekblom et al. (1972b) reduced heart rate ~20% during maximal exercise and found a 6% reduction of VO_2 max, and Gullestad et al. (1996) reduced the HR 28% (by beta-adrenoceptor blockade), which reduced VO_2max and performance 9% and 5.3%, respectively. The discrepancy between the decrease in HR and VO_2max can be attributed to increased stroke volume and therefore partly maintain cardiac output and VO_2max (Ekblom *et al.*, 1972b). Another approach for evaluating the significance of oxygen supply for VO_2max is to examine studies experimenting with exercise of different muscle mass. By adding arm exercise to leg exercise Secher et al. (1977) observed a reduction in blood flow and VO_2 in the exercising legs, and later Volianitis and Secher (2002) did a follow up experiment that demonstrated that when adding leg exercise to arm exercise (maximum effort for 5-6 min) blood flow in arms was reduced by 19.1% and VO_2 by 9.6%. Those two studies also exposed another important issue: the reduction in blood flow observed in the already exercising muscle mass when adding more muscle mass to an ongoing exercise, demonstrated the heart's insufficient pump capacity during whole body exercise. This phenomenon was further examined by Calbet at al. (2004) who simultaneously measured cardiac output and blood flow in arms and legs

during different classic styles of cross country skiing. With this setup they demonstrated that the maximal capacity of conductance of the arms and legs outweighed the maximal pumping capacity of the heart, resulting in restriction of the vasodilatory response during maximal exercise, presumably to maintain perfusion pressure. However, Secher & Voliniatis (2006) discuss whether the diastolic function of the heart, attenuated by a reduced preload, limits cardiac output more than the heart's pump capacity itself during maximal exercise. They found support for their suggestion in the large internal diameters of the heart with relatively thin walls characterized for endurance athletes, and the fact that stroke volume increases after fluid or plasma expanders.

The well documented relationship between oxygen delivery and VO_2max, both in untrained and endurance-trained subjects, establishes cardiac output as the most significant limiting factor in the O_2-uptake pathway during exercise.

6.4 Peripheral factors

The skeletal muscles have been called a sleeping giant, meaning that their metabolic capacity exceeds the capacity of the cardiorespiratory system to supply muscles with oxygen during whole body exercise. The size of the metabolic capacity was first quantified by Andersen & Saltin (1985) who isolated the exercise to only m. quadriceps femoris performing dynamic one-legged knee extensions (1-KE). By developing the thermodilution technique (Andersen *et al.*, 1985) combined with arterial and venous blood gas measurements, they calculated the capacity for blood flow and VO_2 to reach 2.5 and 0.350 $L \cdot kg^{-1} \cdot min^{-1}$, respectively. Using anthropometrical measurements, the active muscle mass during this kind of exercise was estimated to be approximately 2.5 kg. In endurance-trained cyclists mass-specific blood flow and VO_2 of 3.85 and 0.6 $L \cdot kg^{-1} \cdot min^{-1}$ during 1-KE (Richardson *et al.*, 1993) have been measured. These high values fell on the same line and extend previous findings by Andersen & Saltin (1985) regarding the relationship between work rate and blood flow and between work rate and VO_2 during 1-KE. If extrapolating the high mass-specific capacity of blood flow to whole body exercise engaging a muscle mass of 30 kg, the cardiac output should have been approximately 70 $L \cdot min^{-1}$. Assuming a more conservative cardiac output of 30 $L \cdot min^{-1}$, the heart can supply only 12 kg muscle with a blood perfusion of 2.5 $L \cdot kg^{-1} \cdot min^{-1}$. This indicates that the muscular energy turnover is submaximal to the muscular capacity during maximal whole body exercise. Despite this, both the oxidative activity and the percentage of type 1 fiber are is found to be higher in endurance-trained than untrained subjects (Gollnick *et al.*, 1972), and training studies show significant adaptations at the muscular level after endurance training as well. In 1967 Holloszy studied the mitochondria of the gastrocnemius in rats after 12 weeks of daily treadmill running. He found the capacity to oxidize pyruvate and the activities of the oxidative enzymes in the

mitochondria to increase twofold accompanied by a significantly longer running time to exhaustion compared to control rats. In humans, Gollnick et al. (1973) found a doubling of the succinate dehydrogenase and phosphofructokinase activity after 5 months of bicycling, together with a 13% increase in VO_2max. Further, Saltin et al. (1976) found increased activity of the oxidative enzymes succinate dehydrogenase (33%) after 4 weeks of one-legged cycle training in the trained leg together with an increase in one-legged VO_2max (24%), and Henriksson et al. (1977) reported increased activity of two oxidative enzymes (32-35%) and VO_2max (19%) after 8-10 weeks of endurance bicycling training. In terms of fiber-type composition, Gollnick et al. (1973) reported the area of the type I fiber to increase by 23% after the training period and Ingjer (1979) found increased capillarity (29%) after 24 weeks of endurance training that increased VO_2max by 25%. Those examples make it clear that significant improvements at the muscular level occur despite the existence of a muscular metabolic and conductance reserve during whole body exercise.

Richardson et al. (1993) reported that, despite the high muscular blood flow and therefore short transit time of the red blood cells, extraction reached 85% during 1-KE, a value similar to that reported during bicycling, which has significantly longer transit time (Knight *et al.*, 1992). High O_2-extraction together with no leveling off of blood flow and VO_2 at maximal work rate put into question whether the true muscular metabolic capacity is really achieved during 1-KE. To examine this, Richardson et al. (1999a) conducted another 1-KE study with endurance-trained subjects (VO_2max: 65 $ml \cdot kg^{-1} \cdot min^{-1}$), but this time they manipulated the fraction of inspired O_2 (0.12, 0.21 and 1.0). VO_2 and performance reached 17% and 14% higher values during hyperoxia compared to normoxia, strongly indicating that the subjects were not limited by skeletal muscle metabolic capacity during normoxia, but rather by oxygen supply. However, whether increase in flow in excess of that achieved during maximal 1-KE would have increased mass-specific VO_2 is uncertain because the increased flow may reduce the transit time in the capillaries significantly, potentially compromising O_2-extraction.

After 9 weeks of endurance training by sedentary subjects (VO_2max: 37 ml.kg-1.min-1), Roca et al. (1992) found a decrease in mean capillary PO_2 content compared to their baseline values, indicating increased diffusion capacity of O_2 in the trained state. An existence of a diffusion limitation at the muscular level was supported by Knight et al. (1993) who observed an unexpectedly high venous PO_2 when breathing 100% O_2 during ordinary bicycling. According to an O_2 supply limitation hypothesis, they expected femoral venous O_2 to be lowered in proportion to the increase in VO_2. Instead, the finding may indicate existence of diffusion limitation during whole body exercise, although other explanations, like hypoxia-induced decreased haemoglobin offloading or increased perfusion heterogeneity, exist. Wagner (2000a) discusses evidence for O_2 diffusion limitation by pointing to studies reporting that the muscles have a metabolic potential during normoxia that is apparent by

increased metabolic turnover during hyperoxia, and the existence of a significant gradient between the mean capillary (40 Torr) and intramuscular PO_2 (3 Torr) in trained individuals. The finding by Roca et al. (1992) indicates a change in the peripheral / central capacity ratio, and Wagner (2000b) suggested that a switch from metabolic to central limitation takes place after endurance training. Despite agreement that a diffusion limitation from the capillary to mitochondria exists, its significance for VO_2max is not agreed on by all physiologists (Saltin & Calbet, 2006). For example, Saltin et al. (1968) reported 90% O_2 extraction during treadmill running even after 20 days of bed rest during which VO_2max fell 28% in healthy subjects who were previously at normal activity level.

Calbet et al. (2005) has reported lower O_2 extraction for the arms (85%) than the legs (93%) which support previous findings (Rasmussen *et al.*, 1975;Secher *et al.*, 1977;Volianitis & Secher, 2002). These findings indicate that the metabolic capacity is not equal in all the body's skeletal muscles, if evaluated from the perspective of utilization of O_2 supply. However, Calbet (2005) used cross country skiers with endurance-trained arms in their study, and an O_2 extraction of 85% for the arms is comparable to previously reported values for leg O_2 extraction. This observation indicates that relatively high O_2 extraction in arms is achieved after adequate training stimuli, which is also supported by Rasmussen et al. (1975), who reported a 6% increase in extraction after 5 weeks of arm cranking. The underlying mechanism for increased O_2 extraction is not clear from the literature: O_2 extraction is not necessarily directly related to the activity of oxidative enzymes because O_2 extraction is reportedly maintained when the activity of oxidative enzymes decreases 11% after 42 days of bed rest (Saltin *et al.*, 1968) and further Calbet et al. (2005) found no relationship between capillary-muscle O_2 conductance and the activities of CS and HAD in arms. However, Calbet et al. (2005) discuss several other mechanisms that potentially explain the lower O_2 extraction in arms: higher diffusing distances, higher heterogeneity in blood distribution, lower mean transit time and lower diffusing area are listed as the most likely factors. One observation in their study is that lower O_2 extraction is paralleled by an elevated flow / VO_2 ratio in arms compared to legs, which thereby plausibly functions as a compensatory mechanism for meeting the muscular O_2 demand.

In summary, it seems clear that O_2 supply limits muscular metabolic turnover during whole body exercise, but not during exercise with a small muscle mass. VO_2 can be limited at the muscular level because of diffusive resistance from capillary to mitochondria, and it is suggested that untrained subjects are more peripherally limited before than after training. Diffusing resistance may be a part of the mechanism behind lower O_2 extraction observed in arms compared to legs. However, adaptations of both O_2 supply capacity and muscular metabolic capacity occur after endurance training indicating that training status is an important modulator.

6.5 Physiology of COPD patients and rationale for inclusion

One method for elucidating the physiological significance of the metabolic reserve capacity is to study the muscular metabolic capacity in subjects with chronically reduced O_2 supply. In such a situation, what happens to the balance between O_2 supply and muscular metabolic capacity?

In contrast to healthy subjects, COPD patients have significant central limitations due to structural changes in the lungs that cause airway obstruction. These changes include increased total lung volume and increased functional residual capacity caused by increased residual volume. In addition to increased lung volume, COPD patients also have airway obstruction, which puts an increased load on their ventilatory muscles (Corbridge & Irvin, 1983). The obstruction is usually expressed as decreased expiratory volume during the first second during forced vital capacity (FEV_1). Healthy subjects can normally expire 80% of their vital capacity the first second while COPD patients usually have a FEV_1 lower than 70%. The changes in the lungs facilitate arterial hypoxemia and trapping of CO_2 in blood because of a mismatch in the ventilation/perfusion ratio, alveoli hypoventilation and reduced mixed venous O_2 leaving the lungs, all of which escalate during exercise (Barnes & Godfrey, 1997;Lotters *et al.*, 2002). COPD patients are described as ventilatory limited because of this unsatisfying ventilation during strenuous exercise (Sietsema, 2001;Morrison *et al.*, 1987). The obstruction may also add load on the ventilatory muscles and thereby increase the O_2 cost per volume unit air ventilated and function as a steal effect of blood flow from locomotor muscles (Levison & Cherniack, 1968;Sala *et al.*, 1999;Baarends *et al.*, 1997;Harms *et al.*, 1997;Dempsey *et al.*, 2008).

COPD patients may develop pulmonary hypertension, or cor pulmonale, due to sustained hypoxic vasoconstriction and increased pulmonary vascular resistance. In turn this may induce reduced systolic function and increased work of the right ventricle. Finally, increased load on the right ventricle may result in hypertrophy and heart failure (Sietsema, 2001;Stewart & Lewis, 1986;Barbera *et al.*, 2003). Pulmonary hypertension increases during exercise and reduced cardiac output at the same workload compared to healthy subjects has been reported (Jakobsson *et al.*, 1990;Kutsuzawa *et al.*, 1992;Jakobsson *et al.*, 1995;Mannix *et al.*, 1995). Morrison et al. (1987) reported that the right ventricular dysfunction can be related to VO_2max. Therefore COPD patients are centrally limited not only because of changes in their lungs, but also because the disease facilitates structural and functional changes of the heart that superimpose on the central limitation caused by lung dysfunction.

In 1999 Richardson et al. reported finding of a metabolic reserve capacity in COPD patients, which was perhaps not surprising since the disease is centrally localized. But the finding was rather controversial since skeletal muscles of COPD patients reportedly favor anaerobic metabolism over aerobic, and are repeatedly reported to be characterized by reduced oxidative enzymes, reduced percentage type I fibers, and increased glycolytic enzymes and type II fibers (Whittom *et al.*, 1998), (Gosselink *et al.*, 1996;Maltais *et al.*, 1998;Sala *et al.*, 1999;Richardson, 1999). The significance of the muscular changes and whether these changes should be linked to the disease per se or to disuse was therefore enthusiastically discussed in the literature (Richardson *et al.*, 1999a). However, the documentation of a metabolic reserve capacity in COPD patients strongly indicated that they were not abnormally limited by their muscles during whole body exercise. In the same study, work rate increased 25% during 1-KE during administration of O_2 enriched air (100% O_2), indicating an O_2 supply limitation even during exercise with a muscle mass of approximately 2 kg. Comparison of the 25% increase in work rate during 1-KE when breathing 100% O_2 with the 14% increase in endurance-trained subjects (Richardson *et al.*, 2004) indicates that the metabolic turnover during normoxia in the mitochondria of quadriceps is submaximal during 1-KE both in COPD patients and trained subjects, and may be even more submaximal in COPD patients. Furthermore, Richardson et al. (2004) found equal metabolic capacity compared to severely sedentary subjects evaluated from VO_2peak during 1-KE. From this finding one could hypothesize that COPD patients have higher metabolic reserve capacity during whole body exercise than healthy subjects.

Study of the muscular metabolic capacity during exercise with different muscle mass in a COPD patient group will bring insight into the balance between muscular metabolic capacity and O_2 supply limitation. Such information would not only be helpful for rehabilitation of COPD patients, but would also expand knowledge regarding peripheral and central limitations of VO_2max in general.

7 Aims of the study

The purpose of this thesis was to examine the significance of the muscular metabolic capacity for VO_2max and how the balance between muscular metabolic capacity and O_2 supply is altered by training status and differences in the muscle mass involved in the exercise. The specific objectives were:

1. To study the balance between muscular aerobic metabolic capacity, measured as VO_2max during 1-KE exercise, and O_2 supply capacity measured as VO_2max during bicycling in groups with different VO_2max and training status.

2. To study the effect of increased muscular aerobic metabolic capacity on O_2 extraction and blood flow during whole body exercise.

3. To study the effect of exercise intensity on arm O_2 extraction and blood flow.

8 Methods

In studies one and two we examined the exercise capacity by measuring pulmonary VO_2 during maximal 1-KE, 2-KE and bicycling exercise. The knee extensions were performed on a knee extension ergometer for dynamic cycling. In studies three and four we, in addition to pulmonary VO_2, measured VO_2 in the exercising limbs by blood flow and arterial and venous blood gas measurements. The exercise models in study three were one-legged cycling and bicycling on a cycle ergometer, and in study four, double poling on a double poling ergometer.

A total of 53 subjects were included in the studies. Eight of the subjects were established COPD patients with limited exercise capacity, 24 were endurance-trained subjects, while the remaining subjects served as controls by having normal aerobic capacity and activity levels for their age. All subjects were, verbally and in written form, informed of the procedures, risks and rationale of the respective study before they gave written consent to participate. The study protocols were approved by the Regional Ethics committee and performed according to the declaration of Helsinki.

Table 1. Physical characteristic of the subjects (Data are mean ± SD [range]).

Study	n	Subject characteristics	Age (yr)	Height (cm)	Weight (kg)	VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	FEV_1 (% pred.)
I	8 (4♀)	COPD patients	**59 (5)#**	**166 (9)**	**73 (15)**	**16 (5)***	**35 (8)***
			(49-67)	(160-180)	(53-104)	(11-27)	(23-48)
	8 (6♀)	Controls	**51 (7)**	**172 (6)**	**76 (14)**	**32 (3)**	**103 (13)**
			(48-61)	(167-180)	(64-110)	(26-36)	(81-116)
II	9 (1♀)	Endurance-trained	**25 (3)**	**180 (9)**	**74 (9)***	**64 (4)***	-
			(19-31)	(167-199)	(65-92)	(59-69)	
	8 (1♀)	Controls	**27 (4)**	**181 (9)**	**90 (12)**	**46 (4)**	-
			(24-35)	(167-193)	(71-109)	(41-54)	
III	12 (6♀)	Endurance-trained	**24 (3)**	**174 (9)**	**68 (8)**	**57 (8)**	-
			(20-31)	(162-189)	(59-82)	(49-72)	
IV	8 (♂)	Endurance-trained	**24 (7)**	**178 (6)**	**74 (6)**	**66 (5)**	-
			(19-38)	(168-187)	(62-79)	(59-74)	

Asterisk (*) indicates a statistically significant difference between groups in their respective study (P<0.05) and number sign (#) indicates a tendency (P<0.1).

8.1 Study design

8.1.1 Study I

Study I compared the exercise capacity in well established and long-standing COPD patients (COPD GOLD stage III-IV, according to GOLD criteria [GOLD, 2006]) to healthy controls during exercise with different muscle mass. All subjects were familiarized to the exercise tests on four separate days before experiment day. The subjects performed 1-KE, 2-KE and bicycle exercises, which activated muscle masses of approximately 2, 4 and 20 kg, respectively. Each exercise test lasted 9-15 min with 3-min stepwise increments in intensity until exhaustion. Intensity increments for COPD patients were 2W/leg during knee extensions and 10 W during bicycling; increments for the controls were 4 W and 20 W, respectively. Pulmonary measurements of VO_2, blood pressure and HR were recorded continuously from 10 min before exercise until exhaustion (Fig. 6). One hour prior to the first test, subjects were catheterized in arteria radialis for measurement of blood pressure and HR, and drawing of blood samples. Blood samples were analyzed for lactate, pH and blood gases and were drawn immediately before exercise and at exhaustion. The order of 1-KE and 2-KE exercises and between right and left leg performing 1-KE were counterbalanced, while bicycling, which was assumed to be the most fatiguing exercise, was performed as the final test. All tests were performed in one day with at least 1 h rest between tests. To calculate the mass-specific VO_2, the highest VO_2 achieved for each exercise modality was divided by the exercising muscle mass (see calculations for details).

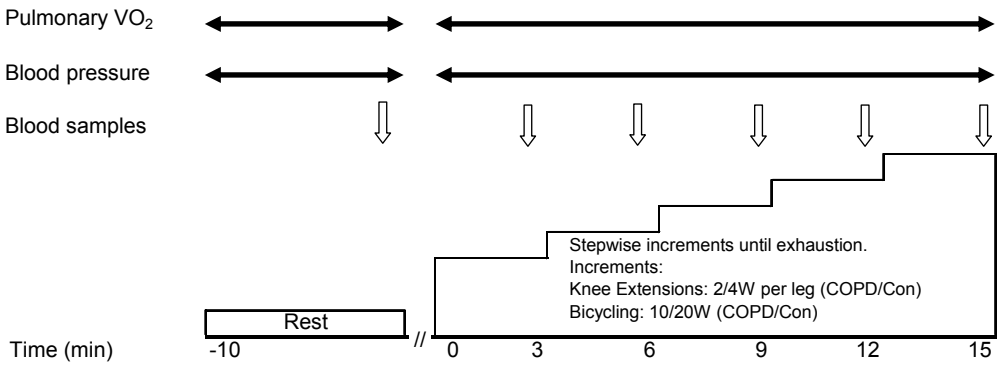

Figure 6. Schematic protocol, study I.

8.1.2 Study II

In this study we applied the same protocol as for study I on endurance-trained subjects and subjects with normal physical activity, but without arterial blood samples. After three familiarization days, the subjects reported to the laboratory at three different times in one day to perform 1-KE, 2-KE and bicycling, with at least 2 hour's rest in between. Subjects initially sat relaxed at the ergometer for 10 minutes to obtain resting values of the parameters to be tested and thereafter performed a 5-min exercise-specific warm up at a workload of 20W per leg before the KE tests, and 100W bicycling before the bicycling test. The stepwise increment was 5W per leg during knee extensions and 20W/25W (women/men) during bicycling until exhaustion (6-12 min), defined as inability to maintain a cycling frequency of 60 rpm. Counterbalancing and test order were as for study I. Pulmonary oxygen uptake (VO_2), heart rate (HR) and the oxygen saturation of haemoglobin (SpO_2) were measured continuously before (10 min) and during exercise (Fig. 7). Blood samples analyzed for lactate concentration were taken from a fingertip immediately before warm-up and at exhaustion.

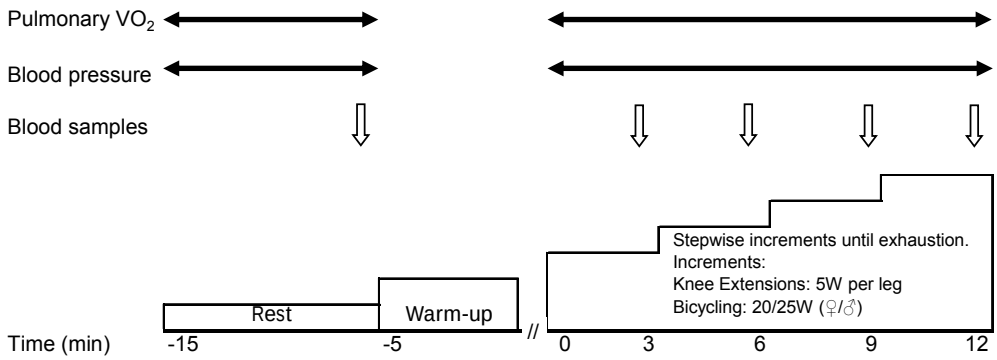

Figure 7. Schematic protocol, study II.

8.2.1 Study III

In study III we examined the effects of 7 weeks of one-legged endurance training in twelve endurance-trained subjects. Before and after the training period the subjects tested pulmonary VO_2max during ordinary bicycling. In addition, after the training period they performed one-legged cycling and ordinary bicycling during submaximal and maximal exercise tests. Biopsies from vastus lateralis for analysis of oxidative enzymes were obtained from both legs after the training period for all subjects.

Eight of the twelve subjects volunteered for submaximal and maximal bicycling tests during which the oxygen uptake in legs was determined from measurements of blood flow times aterio – veneous oxygen differences (Fig. 8). To evaluate blood flow and O_2 extraction with regard to physiological adaptations from the training period, strain gauges were applied in the crank arms of the bicycle to ensure equal power output in the legs. With this setup modification, we could compare the metabolic turnover in the trained leg with that of the control leg at equal power output.

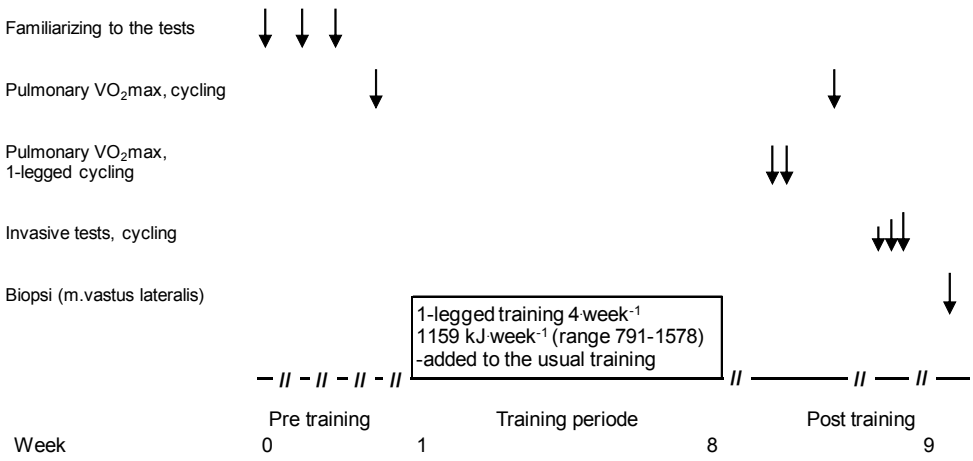

Figure 8. Schematic protocol, study III.

8.2.1.1 Protocols

Tests with pulmonary VO_2 measurements

Subjects did not perform strenuous exercise for 24 hours prior to the tests. Before the training period, maximal pulmonary oxygen uptake was determined during bicycling on an ergometer (Excalibur Sport, Lode B.V., Groningen, The Netherlands) to exhaustion, defined as inability to maintain a pedal frequency of 80 revolutions per minute. Prior to the first test before the training period, all subjects reported to the laboratory on 3 separate days for familiarization with the test ergometer and test procedure. The test consisted of stepwise increments of exercise load each minute until the final step, which was maintained for approximately 1½ min. The increments were 20 W for women and 25 W for men. VO_2 was measured during the last step(s) and heart rate at exhaustion was registered. Blood samples from rest while sitting at the ergometer and at exhaustion were analyzed for lactate concentration.

The VO_2max test was repeated after the training period. The subjects also performed one-legged VO_2max tests with the trained and untrained leg separately; the test procedure was as described for bicycling except that intensity increments were 10 and 15 wattsfor women and men, respectively. The order of untrained and trained leg was counterbalanced. Before all three maximal tests, the subjects performed three submaximal exercises at 80/100W (women/men), 120/150W and 160/200W during bicycling and 40/50W, 60/75W and 80/100W during one-legged cycling. The tests were done in order of increasing workload, but were counterbalanced between control and trained leg as well as one-legged cycling and bicycling. Each submaximal exercise lasted 8 minutes with 15 min rest between each test. Rest in between the control and trained leg one-legged cycling tests and between one-legged cycling and cycling tests were ~1½ hours. Constant cycle frequency of 80 RPM was achieved by visual feedback from a PC monitor. Heart rate was monitored from 5 to 7 minutes, expired air for VO_2 analysis was obtained from the two last minutes, and blood samples for analysis of lactate were taken within the final 30 seconds of exercise.

Tests with invasive VO_2 measurements

The invasive measurements were taken during three cycling tests: two 8-min submaximal exercises at 80/100 (women/men) and 120/150W, and one strenuous exercise of 210±22 (women) or 303±24W (men) (210/303W) until exhaustion (6-9 min). Leg blood flow was measured simultaneously in both legs immediately after the test, and arterial and venous blood samples were drawn at the 3rd and 6th min. Arterial blood pressure was measured continuously.

8.2.1.2 Training

During the training period the subjects reported to the laboratory 4 times per week for 7 weeks for supervised one-legged training on a mechanically braked ergometer with fixed flywheel (Monark 818E, Monark Exercise AB, Sweden). The training was counterbalanced for left and right leg and the cycling frequency was kept at 80 RPM. The control leg rested on a chair beside the ergometer. Average training volume was 1159 $kJ \cdot week^{-1}$ (range 791-1578) at a workload of 108W (76-160) corresponding to 72% (70-77) of the one-legged maximal heart rate (HR). HR was monitored by a Polar heart monitor (Polar Electro Oy, Kempele, Finland). Training bouts consisted of a 10-min warm-up period followed by continuous exercise at a constant workload. The four weekly training bouts varied in intensity (59-90% of HR) and duration (40-100 min). The training load and volume were increased progressively according to the HR response. The control leg was trained several sessions during the training period to avoid test differences due to cycling technique. During the training period the subjects continued and reported their regular pre-study physical activity level for the purpose of maintaining cardiovascular capacity.

8.2.2 Study IV

In study IV we examined blood flow distribution and O_2 extraction in arms and legs during double poling at two submaximal intensities performed in one day on a double poling ergometer. Each submaximal intensity trial lasted 8 min and was performed twice. Subjects rested for 10 min between the exercises of equal intensity and 30 min between the two different intensities. The workloads were 82 (range 66-97 watts) and 117 (79-143) watts for the low and moderate intensity, respectively (Fig. 9). VO_2max achieved during double poling was 92% (4%) (mean $\pm$ S.D.) of the running VO_2max. The workloads corresponded to mean 54% (5%) and 76% (7%) of the VO_2max achieved for poling. For calculations of leg and arm VO_2, blood flow was measured (determined by the thermodilution technique in one arm and one leg). The blood flow measurements in leg and arm were performed simultaneously at the 3rd and 6th min, immediately after blood samples for analyses of blood gases and metabolites were drawn. Pulmonary measurements of VO_2 were performed from 2.5 min to 6 min. Blood pressure was measured continuously for plotting of blood pressure curves, from which blood pressure and HR were derived. Video recordings to analyze elbow, hip and knee angles were performed for each subject. In addition, pole angle, vertical displacement of the pelvis, horizontally applied poling force and horizontal displacement of the trolley were sampled continuously on a PC. See "Test apparatus and procedures" for details.

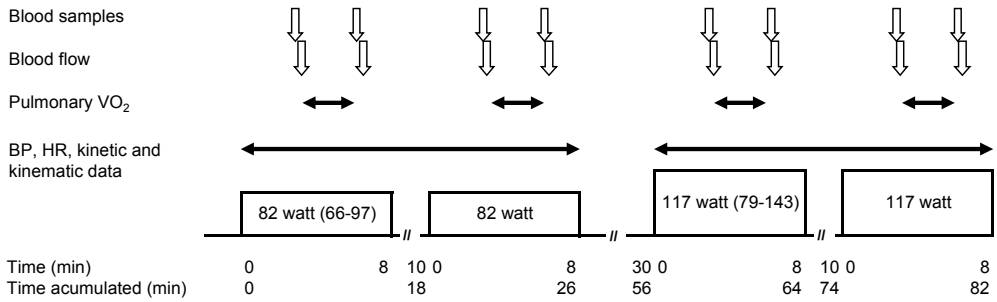

Figure 9. Schematic protocol, study IV.

8.3 Test apparatus and procedures

8.3.1 Ergometers

Knee extensor ergometer

In studies I and II knee extension exercises were performed on a custom-built electromagnetically braked knee extensor ergometer as previously described by Andersen and Saltin (1985) and modified by Hallén et al. (1996). This exercise form is previously reported to activate only the m. quadriceps during extension of the knee (Richardson et al., 1998). Nevertheless, by administration of a telescopic function of the crank arm connecting the rods we ensured no transmission of forces from the leg to the crank arm during flexion of the knee (Fig. 10 and 11). However, the subjects were instructed and trained during the familiarizing period to relax their non-exercising muscles including their hamstrings during the backward movement of their lower leg. The ergometer was operated by a computer and visual feedback of the extension – relaxation cycle frequencies as well as force tracings from the legs were displayed on a PC monitor. Data regarding forces from strain gauges (U2A 50 Hottinger Baldwin Mestechnik, Darmstadt, Germany) in the crank arm rods, angle position of the crank arms, and revolution frequency was collected (sample rate 100Hz) to calibrate the amount of brake administered on the flywheel to achieve a given power output. To simulate momentum, the electromagnetic brake was turned off between the extension cycles of the leg during 1-KE (during the backward movement of the leg), and when the crank arms were around the horizontal position for 2-KE. This minimized accelerations and decelerations and ensured a smooth speed of the flywheel, even during high power outputs. A four point safety belt was employed to help the subjects avoid muscle activity needed for stabilization. The chair of the ergometer was individually adjusted to the thigh length of the subjects.

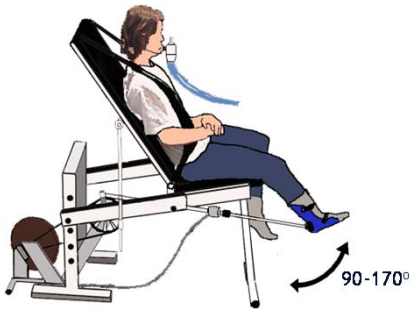

Figure 10. Knee extensor ergometer. The connection rod between the leg and crank arm has a telescopic function which ensured no resistant during the backward phase of the leg movement.

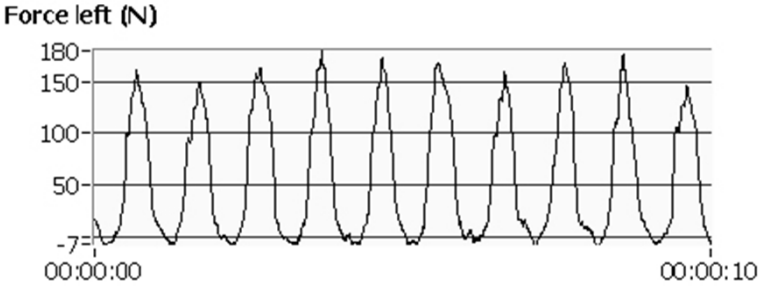

Figure 11. Force tracings from 1-KE illustrate the effect of the telescopic function of the crank arm connecting rods, which cause minor negative force components. Data sampling frequency; 100 Hz.

Cycle ergometers

In studies I and II bicycling was performed on a mechanically braked cycle ergometer (Ergoline 90 Jaeger Toennies, Würzburg,Germany and Monark 818E, Monark Exercise AB, Sweden, respectively). In study III testing was performed on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode B.V., Groningen, The Netherlands). The ergometer was customized with a fixed flywheel in study III to enable one-legged cycling. The force from strain gauges in the crank arms, cycle frequency and power output were sampled on a PC. During all tests cycling frequency was displayed a monitor. Force distribution between the legs was calculated real time and visualized during the invasive bicycle tests. The accuracy of the ergometer was controlled by a calibrator (Mod 17800, VacuMed, CA, USA) by applying the procedure described by Barstow et al. (1996). The training sessions in study III were performed on a mechanically braked ergometer with fixed flywheel (Monark 818E, Monark Exercise AB, Sweden).

Double poling ergometer

In study IV double poling was performed on a modified (Holmberg & Nilsson, 2008;Nilsson *et al.*, 2004) row ergometer (Concept II C, Concept Inc., Morrisville, Vt.,USA), with custom applied devices for measurements of kinematic and kinetic characteristics (Fig. 12). During poling, continuous measurements of pole and knee angles, vertical displacement of the pelvis, horizontally applied poling force and horizontal displacement of the pole trolley and the force in the z-direction (G) by a force plate (AMTI Biomechanics type SG-9, Massachsetts 02158, USA) connected to an amplifier (Gain 4000; low pass filter 1050Hz) applied beneath the ergometer's standing platform were performed. The horizontally applied poling force was measured by a strain gauge arranged at the

back of the horizontal slide of the trolley (U2A 200 Hottinger Baldwin Mestechnik, Darmstadt, Germany) and by integrating this force to measurements of the horizontal displacement (custom device) of the "pole-trolley" we confirmed the power output of the ergometer. All data were simultaneously and continuously sampled on a computer at 100 Hz.

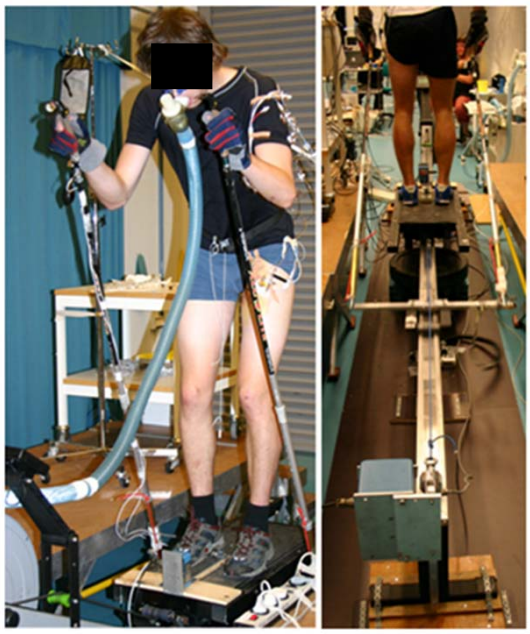

Figure 12. Set-up of the double poling ergometer with a subject prepared for measurements of both pulmonary and invasive oxygen uptake.

A video camera (Handycam Vision DCR TRV900E, Sony Inc., Japan) for continuous video recordings to sample elbow, hip and knee angles was arranged at the right side of the subjects. The video tapes were digitized in Adobe Premiere Pro (Adobe Systems Incorporated, Version 2.0, Stingray) and thereafter analyzed frame by frame in the HU-M-AN (Version 5.0 2D-3D) software (HMA Technology Inc.). Averages from the recordings of the 1th, 4th and 7th min were used in the final kinematic analyses.

8.3.2 Pulmonary oxygen uptake

Douglas bag method

In study III subjects breathed through a mouth piece connecting to a two-way non re-breathing valve (2700 series, Hans Rudolph, inc., Kansas City, USA) while the nose was sealed with a nose clip. Pulmonary expired gases were collected in Douglas bags through a flow turbine (K 520, K LEngineering, Calif., USA). The flow turbine was configured to start and stop the pulmonary gas sampling into the Douglas bags exactly at the end of expiration. The Douglas bags were analyzed for the concentration of O_2 (Ametek S-3A, Pittsburgh, USA), CO_2 (Ametek CD-3A, Pittsburgh, USA), volume (K 520, K L Engineering, Calif., USA) and values were corrected for pressure (Wika, Klingenberg, Germany) and temperature (Pt 120). The metabolic calculations were done using custom designed software (Labview, National Instruments, Texas, USA). The O_2 analyzer was calibrated with room air (20.93% O_2) and linearity was checked with a known O_2 concentration (12.00%). The CO_2 analyzer was calibrated with room air (0.03% CO_2) and with a known CO_2

concentration (5.00%). Calibration was performed before each test. Ventilation ensured that the same gas concentrations as those found outdoors were achieved in the laboratory. The accuracy of the volume turbine was verified with a 3 L syringe (model 5530, Hans Rudolph, Kansas City, USA), while temperature and pressure sensors were controlled by a mercury thermometer and barometer (Leybold, Hürth, Germany).

Computerized metabolic systems

In studies I, II and IV pulmonary gas exchange was measured breath by breath by a computerized metabolic system. Subjects breathed through a mouthpiece connecting to a two-way non re-breathing valve (studies II, III and IV) (2700 series, Hans Rudolph, inc., Kansas City, USA), or a mouthpiece equipped with integrated flow volume and gas measurements (study I), while the nose was sealed with a nose clip. For study I gas exchange was measured with either an Oxycon Pro (n=10), Oxycon Alpha (n=1) (both; Eric Jaeger, Hoechberg, Germany) or Vmax29 (n=5) (SensorMedics Corporation, USA) oxygen analyzer. The use of different exercise spirometry systems during the test period was due to system breakdown. In studies II and IV pulmonary gas exchange was measured with an Oxycon Pro equipped with mixing chamber (Eric Jaeger, Hoechberg, Germany). The gas analyzers were routinely calibrated against certified calibration gasses of known concentrations (O_2: air and CO_2: 5%), and the flow turbines were calibrated with a 3 L calibration syringe (5530 series, Hans Rudolph, Kansas City, USA).

8.3.3 Invasive VO_2 and blood variables

In studies III and IV blood samples from catheters were analyzed for ctO_2, sO_2, PO_2, PCO_2, Hb, pH, $pO_2$50 and La^- by an ABL analyzer (ABL, Radiometer, Copenhagen, Denmark). Paralleled analyses were performed routinely as well as calibration of the analyzer.

Lactate from fingertip

In studies II and III an enzymatic lactate analyzer (YSI 1500 Sport, Yellow Springs Instruments, Ohio, USA) was used for analysis of lactate. The lactate analyzer was calibrated with a 5 $mmol \cdot L^{-1}$ lactate standard and linearity was controlled with a 15 $mmol \cdot L^{-1}$ lactate standard, according to the instruction manual.

Blood pressure and Heart rate

In studies I, III and IV arterial blood pressure was measured with a pressure gauge (Baxter B.V. Uden, Holland) connected to a transducer amplifier (GOULD instrument systems, Valley View, USA) and collected on a PC with a sampling rate of 100Hz. Blood pressure was calibrated using a column of

water equal to 50 Torr. Heart rate was derived from blood pressure curves when available (studies I, III and IV), otherwise it was recorded by a Polar HR monitor (Polar Electro Oy, Kempele, Finland).

Blood flow

On the day of the experiment, subjects reported to the laboratory 2 hours prior to the experiment. While subjects lay in supine position one catheter was placed under local anaesthesia (2% lidocain) in the brachial artery (studies I and IV) or arteria femoralis (study III) and one catheter was placed in vena femoralis (studies II and IV) using the Seldinger technique. The femoral venous catheters (flow–through model 93–505, Edslab Baxter A/S, Allerød, Denmark / 93.505 Edwards Lifesciences LLC, Irvine CA, USA), were inserted 2 cm below the inguinal ligament and advanced ~7 cm in anterograd direction. In study IV a Swan-Ganz triple-lumen catheter (Model132F5) was inserted into the subclavian vein 5 cm before the merger with jugular vein. Thermistors (Edslab 94-0.30-2.5F, Baxter A/S, Allerød, Denmark) were inserted through the catheters and placed 8 cm beyond the tip of the catheters. The catheters were connected to a three way stopcock for venous blood sampling and injection of ice cold saline. Following preparation, subjects rested in supine position for ~30 min prior to exercise.

Blood flow was measured by constant infusion thermodilution technique (Gonzalez-Alonso *et al.*, 2000;Andersen & Saltin, 1985). Ice-cold saline was infused into the femoral vein at a constant rate of 20-120 ml/min^{-1} for 15-25s, depending on blood flow, to achieve a drop in blood temperature of 0.4–0.9°C. During submaximal tests flow measurements were taken in the 3rd and 6th minute, and during the maximal test in the 3rd and 6th minutes and immediately prior to exhaustion. Femoral venous blood and infusate temperature were sampled continuously before and during ice-cold saline infusion Infusate temperature was measured at the site of entry of the catheter with a flow–through thermistor, while venous blood temperature was measured with the thermistors placed in the veins. The thermal data was collected on a PC with a sampling frequency of 100Hz (Maclab 16/s ADInstruments, Sydney, Australia or alternatively Labview, National instruments, Texas, USA). The thermistors were calibrated after the experiments by comparing with an analog thermometer of high resolution at two different temperatures: 36 and 39 degrees celsius. Temperature data was corrected if different from the analog thermometer. All analyses of the temperature curves were performed using a custom designed software.

8.3.4 Calculations

Muscle mass calculations

The knee-extensor muscle mass was estimated anthropometrically (Jones & Pearson, 1969). The thigh volume (V), excluding subcutaneous fat, was calculated from the formula: $V = L \cdot (12\pi)^{-1}(O_1^2 + O_2^2 + O_3^2) - (S-0.4) \cdot 2^{-1} \cdot L \cdot (O_1^2 + O_2^2 + O_3^2) \cdot 3^{-1}$ where L is the thigh length estimated from the top of patella to spina iliaca anterior superior, O_1, O_2 and O_3 are the circumferences at the points 1/4, 1/2 and 3/4 between the patella and the spina iliaca anterior superior, and S is the mean skinfold thickness posterior and anterior at the middle measuring point. Skinfold thickness was determined by a Harpenden skin-fold calliper. The knee extensor muscle mass was then calculated as: $Mass = 0.307 \cdot V + 0.353$ and then corrected as suggested by Raadegran et al. (1999): $Mass_{corr} = 0.792 \cdot Mass - 0.382$. The active muscle mass during bicycling was estimated from gender and weight and corrected for age in accordance with Gallagher et al. (1997) -- women: active muscle mass = $(Mb \cdot 0.26 - (Age - 20) \cdot 0.03_{mr})$); men: active muscle mass = $(Mb \cdot 0.29 - ((Age - 20) \cdot 0.05_{mr}))$ where Mb is the total body mass and "mr" is the annual muscle mass reduction factor.

Blood flow calculations

Blood flow was calculated according to the thermal balance principles described by Andersen & Saltin (1985). The coefficient of variation for this method is reported to range between 3% and 6% (Andersen & Saltin, 1985).

Invasive VO_2, lactate balance and vascular conductance calculations

Arterial-venous oxygen differences (a-v O_2diff) were calculated from the differences in arterial and venous oxygen content. Blood flow was the average of the two or three measurements during each exercise workload. Oxygen uptake was calculated as the product of blood flow and a-v O_2diff, and delivery O_2 was the product of arterial O_2 content and blood flow. Lactate balance was calculated as the product of plasma flow and venous-arterial plasma lactate differences (studies III and IV). (Plasma flow was given as the product of venous blood flow and blood plasma concentration [1-hematocrit]). In study IV the arm and leg VO_2 was multiplied by a factor of two to get VO_2 for both arms and legs .

Leg vascular conductance was calculated as blood flow divided by the difference in mean arterial – the estimated leg venous blood pressure. The leg venous blood pressure was considered to be 4 mmHg (0.53 kPa) at rest and for the three invasive submaximal cycle exercise tests as suggested by Calbet et al. (2004).

Double poling ergometer

The leg work done per poling stroke was calculated as the product of the vertical displacement of the hip and the force in the z-direction divided by time. The horizontal work per pole stroke was calculated as the product of the horizontal force and the horizontal displacement of the trolley divided by time. The range of motion angle was calculated by subtracting the average minimum angle from the average maximum angle for the strokes during the respective time periods (1th, 4^{th} and 7^{th} min).

8.3.5 Muscle biopsies

In study III biopsies were taken from m. vastus lateralis of both legs within 1 week after the final exercise test. Percutaneous needle biopsy under local anesthesia (2% lidocain) was performed with manual suction (5 mm Pelomi needle, Albertslund, Denmark). Muscle biopsies of 50-200 mg were taken from vastus lateralis and rinsed in saline before fat and connective tissue were carefully removed. Samples for homogenization and immunohistochemistry were then frozen in isopentane on dry ice and stored at -80ºC until analysis. Citrate synthase (CS) and 3-hydroxyacyl-CoA dehydrogenase (HAD) activity were determined by using fluourometric methods with NAD-NADH coupled reactions as described by (Essen-Gustavsson & Henriksson, 1984).

8.3.6 Software for instrument control, data sampling and analyses

Control and communication with instruments and ergometers, data sampling and analysis, except for part of the blood flow measurements in study III and the video data in study IV, were done by software customized for each study's requirements (Labview, National Instruments, Texas, USA).

8.4 Statistics

Study I: variables were tested for a significant difference between the groups by homoscedastic t-tests (studies I and II) and between control leg and trained leg or arms and legs by paired t-tests (studies III and IV). Significance level was set at $P<0.05$ (2-tailed). In the thesis, comparisons of multiple groups is done for studies I and II. For these comparisons one-way ANOVA is used to determine overall differences, with a Tukey-Kramer Multiple Comparisons Test as post test. Significance level was set at $P<0.05$.

9 Results & Discussion

The most important findings of the studies are presented in this section. The presentation form will differ from the original papers presented at the end of this thesis. Please refer to the individual articles for supplemental data.

9.1 Studies I and II

The main findings from studies I and II were that in healthy subjects -- which included endurance-trained, untrained, young and old -- the muscular metabolic reserve during whole body exercise was relatively similar despite huge differences in muscular mass-specific metabolic capacity and VO_2max, indicating that the balance between muscular metabolic and oxygen supply capacity is relatively independent of training status. The COPD patients differed from the other groups by having a relatively higher muscular metabolic reserve capacity during whole body exercise. The exercise capacity was examined during 1-KE, 2-KE and bicycling in these two studies.

For whole body VO_2max, work rate and muscular mass-specific VO_2max, the endurance-trained subjects (ET) achieved the highest values regardless of exercise mode (1-KE, 2-KE or bicycling) ($p<0.05$). The exercise capacity was therefore highest in ET evaluated from these variables while the lowest values were not surprisingly achieved by the COPD patients ($p<0.05$). Interestingly, the mass-specific VO_2 achieved during 1-KE was close to equal for the two control groups, although they differed 24 yr in age and presumably also activity level. Comparing COPD patients with their controls, the difference in VO_2, work rate and mass-specific VO_2, increased as the exercising muscle mass increased ($p<0.05$). For the ET the differences from their control group were relatively independent of exercise mode (Table 2, fig. 13 and 14).

Table 2. Characteristics and variables for the subjects participating in studies I and II (Data are mean ± S.E).

		Study I		Study II	
	Subjects (n) Exercise	Controls (8)	COPD (8)	Controls (8)	Endurance trained (9)
VO_2 ($ml \cdot min^{-1}$)	1-KE	976 (76)	786 (77)#	1387 (76)	1755 (72)*
	2-KE	1468 (124)	916 (75)*	2234 (113)	2765 (98)*
	Bicycling	2453 (210)	1185 (131)*	4115 (150)	4794 (226)*
Work rate (Watt)	1-KE	29 (2)	12 (1)*	43 (2)	54 (2)*
	2-KE	54 (4)	21 (2)*	89 (3)	108 (4)*
	Bicycling	169 (14)	53 (4)*	298 (7)	375 (21)*
Active muscle mass (kg)	1-KE	2.0 (0.5)	1.7 (0.3)*	2.8 (0.3)	2.5 (0.3)
	2-KE	4.0 (1.0)	3.4 (0.6)*	5.6 (0.7)	5.1 (0.7)
	Bicycling	20 (4.6)	19 (5)*	26 (4)	21 (3)*
Mass-specific VO_2 ($ml \cdot min^{-1} \cdot kg^{-1}$)	1-KE	345 (25)	264 (32)#	386 (28)	570 (27)*
	2-KE	295 (24)	175 (24)*	347 (28)	488 (17)*
	Bicycling	104 (6)	44 (7)*	138 (6)	206 (5)*

Table 2 shows the highest values of VO_2, work rate and mass-specific VO_2 (VO_2 – VO_2 at rest / active muscle mass) achieved for the four groups in studies I and II during exercise with different active muscle mass. These groups are different in age (between studies), training status and central limitations in regard to lung function (FEV1; see Table 1). Asterisk (*) indicates a statistical difference between groups within their respective study (P<0.05) and number sign (#) indicates a tendency (P<0.1).

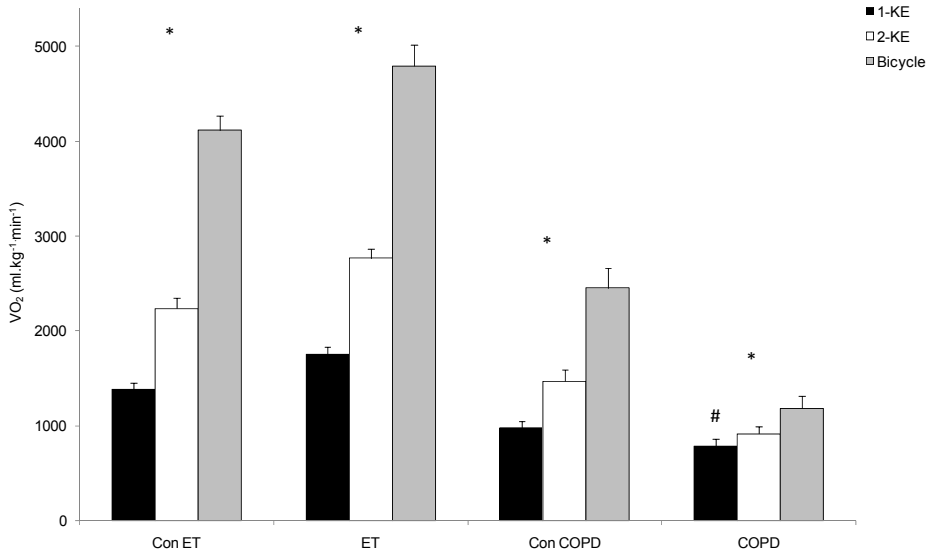

Figure 13. VO_2max achieved for 1-KE, 2-KE and bicycling. Bars are mean ± S.E. ET; endurance trained, Con ET; controls ET, COPD; chronic obstructive pulmonary disease patients, Con COPD; controls COPD. Asterisk (*) indicates a statistical difference from the other groups for all the three exercise modes (p<0.05) and number sign (#) indicates tendency of difference between COPD and Con COPD (p=0.10). N=8 for all groups except ET; n=9.

The muscular metabolic capacity is quantified as the mass-specific VO_2 during 1-KE. By this method we assume that oxygen supply is not limiting oxygen uptake in the knee extensors as reported in the literature (Andersen & Saltin, 1985;Richardson *et al.*, 1999a;Richardson *et al.*, 1999b;Richardson *et al.*, 1993). By comparing the mass-specific VO_2 during 2-KE and bicycling with that of 1-KE we can

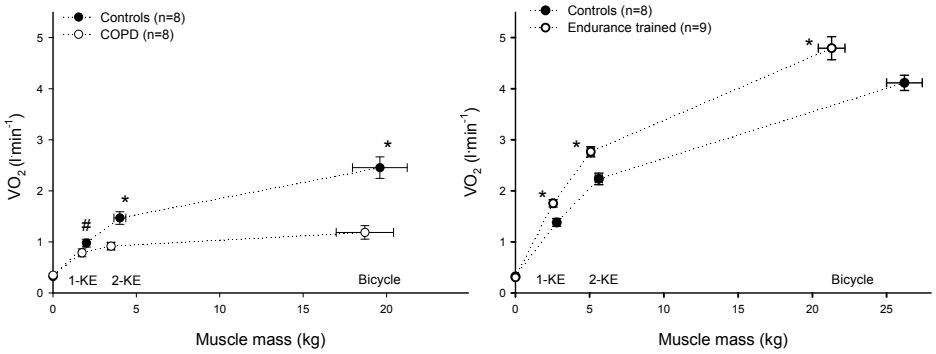

Figure 14. Oxygen uptake vs. muscle mass during 1-KE; one-legged knee extensor exercise, 2-KE; two-legged knee extensor exercise and bicycling for study I (left) and II (right). Number sign (#) indicates P<0.1 and asterisk (*) P<0.05.

evaluate to what extent the O_2 supply can support the O_2 metabolic capacity as the active muscle mass increases. During 2-KE and bicycling the COPD patients, the control of the COPD patients, the controls of the ET, and the ET achieved 67% and 17%, 85% and 31%, 90% and 37% and 87% and 37%, of the 1-KE mass-specific VO_2, respectively. Within the groups the mass-specific VO_2 was different between the three exercise modes ($p<0.05$). Otherwise, relative to the 1-KE exercise, only the COPD patients achieved lower mass-specific VO_2 than the other groups both for 2-KE and bicycling (Fig. 15).

The metabolic reserve was therefore in the range of 10% - 15% and 69% - 63% for the healthy groups during 2-KE and bicycling, while it was 33% and 83% for the COPD patients, respectively. Therefore, the COPD patients differ from the other groups by having lower exploitation of their muscular metabolic capacity during both 2-KE and bicycling. This is not surprising for bicycling in regard to ventilation limitations of COPD patients (no ventilation reserve during bicycling: ventilation = 105%±5% of maximal voluntary ventilation), which probably restrict the O_2-supply, and arterial O_2-saturation of 84% (9%). In comparison, the control group of the COPD patients achieved 91% (3%) of MVV and 96% (5%) O_2-saturation.

As discussed above; the amount of the muscular metabolic capacity that is exploited when the exercising muscle mass increases indicates how well the cardiopulmonary system can supply the

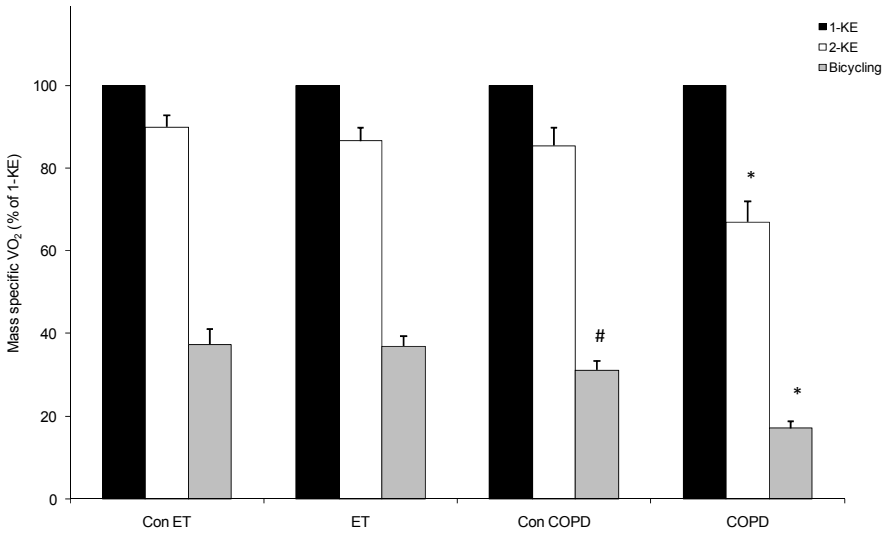

Figure 15. Utilization of the metabolic capacity relative to the 1-KE mass-specific VO_2. Bars are mean ± S.E. ET; endurance trained, Con ET; controls ET, COPD; chronic obstructive pulmonary disease patients, Con COPD; controls COPD. Asterisk (*) indicates a statistical difference from every other group and number sign (#) indicates tendency of difference between Con COPD and ET (p=0.09). N=8 for all groups except ET; n=9.

muscles with oxygen. For the healthy subjects, mass-specific VO_2 was 10%-15% lower for 2-KE than 1-KE. The muscular perfusion capacity during 2-KE should not be sufficient to challenge the maximal cardiac output. Why then doesn't the net VO_2 double in the healthy groups when the muscle mass doubles from 1-KE to 2-KE? One possibility is that pulmonary measurements are inadequate for accurately quantifying the mass-specific VO_2, because pulmonary VO_2 during knee extensions may increase more than expected from the increase in power output, due to engagement of stabilizing muscles not directly involved in the external work (Andersen *et al.*, 1985). Based on this knowledge we corrected the VO_2 data to avoid an exponential increase in VO_2, but this was only observed for some subjects in study II, probably because of an advantage of the custom built ergometer which minimized the need for both stabilizing muscle activity and hamstring activation. Of note, Richardson et al. (1995a) reported 5 times higher nor-adrenaline and adrenaline spillover when adding arm exercise to 1-KE exercise, although blood flow was not compromised. It could be that an increased sympathetic activity during 2-KE restricts blood flow sufficiently to attenuate mass-specific VO_2, despite a significant cardiac reserve in the present studies. However, without invasive measurements of leg blood flow and cardiac output, explanations for the 10%-15% difference in mass-specific VO_2 between 1-KE and 2-KE will remain speculative. Regardless, the COPD patients had an even more pronounced reduction (33%) in mass-specific VO_2 during 2-KE compared to 1-KE. This difference in mass-specific VO_2 indicates that their muscles suffer more from restricted O_2-supply, and that a muscular metabolic reserve is appearing when exercising with a muscle mass of only 3.4 kg. The question thus arises: could it also be that the COPD patients are O_2-supply limited during 1-KE, meaning that we have underestimated their skeletal muscular mass-specific metabolic capacity? The finding of Richardson et al. (1999) may not support this, because breathing a helium and O_2 mixture (intended to reduce O_2-supply needed for ventilation) didn't increase COPD patients' work rate during 1-KE but did so during bicycling.

One interesting observation was that the HR in the COPD patients was 14% and 24% lower compared to their control group at exhaustion for 2-KE and bicycling, respectively ($p<0.05$). At the same time BP was 159 and 143 Torr during 2-KE for the COPD patients and their controls, respectively ($p=0.14$), and tended to be 14% higher in the COPD patients during bicycling ($p=0.054$). The submaximal values for both VE and VO_2 in COPD patients during 2-KE, indicate both a ventilatory and cardiac output reserve. However, the SaO_2 fell to 88% and 84% during 2-KE and bicycling, respectively. The combination of low HR and high BP could therefore be an indication of a blood flow down regulation mechanism, caused by, for example hypoxemia-induced pulmonary arterial vasoconstriction (Stewart & Lewis, 1986;Weitzenblum, 1994) or hypoxemia-induced sympathetic vasoconstriction and vagal

bradycardia (Sun & Reis, 1994) or a metaboreflex in the respiratory muscles which is thought to initiate sympathetic outflow and vasoconstriction (Dempsey *et al.*, 2006).

In hypoxia (SaO_2; 73%) for healthy subjects it is reported that when HR and cardiac output decreases 20%, BP increases 10% during maximal exercise, and that vagal blocking resettles HR but not cardiac output or BP (Boushel *et al.*, 2001;Bogaard *et al.*, 2002) and further, that cardiac output is unaffected by ß-blockade during hypoxia (Bogaard *et al.*, 2002). These studies indicate that cardiac output is down-regulated during hypoxia. Could it be that the observed HR and BP in the COPD patients reflect the same mechanism? Of course it is unknown whether the SaO_2 in the COPD patients was low enough to trigger protection mechanisms to avoid toxic hypoxia in study I, but in support of this hypothesis, arterial chemo receptors of the carotid and aortic bodies increase their firing rate when PO_2 falls below 60 mmHg (SaO_2 approx.91%). Furthermore, it is likely that sensors in the medulla oblongata play an important role in the activation of a stereotype autonomic response causing both a peripheral vasoconstriction and vagal bradycardia (Sun & Reis, 1994). Although the discussion above is highly speculative, the point is to exemplify that other possible mechanisms exist that may be relevant for explaining our findings of reduced heart rate together with high arterial blood pressure in the COPD patients. However, a less complex mechanism may reduce O_2-supply in COPD patients: a reduced pump capacity of the heart may simply be induced by dynamic hyperinflation (trapping of air in the lungs), which deranges ventricular filling and ejection, disturbing cardiac output (Sietsema, 2001;Montes de *et al.*, 1996).

The findings in study II indicate that the balance between muscular metabolic capacity and O_2-supply in endurance-trained subjects is not altered compared to normal physically active subjects. This could be observed by the similar exploitation of the muscular metabolic capacity during both 2-KE (87% and 90%, respectively) and bicycling (37% and 37%, respectively). This conclusion is strengthened by inclusion of the control group of the COPD patients (study I), which consisted of sedentary subjects with significantly lower VO_2max (Fig. 16), and by including the data by Slettaløkken et al. (2010), who applied the same exercise modes as in studies I and II on congestive heart failure patients, coronary artery disease patients and a control group. For the groups in Slettaløkken et al. (2010), the muscular mass-specific VO_2 as measured during 1-KE ranged from 272 to 340 $ml \cdot kg^{-1} \cdot min^{-1}$ (ns). During 2-KE they exploited 91%, 82% and 86% of their mass-specific VO_2 as measured during 1-KE, and during bicycling they achieved 24% - 31%, respectively, with no statistical differences between groups.

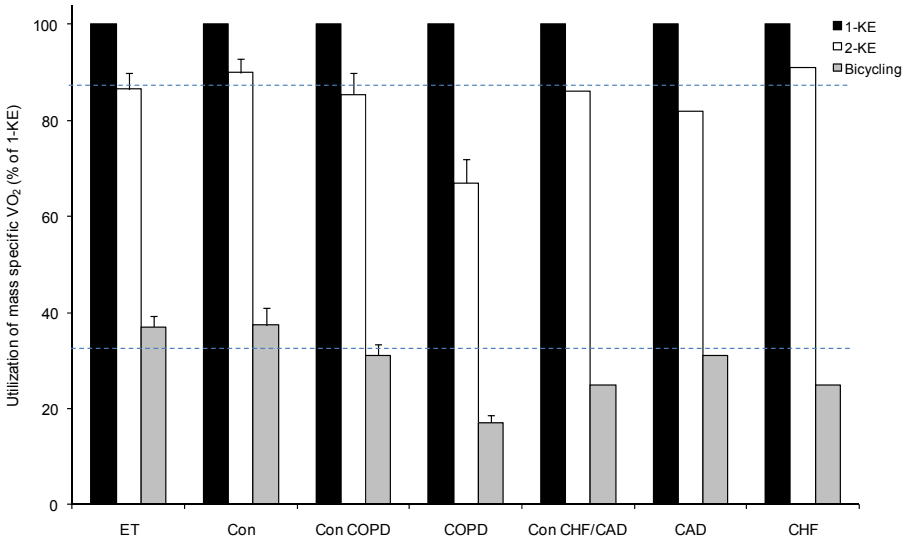

Figure 16. Average utilization of the muscular mass-specific VO_2 during 2-KE and bicycling for different groups. Muscular mass-specific VO_2 was calculated from VO_2max achieved during 1-KE. 1-KE; one-legged knee extensions, 2-KE; two-legged knee extensions, ET; endurance trained study II, Con; controls ET, Con COPD; controls COPD study I, CAD; coronary artery disease patients CHF; congestive heart failure patients, Con CHF/CAD; controls to CAD and CHF. Data from the three last groups are adapted from Slettaløkken et al. (2010). Broken lines indicate the averages for 2-KE (upper) and bicycling (lower).

The four healthy groups from these three studies exploited 86%-91% of the muscular metabolic capacity during 2-KE, and 25% - 37% during bicycling, despite a large range of VO_2max values(32 – 64 $ml \cdot kg^{-1} \cdot min^{-1}$). Hence, the difference in exploitation of the muscular metabolic capacity during bicycling was 12% while VO_2max varied up to 100%. These findings therefore support the hypothesis by Taylor and Weibel (1981) that each step in the O_2 pathway is built in a reasonable manner and is matched to the functional capacity of the organism. However, the severity of the central limitations of the COPD patients remains significant: judging from the relative metabolic reserve during 2-KE and bicycling, the COPD patients still have the most pronounced central limitation, even after including a heart failure and an angina group. Therefore, COPD patients may be a challenge to the Taylor and Weibel (1981) hypothesis in that their disease reduces their O_2 supply relatively more than their muscular metabolic capacity. Indeed, in our studies, COPD patients had the highest metabolic reserve capacity (83%) not only during bicycling, but also during exercise with a muscle mass of only 3.4 kg/2-KE (34%). However, the analysis can be turned on its head by saying that even by inclusion

of the COPD patients the Taylor and Weibel hypothesis (1981) remains quite robust. This point of view is supported by noting that although the mass-specific metabolic capacity ranges from 264 to 570 $ml \cdot kg^{-1} \cdot min^{-1}$ among the groups, the exploitation of the metabolic capacity during bicycling ranges from only 17% to 37% (Fig. 16). Of note in this regard is the significant difference in aerobic fitness of 400% evaluated from VO_2 max (16-64 $ml \cdot kg^{-1} \cdot min^{-1}$) in these groups. Plotting the data for the mass-specific VO_2 for 1-KE against mass-specific VO_2 for bicycling indicates predictability between the two variables, although some heterogeneity exists within the groups. Finally, the relationship still remains if calculated from VO_2 without muscle mass corrections (Fig. 17).

In summary, the muscular metabolic capacity differs significantly among the ET and the other groups, probably mainly due to training status. However, the relatively equal exploitation of the metabolic capacity for the healthy groups during bicycling indicates that the balance between muscular metabolic capacity and oxygen supply capacity is in balance in those groups of various training status. The COPD patients have a relatively higher skeletal muscular mass-specific metabolic reserve during whole body exercise compared to the other groups. This is due to a combination of a relatively preserved skeletal muscular metabolic capacity and their lung disease which limits muscular oxygen supply in an abnormal way.

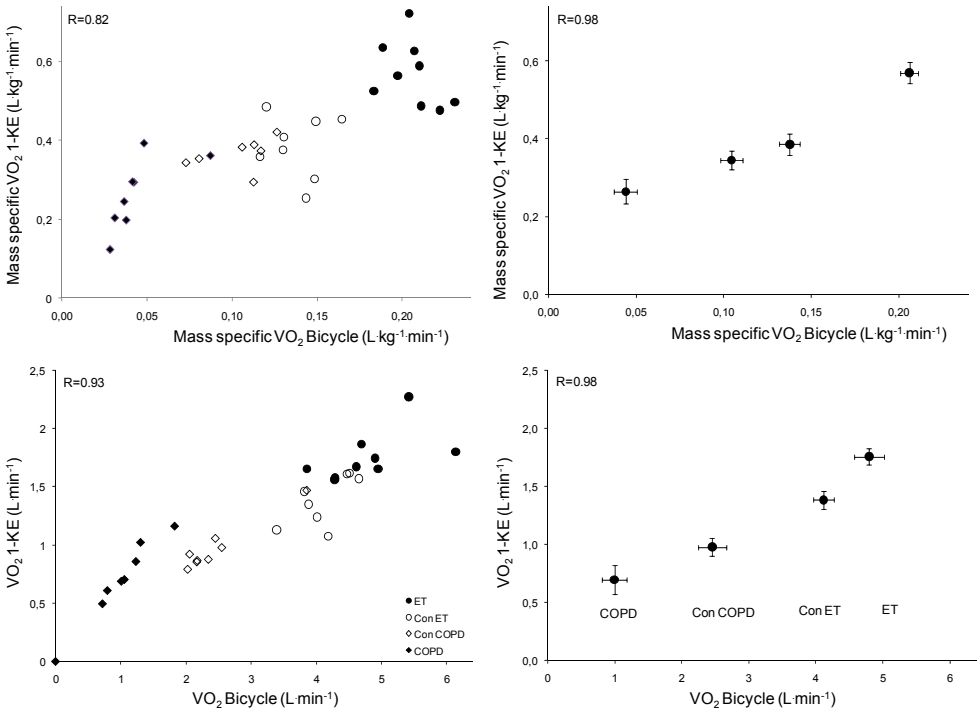

Figure 17. Highest achieved oxygen uptake during 1-KE; one-legged knee extension exercise, plotted against highest achieved oxygen uptake during bicycling. The upper figures show mass-specific VO2, while the lower show whole body VO2. The left figures show individual data from studies I and II, while the right figures show the mean of the groups ± SE. COPD; chronic obstructive pulmonary disease patients, Con COPD; controls of COPD patients (study I), ET; endurance-trained subjects, ConET; controls of the ET (study II).

9.2 Study III

Seven weeks of one-legged endurance training in endurance-trained subjects did not increase their VO_2max for bicycling although one-legged VO_2max was higher with the trained than with the control leg after the training period. During high intensity bicycling exercise with the two legs working at precisely the same intensity, VO_2 was higher in the trained leg as a consequence of both higher blood flow and O_2 extraction. However, that the enhanced VO_2max in the trained leg had no implication for bicycling VO_2max provides additional support for a central limitation to VO_2max during whole-body exercise. It also indicates that the training status of the exercising muscles may influence oxygen delivery and uptake when muscles with different training status (oxidative capacity) exercise at the same time, for instance during simultaneous leg and arm exercise as indicated in Study IV.

Effect on muscle oxidative enzymes

After training, citrate synthase activity was 30% (12%) higher ($P<0.05$) and 3-hydroxyacyl-CoA dehydrogenase activity tended to be higher by 23% (14%) ($P=0.12$). Hence, one-legged endurance training in addition to regular endurance training affects the oxidative capacity in endurance-trained subjects. The large interindividual variation in training response on these variables may be an effect of variation in the training status among the subjects, despite the fact that they all were endurance trained.

Effect on performance and physiological response during one-legged cycling and bicycling

During submaximal one-legged cycling, pulmonary VO_2 and HR were not different between the control and the trained leg, and there was no difference between the legs in lactate and ventilation at the lowest workload. At the two highest submaximal workloads (120/150W and 160/200W), lactate was significantly lower in the trained leg (19% [6%] and 24% [7%], respectively, $p<0.05$). Ventilation was 3.4% (4.2%) lower ($p<0.05$) at 120/150W workload, and tended to be lower (3.6%, $p=0.08$) at 160/200W workload (Fig. 18).

During maximal one-legged cycling subjects achieved 6.7% (2.3%) higher VO_2, 13.1% (3.0%) higher lactate, 9.7% (4.0%) higher ventilation, 3.2% (0.7%) higher HR, 9.5% (2.0%) higher power output and 30.0% (6.6%) longer time to exhaustion with the trained leg compared to the untrained leg ($p<0.05$ for all variables) (Fig. 18). The lower lactate combined with no difference in VO_2 and HR during the submaximal exercises indicate that the increased work capacity during the maximal exercise is caused more by muscular adaptations than cardiovascular adaptations.

For the two highest submaximal bicycling workloads, there was a tendency for lower lactate (~10%) after the training period (p=0.12-0.07), while there were no differences in VO_2, ventilation or HR before and after the training period (Fig. 18).

During maximal bicycling exercise, only lactate was lower after training (11% [1.9%], $p<0.05$), otherwise there were no differences (Fig. 18).

The relatively small difference in VO_2max between the trained and untrained leg in the present study compared to previous studies can probably be explained by differences in methods and training status of the subjects. Davies and Sergeant (1975) and Klausen et al. (1992) reported 14% and 19% increase in one-legged VO_2 max after 4 and 8 weeks of one-legged training, respectively. However, both these studies reported the increase in one-legged VO_2 max as the difference between pre and post training for the trained leg, which did not control for a familiarizing effect. Within the previous studies only Gleser (1973) and Saltin et al. (1976) compared the trained and untrained leg separately after the training period. These studies reported 8% and 14% higher VO_2max in the trained compared to the untrained leg, respectively. However, the training status of the subjects in these studies was lower based on the reported VO_2max of 46 $ml \cdot kg^{-1} \cdot min^{-1}$ (similar in both studies). This may explain why they found a more pronounced training response compared to the present study.

The results from both the submaximal as well as maximal bicycling exercise support the finding during the one-legged exercise, that the training resulted in only local muscular adaptations. These adaptations were significant and gave lower lactate during submaximal exercise both with one and two legs and after maximal exercise with two legs. However, during maximal one-legged exercise, lactate increased in concert with increased one-legged VO_2max and exercise capacity. That the increased VO_2max in the trained leg had no implication for bicycling VO_2max lends support to existence of a central limitation to VO_2max during whole-body exercise. From these data we can conclude that, even in endurance-trained subjects, extra one-legged training increases muscular metabolic capacity. The results also suggest that the increased metabolic capacity can be linked to an increased oxidative metabolism.

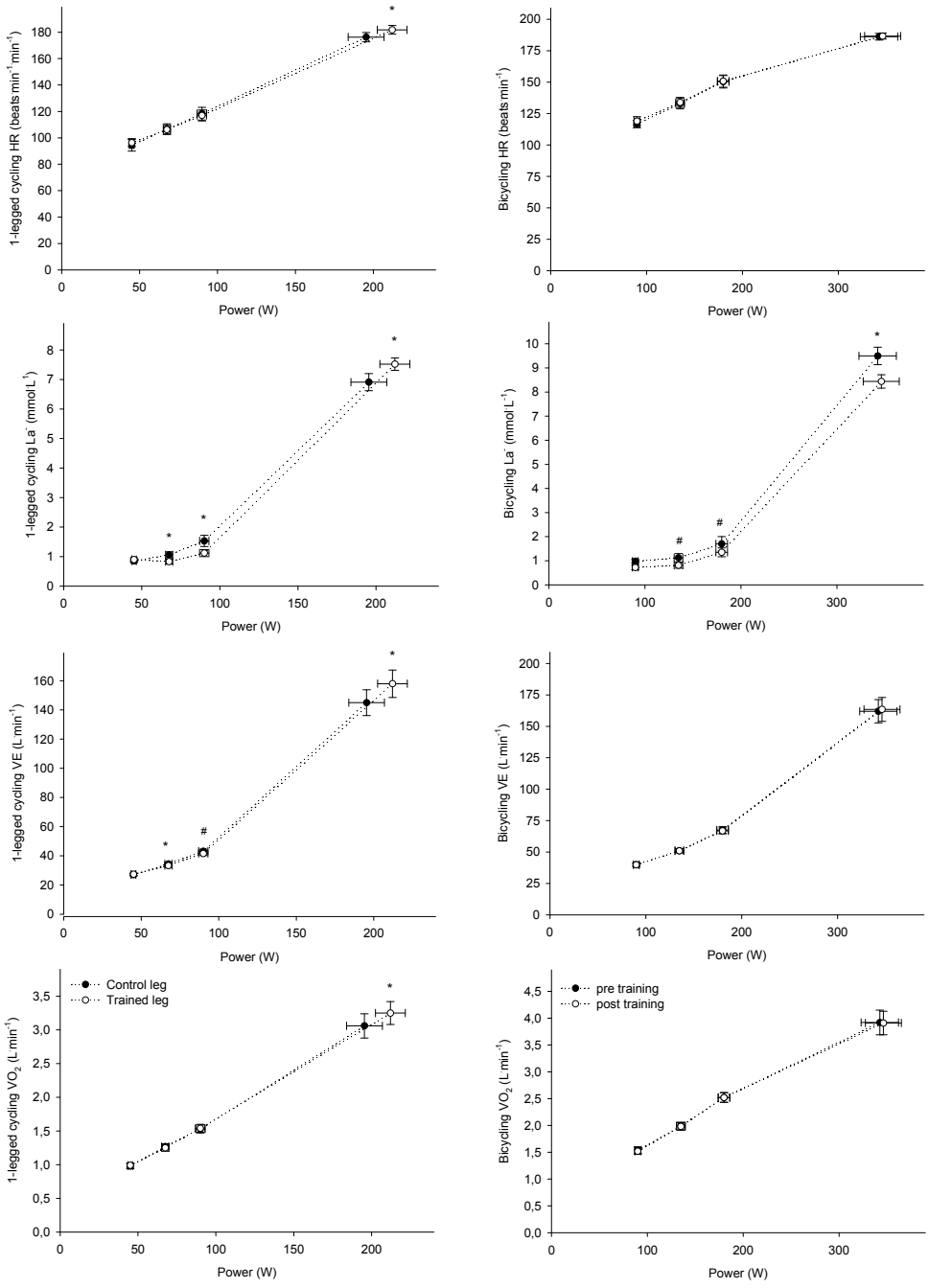

Figure 18. Effects of increased workload (power) during one-legged cycling and bicycling HR; heart rate, VE; ventilation, La-; blood lactate and VO_2; pulmonary oxygen uptake, n=12. Asterisks and numbers sign indicate p value less than 0.05 and 0.1 between the trained and control leg or pre and post training, respectively.

Effects on a-v O_2diff, blood flow and VO_2 during bicycling with equal power output for each leg

There were no differences in a-v O_2diff, blood flow or VO_2 between control and trained leg during the two lowest exercise intensities. During the high intensity exercise (>90% of VO_2max) the a-v O_2diff was 4.2% (1.2%) higher ($p<0.05$) and blood flow was 16.0 % (7.0%) higher ($p=0.06$) in the trained compared to the untrained leg (Fig. 19). Oxygen extraction tended to be 1.3% (0.7%)($p=0.1$) and was 3.5% (1.1%) higher in the trained leg during the two highest workloads ($p<0.05$)(Fig. 20). The higher extraction and blood flow resulted in 21.1% (7.8%) higher VO_2 in the trained leg compared to the control leg during high intensity bicycle exercise ($p<0.05$). Results from the pulmonary one-legged cycle tests and the bicycling tests suggested that the trained leg released less lactate than the control leg at the same exercise load; therefore it was interesting to examine whether lactate difference was detectable between the legs during bicycling. Unfortunately, technical problems resulted in an incomplete lactate balance data set (n=6-7). However, the mean lactate release was lower for the trained leg during the low and moderate intensity exercises ($p=0.19$ and 0.12; n=7), which may be a type II error. For the high intensity exercise the legs were more similar, possibly indicating that the higher oxidative capacity didn't affect the anaerobic metabolism during high intensity exercise (n=6) (Fig. 21).

Despite the 16% higher blood flow in the trained leg during the high intensity exercise, the extraction was also 3.5% higher compared to the control leg. This finding contradicts some previous findings: in cross country skiers it has been suggested that more blood for a given VO_2 is supplied to the arms as a compensatory mechanism for lower O_2 extraction than the legs (Calbet *et al.*, 2005). Furthermore, the reported two- to threefold higher blood flow per kg active muscle during 1-KE (muscle mass 2-3 kg) compared to bicycling (whole-body exercise) compromises extraction probably because of a shorter mean transit time (Andersen & Saltin, 1985;Richardson *et al.*, 1995b;Richardson *et al.*, 1993;Richardson & Saltin, 1998;Richardson *et al.*, 1999a). Therefore, because increased flow is associated with lower extraction, the present study is novel in that both extraction and blood flow are higher in the trained leg.

With power output being similar in the control and trained leg, and oxygen uptake being higher in the trained leg, it is logical that the metabolic stress was higher in the control leg, which is supported by the lactate data. This supports the finding by Gonzalez-Alonso *et al.* (2001), who reported that red cell deoxygenation is important for regulation of blood flow. In a bicycling scenario, this mechanism may be more important than the metabolic stress per se. The 30% higher activity of CS in the trained leg indicates that part of the higher extraction in the trained leg may be linked to higher oxidative capacity of the mitochondria (HAD in trained leg was 123% of the control leg, $p=0.12$). However, we

cannot exclude the possibility that adaption of the capillaries has also taken place thus increasing mean transit time of haemoglobin and facilitating O_2 offloading.

In summary, one-legged endurance training in addition to the regular endurance training in endurance-trained subjects increased muscle oxidative enzymes, and one-legged, but not bicycling, VO_2max. During submaximal exercise, only lactate metabolism and ventilation were affected, with no differences in HR, pulmonary VO_2, leg VO_2, a-v O_2diff or blood flow. During high intensity exercise, despite the fact that pulmonary VO_2max was not affected, leg VO_2, blood flow and O_2 extraction were higher in the trained leg than the control leg. The higher blood flow and higher O_2 extraction in the trained leg during intense bicycling indicates that not only O_2 supply, but also muscular metabolic capacity, influences muscular oxygen uptake. The higher blood flow in combination with lower venous O_2-content is compatible with the hypothesis that red cell deoxygenation may play a role in control of local tissue perfusion by overriding an increase in sympathetic vasoconstrictor activity during exercise. The unchanged submaximal HR and pulmonary VO_2max during bicycling indicate no increase in cardiac output, suggesting that blood flow was redistributed to the trained leg at the expense of the control leg during high intensity exercise.

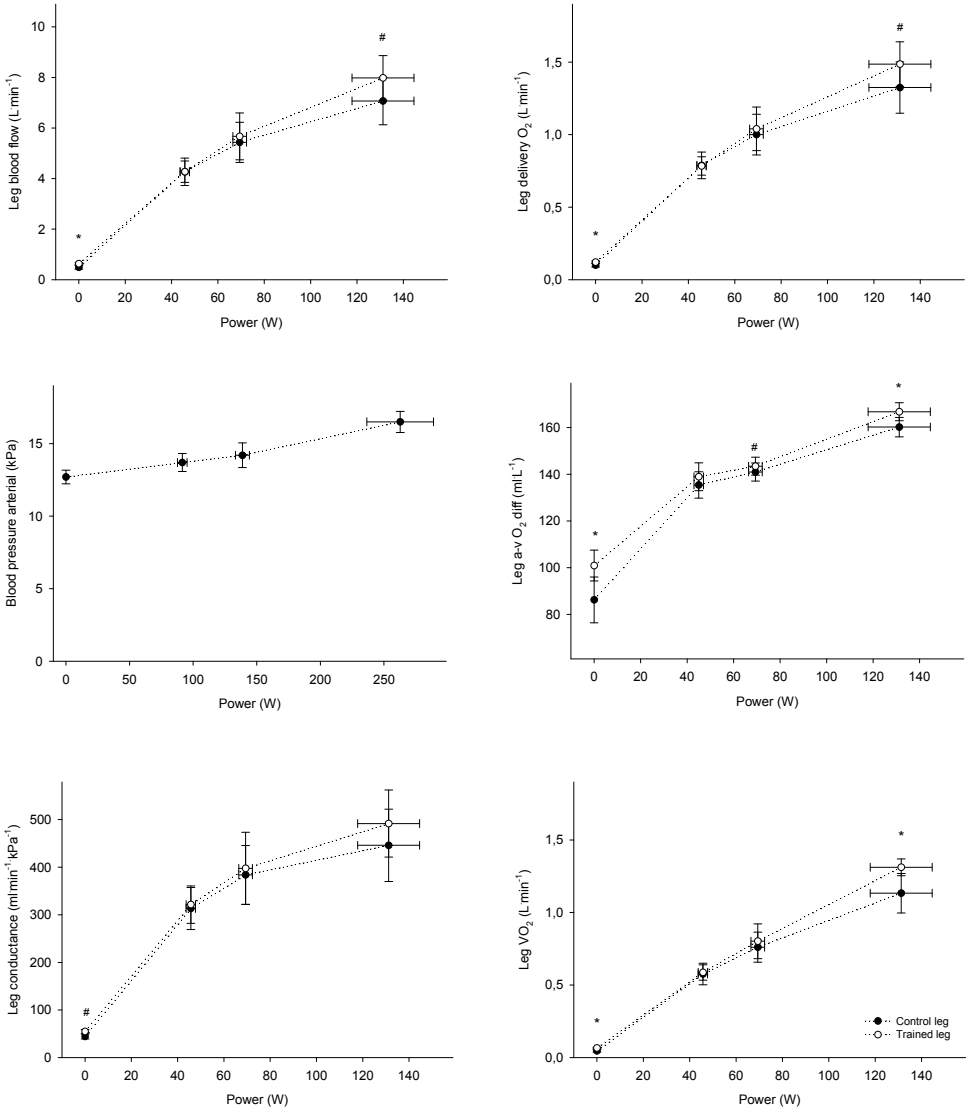

Figure 19. Leg blood flow (a); blood pressure (b) ; leg conductance(c) ; leg delivery O_2 (d) ; leg arterial-venous O_2 diff (e) and leg oxygen uptake (f) during submaximal cycling exercises at different power outputs after 7 weeks of one-legged endurance training. Data are mean±SE (n=7-8). Asterisks and numbers indicate p value less than 0.05 and 0.1 between the trained and control leg, respectively.

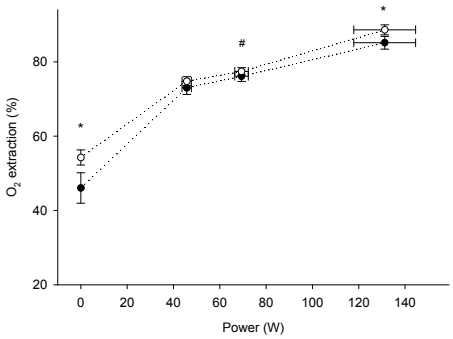

Figure 20. Leg oxygen extraction during cycling at different intensities. Data are mean±SE (n=7-8). Asterisks and numbers sign indicate p value less than 0.05 and 0.1 between the trained and control leg, respectively.

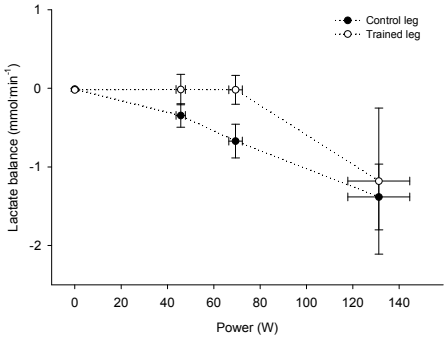

Figure 21. Lactate release during cycling at different workloads. Data are mean±SE (n=6 during the highest workload, otherwise n=7-8).

9.3 Study IV

This study contributes to this thesis by introducing double poling, a cross country ski technique that involves both arms and legs and hence a large muscle mass. During double poling the workload is shared between the arms and legs and the relative distribution between the upper- and lower body is determined by the skiers' technique. During low workload, the arms and legs contribute equally, as shown by similar oxygen uptake in arms and legs. However, when the workload is increased, the double poling technique changes towards more emphasis on leg exercise, as indicated by 33% larger increase in oxygen uptake in legs than arms. The different responses of arms and legs were associated with changes in technique toward more mechanical work done by the legs due to increased knee flexion during each poling stroke. Hence, even if a large contribution from the arms is advantageous from a biomechanical point of view, arm muscle mass is probably too small at higher workloads and therefore more of the work must be carried out by the legs. The increase in leg VO_2 is a consequence of both increased leg blood flow and O_2 extraction, while arm VO_2 increases only as a result of increased blood flow.

A-v O_2 diff, extraction, blood flow, VO_2 and lactate

During the low and moderate intensity workloads the a-v O_2 diff was 15% (4.1%) and 26% (3.8%) and O_2 extraction 8.2% (2.2%) and 15% (2.2%) higher in the legs than the arms ($p<0.05$), while there were no significant differences for blood flow. For VO_2, there was no significant difference between arms and legs at the low workload, while it was 78% (33%) higher in the legs than the arms at the highest workload ($p<0.05$) (Fig. 22).

The a-v O_2 difference was similar for the arms at the two exercise loads, while it increased 12% (1.2%) from the low to the moderate exercise for the legs ($p<0.05$). Similarly, O_2 extraction didn't change with workload for the arms, while it increased 8.1% (0.9%) for the legs ($p<0.05$). Blood flow increased 20% (6.8%) and 44% (10.0%) from the low to the moderate workload for arms and legs, respectively ($p<0.05$) and VO_2 therefore increased 23% (5.7%) and 54% (8.0%) for arms and legs when the workload increased from 82 to 117 Watts ($p<0.05$) (Fig. 22). The increases from low to moderate workload for a-v O_2 diff and VO_2 were 13% (11.1%) and 40% (12.4%) higher for the legs than the arms ($p<0.05$), while the increase in blood flow was not significantly different between the extremities ($p=0.14$).

At the low workload, arms released 1.62 (0.26) $mmol \cdot min^{-1}$ lactate while the legs had an uptake of 0.92 (0.24) $mmol \cdot min^{-1}$ ($p<0.05$). At the moderate workload the lactate release in arms tended to increase to 2.35 (0.42) $mmol \cdot min^{-1}$ ($p=0.07$), while the legs doubled their uptake to 1.87 (0.22) $mmol \cdot min^{-1}$ ($p<0.05$, included between arms and legs) (Fig. 23). At both workloads the arms released potassium while there was no significant arterial-venous difference for potassium over the legs (Fig. 24).

Kinetic and kinematic observations

The increase in oxygen to the legs corresponded with increased mechanical work by the legs to counteract gravity (70 [6.5] to 100 [9.1] J per stroke) ($p<0.05$)

Kinematic changes associated with increased workload by the legs included increased knee angle range of motion from 51 (8.9)° to 58 (9.9)° and vertical displacement of the hip from 10 (1.0) to 14 (1.0) cm ($p<0.05$). Trunk angle however, did not change from low to moderate intensity. Further, the elbow angle range of motion tended to increase from 71 (11.3)° to 75 (10.9)° ($p=0.07$) and the net horizontal poling displacement increased from 1.41 to 1.47 m, while the poling cycle time was reduced from 1.41 to 1.34 seconds as sub-maximal workload increased ($p<0.05$).

It has previously been show that the arms extract less of the supplied O_2 during arm exercise compared to leg exercise (Secher *et al.*, 1977;Volianitis & Secher, 2002;Calbet *et al.*, 2005;Clausen *et al.*, 1973). In our study the oxygen uptake in the legs increased by increasing both blood flow and O_2 extraction, while the oxygen uptake in the arms increased only by elevation of blood flow. This supports previous findings where oxygen uptake increases during arm cranking without any increase in O_2 extraction in normal fit subjects (Ahlborg & Jensen-Urstad, 1991;Volianitis *et al.*, 2004) In competitive rowers on the other hand (VO_2max; 68 $ml \cdot kg^{-1} \cdot min^{-1}$), extraction increases when the VO_2 in arms increases (Volianitis et al., 2004). However, from our study it seems that during combined leg and arm exercise, O_2 extraction do not increases even in fit individuals (VO_2max ; 66 $ml \cdot kg^{-1} \cdot min^{-1}$). This is despite the fact that fit skiers (VO_2max; ~70$ml \cdot kg^{-1} \cdot min^{-1}$) are capable of increasing extraction in arms (by 7%-10%) from double poling to diagonal stride technique and from submaximal to maximal diagonal stride (Calbet *et al.*, 2005;Bjorklund *et al.*, 2010).

Interestingly, the oxygen uptake in arms is higher during submaximal double poling than during maximal diagonal striding (Calbet *et al.*, 2005), which indicates that the intensity in arms during the moderate workload was close to maximal in our subjects. However, the blood flow was also higher during double poling compared to the diagonal stride in the skiers in the study by Calbet et al.,, indicating that part of the lower extraction may be due to a blood flow over perfusion, as discussed

for exercise of small muscle masses like 1-KE (Richardson *et al.*, 1999a). Applying this data to the findings in study III does not result in a good fit; in study III the higher blood flow in the trained leg was explained by a regulatory effect due to the higher O_2 extraction, while in study IV, the arms have a high perfusion together with a relatively low extraction. Furthermore, flow increased in arms from low to moderate workload without changes in extraction, indicating that blood flow increases because of another mechanism than O_2 deoxygenation of the haemoglobin molecule (Gonzalez-Alonso *et al.*, 2001).

The arms also had a net release of potassium, while there was no net uptake or release in the legs. The potassium balance over the legs was not different between workloads. Potassium is part of the signaling system in the muscle as opposed lactate, which is an intermediate in the energy metabolism. The potassium efflux over the sarcolemma is connected to the depolarization of the membrane. The high potassium release and the low extraction may have a common physiological explanation. If the arm muscles exercise close to their maximal capacity the local vasodilatory signals elicited by the muscles are probably close to maximal. Also because of the high activation of the arm muscle, potassium efflux due to plasma membrane depolarization is large. However, because of the small muscle mass the central cardio-respiratory stress is moderate, and thus the sympatho-adrenergic activation is moderate. Hence, both adrenergic stimulation of the sodium-potassium pump and adrenergic vasoconstriction may be suboptimal and cause a low potassium reuptake and an overperfused muscle. Although speculative, the latter will cause a high oxygen delivery compared to the capacity of the muscles and hence a low extraction. Potassium reuptake over sarcolemma is accomplished by the sodium-potassium pump (Na^+/K^+-ATPase). Hence, the fact that the lactate and potassium response is different when workload is increased is not unexpected.

In summary the double poling at moderate workload in our study may be close to the maximal capacity of the arms to perform this type of exercise, as evidenced by the high arterial lactate concentration and potassium release from the arms. The increase in workload was thus met by a change in technique, implying more work done by the legs, which is supported by both the kinematic data and the higher increase in VO_2 in the legs. The similar O_2 extraction together with net potassium release at both low and moderate workload may indicate another mechanism in bloodflow regulation than haemoglobin deoxygenation. From a skiers perspective is it likely that the demand on the arms would be reduced with the diagonal stride. Therefore, in the field the diagonal stride would probably be the skiers' choice at least at moderate workload.

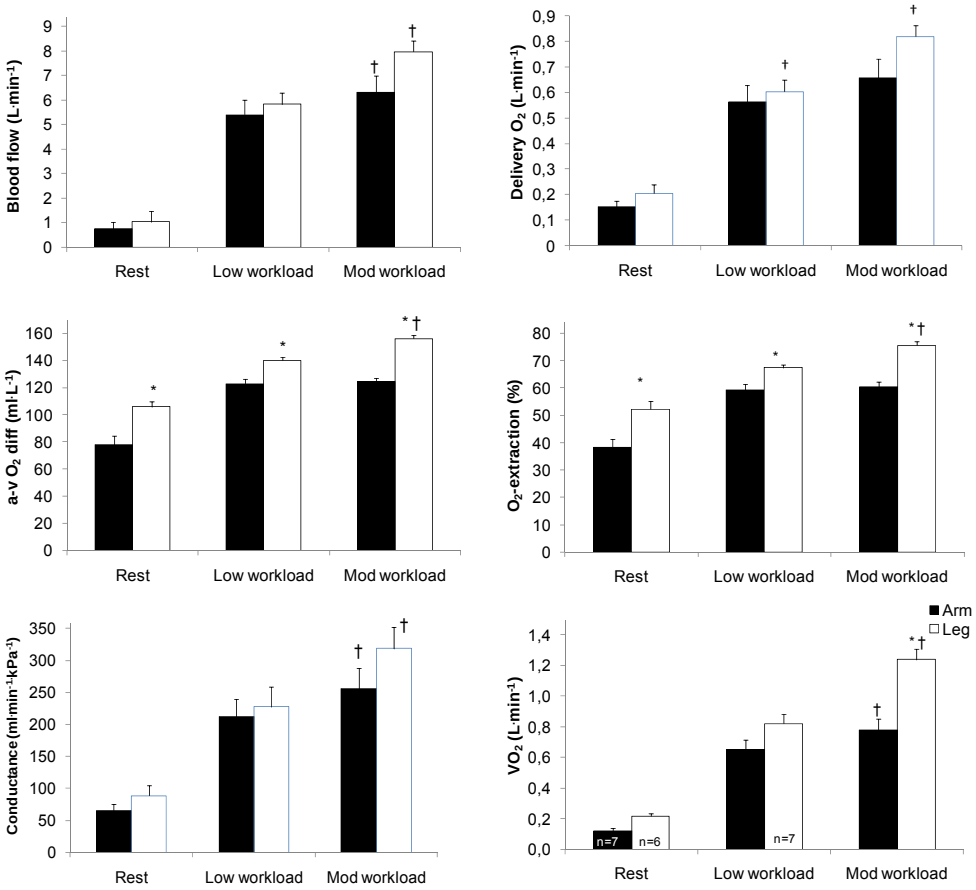

Figure 22. Blood flow, arm and leg a-v O_2 diff; arterial – venous oxygen difference, conductance; vascular conductance, Delivery O_2; delivery oxygen, O_2-extraction; oxygen extraction and VO_2; oxygen uptake plotted at different workloads. Asterisks indicates statistical significant difference between arm and leg (P<0.05) and cross sign statistical significant difference between workloads (P<0.05). N is as indicated otherwise and for a-v O_2 diff n=8.

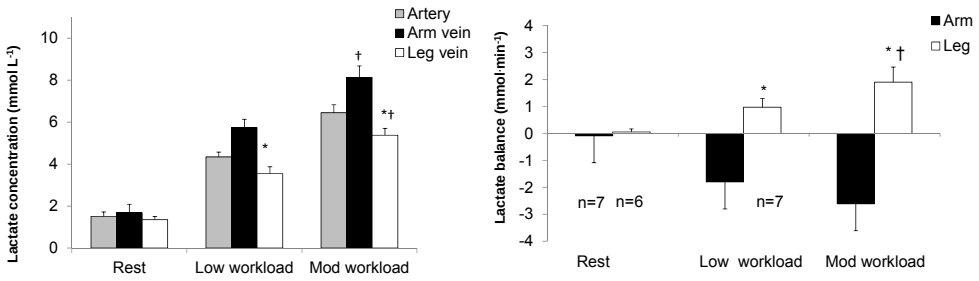

Figure 23. Left: Arm and leg arterial and venous lactate concentration at rest and during easy and moderate workloads. The venous lactate concentration was always different from the arterial during work (P<0.05) and tended to be lower for legs during rest (P=0.07). Right: Lactate balance in arm and leg at rest and during easy and moderate workloads. Negative values means net release, and vice versa. Both the arms and legs had a significant release or uptake during both workloads (p<0.05). Asterisks indicate a p value less than 0.05 between arm and leg, and cross sign between workloads. For the right figure n at rest is as indicated and for leg during the easy workload n=7; otherwise n=8.

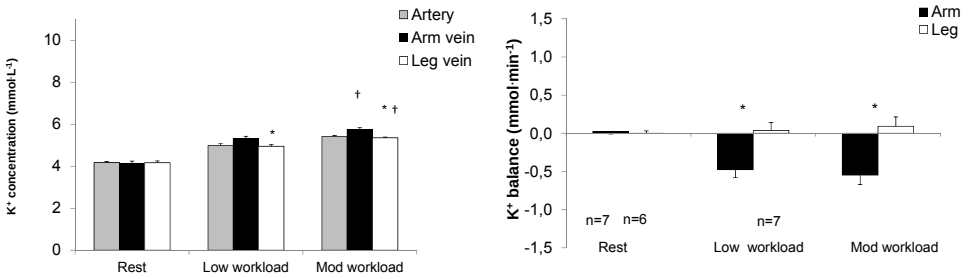

Figure 24. Left: Arm and leg arterial and venous potassium (K^+) concentration at rest and during easy and moderate workloads. The venous lactate concentration in the arms was higher than the arterial and was lower than the legs during work (P<0.05), while the legs not was different from the arterial concentrations. Right: K^+ balance in arm and leg at rest and during easy and moderate workloads. Negative values means net release, and vice versa. Arms had a significant release during both workloads and was different from the legs (p<0.05). Asterisks indicate a p value less than 0.05 between arm and leg. For the right figure n at rest is as indicated and for leg during the easy workload n=7; otherwise n=8.

10 Conclusions

1. The balance between skeletal muscular oxidative capacity and capacity of the cardiovascular system to supply oxygen is relatively unchanged in endurance-trained subjects compared to controls with normal physical activity levels. Further, the balance is still relatively unchanged when including groups of different ages and training status. However, in COPD patients where the capacity to supply oxygen is severely reduced, this balance is disturbed.

2. The COPD patients have a relatively higher metabolic reserve capacity not only compared to their control subjects, but also compared to young endurance-trained subjects. Therefore, COPD patients are relatively less limited by their skeletal muscular metabolic capacity than healthy subjects during whole body exercise.

3. Increased oxidative capacity in one leg, induced by one-legged training, increased one-legged cycling VO_2max but not bicycling VO_2max. Hence, oxidative capacity of the muscles does not limit VO_2max during whole body exercise. During high intensity bicycling with both legs working at precisely the same power output, the trained leg had higher O_2 extraction and blood flow resulting in higher VO_2 compared to the control leg. This indicates that increased muscular oxidative capacity not only increases O_2 extraction, but also facilitates O_2 delivery by influencing blood flow distribution. Together, these results indicate that increased oxidative capacity of the muscles has little or no influence on VO_2max during whole body exercise, but merely influences distribution of oxygen delivery and uptake between the different exercising muscles.

4. When workload increased during simulated cross country skiing with the double poling technique, VO_2 increased more in legs than in the arms. O_2 extraction increased only in the legs and only the arms had a net release of lactate. This indicated that the arm muscles were working close to their maximal oxidative capacity without exhausting the maximal cardiac output and that the arms probably were overperfused in a similar way as m. quadriceps during one-legged knee extension exercise. In this situation the oxidative capacity of the arms limits their exercise capacity, even during whole body exercise.

11 Perspectives

As suggested from studies I and II, there seems to be a balance in muscular metabolic and oxygen supply capacity adaptations independent of training status. The healthy groups included young endurance-trained subjects, young normal physically active subjects and middle-aged healthy subjects. The wide range of activity in the healthy group indicates that both central and peripheral factors adapt to the level of physical activity and therefore their relative proportion stays relatively unchanged. However, study III revealed that adding endurance training of a small muscle group in addition to the regular training in endurance-trained subjects can disturb the balance by increasing only the muscular metabolic capacity. In this regard it would be interesting to measure whether the ratio of the muscular mass-specific capacity to oxygen supply capacity was different between the trained and control leg in study III. This could have been done by use of the one-legged knee extension model and bicycling as in studies I and II, but the increased ratio of one-legged cycling VO_2max/whole body cycling VO_2max for the trained leg suggest that an increased mass-specific capacity has taken place. However, elite endurance-trained athletes who are close to, or at their potential of O_2 supply capacity, can theoretically benefit from an increase in their muscular metabolic capacity. This can be achieved by use of endurance training of relatively small muscle groups that are central to their sport activity. An advantage of using small muscle groups could be that the workload and metabolic turnover of the active muscle mass can be increased compared to whole body exercise, therefore increasing the muscular training stimulus, as indicated in study III. However, we cannot conclude that training intensity was more important than the increase in total training volume for the trained leg. Nevertheless, adding specific endurance training of the arms to the regular endurance training, for example, may be a way to increase the O_2 extraction in arms in cross country skiers to a level seen in legs (study IV).

In accordance with the Fick's equation, increased extraction should be reflected as increased whole body VO_2max, but as may be indicated in study III, this will depend on the muscle mass in which the adaptations have taken place. For example, the phenomenon of distribution of blood flow away from the untrained leg (steal effect), as indicated in study III, suggests that local muscular training must be performed on the majority of muscles important for the sport activity in order to confer an advantage on whole body VO_2max or maximal aerobic power (Abbiss *et al.*, 2011). However, increased whole body VO_2max per se is probably not the only advantage in this context. Training of small muscle mass can increase exercise performance by increasing the ability to work at a higher percentage of VO_2max for a longer duration. However, if the hypothesis of maintenance of the

balance between peripheral and central capacity is correct, then such small muscle mass training has to be continued to avoid a decline over time due to a “balance resettling”.

Small muscle mass training may be even more appropriate for COPD patients than healthy people due to their O_2 supply limitation. Indeed, it has been reported that cycling training with one leg at a time increases whole body VO_2max more than two-legged cycling for COPD patients (Dolmage & Goldstein, 2008). However, we found evidence for O_2 supply limitations with a very small muscle mass (3.4 kg) suggesting that it could be an even greater advantage to reduce the exercising muscle mass further to optimize muscle O_2 supply during training and thereby train with a high muscular mass-specific VO_2. However, it can be that the metabolic stress during exercise is an important stimulus for increasing the muscular metabolic capacity compared to high O_2 availability per se, both in COPD patients and in healthy subjects. This means that the relative training intensity should be high, but that is for future studies to examine.

At the beginning of the work for this thesis, the meaning of the expression “metabolic reserve capacity” was reflected on because it suggests that the capacity is overbuilt for whole body exercise, challenging the hypothesis that all steps in the O_2 pathway are built in a reasonable manner. However, as discussed previously, the metabolic turnover will approach its capacity during high intensity exercise when the exercising muscle mass is low. Furthermore, a high metabolic capacity per se ensures that the muscles are able to exploit the supplied oxygen efficiently regardless of differences in O_2 transit time through the capillaries in subjects of different training and health status. For example, in COPD patients, the relatively preserved muscular metabolic capacity may reflect a compensatory mechanism to the central limitations of O_2 supply. Furthermore, the relatively low O_2 extraction in arm during double poling may support that a high muscular oxidative capacity is important to exploit the supplied O_2 even during whole body exercise.

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ERRATA

Thesis

Page 9, 3rd paragraph, #8: like **hyperoxia**-induced

Page 12, 1st paragraph: #1: **1999b**; #6: Richardson et al. (**1999b);** #8: **Richardson (1999);** #14 Richardson et al. **(1999a)**

Page 14, 2nd paragraph, #2: exercise capacity, **29** were endurance-trained

Page 19, 3rd paragraph, #3: immediately after ~~the test~~ arterial and venous

Page 31, figure 13: Y-axis label name **VO_2 ($ml \cdot min^{-1}$)**

Page 33, 1st paragraph, #22: Richardson et al. (**1999b)**

Page 55, reference missing: Hallen J, Saltin B, & Sejersted OM (1996). K+ balance during exercise and role of beta-adrenergic stimulation. *Am J Physiol* 270, R1347-R1354.

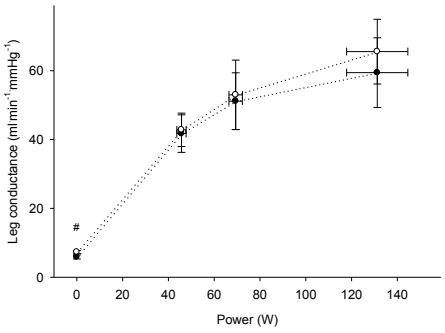
Paper II

Page 684, 3rd paragraph, #15: (age **51** year; VO_2max during…)

Paper III

Since submission to the doctorial committee paper III has been accepted for publication in a revised form. In regard to the methods and results the following changes should be noticed:

Page 20, figure 2c:



Page 21, figure 3: Y axis left and right side label unit **(μ**$mol \cdot g^{-1}$d.w.$\cdot min^{-1}$)

Page 23, table 1: VEmax; All subjects, **162 (32.0)**; Invasive study, **167 (32.2)** (Both; All)

13 Paper I-IV

I Rud B, Christensen CC, Ryg M, Edvardsen A, Skumlien S, & Hallen J (2008). Higher skeletal muscular metabolic reserve capacity in COPD patients than healthy subjects. *Scand J Med Sci Sports*.

II Rud B & Hallen J (2009). Is the balance between skeletal muscular metabolic capacity and oxygen supply capacity the same in endurance trained and untrained subjects? *Eur J Appl Physiol* **105**, 679-685.

III B. Rud, Ø. Foss, P. Krustrup, N.H. Secher and J. Hallén. One-legged endurance training: leg blood flow and oxygen extraction during cycling exercise (Paper in revision).

IV B. Rud, N. H. Secher, J. Nilsson, G. Smith, and J. Hallén. Metabolic balance between the arms and the legs during simulated skiing (Paper in preparation).

Paper I

Rud B, Christensen CC, Ryg M, Edvardsen A, Skumlien S, & Hallen J (2008). Higher skeletal muscular metabolic reserve capacity in COPD patients than healthy subjects. Scand J Med S*ci Sports*.

Scand J Med Sci Sports 2008
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DOI: 10.1111/j.1600-0838.2008.00846.x

SCANDINAVIAN JOURNAL OF
MEDICINE & SCIENCE
IN SPORTS

Higher skeletal muscular metabolic reserve capacity in COPD patients than healthy subjects

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Accepted for publication 18 May 2008

We investigated the interaction between skeletal muscle exercise capacity and central restrictions using exercise modalities, which recruit differing levels of muscle mass in eight patients chronic obstructive lung disease (COPD) (FEV_1 % of predicted; 35 [SE 4%]) and eight healthy controls. Subjects performed conventional bicycling, two-leg knee extensor (2-KE) and single-leg knee extensor (1-KE) exercises. Maximal values for pulmonary $\dot{V}O_2$ (VO_{2max}), power output, blood lactate, heart rate, blood pressure, and arterial oxygen saturation of hemoglobin were registered. VO_{2max} in controls was 2453 (210), 1468 (124), and 976 (76) mL/min during bicycling, 2-KE and 1-KE, respectively. The COPD patients achieved 48% ($P<0.05$), 62% ($P<0.05$), and 81% ($P=0.10$) of the control values. The mass-specific VO_{2max} (VO_{2max}/exercising muscle mass) during 1-KE was 345 (25) and 263 (30) mL/kg/min ($P<0.05$) in controls and COPD patients, respectively. During 2-KE the controls and COPD patients achieved 85% (4%) and 67% (5%) ($P=0.06$) of the mass-specific $\dot{V}O_2$ during 1-KE, while during bicycling they achieved 31% (2%) and 17% (1%) ($P<0.05$), respectively. The COPD patients have central restrictions when exercising with a relatively small muscle mass (2-KE) and have a higher muscular metabolic reserve capacity than controls during whole body exercise.

The reduced exercise capacity in severe chronic obstructive pulmonary disease (COPD) patients has been attributed to pulmonary and cardiovascular dysfunction. However, it is also recognized that, in the wake of the disease, COPD patients develop altered skeletal muscle morphology resulting in a shift toward a reduced percentage of type-I fibers, capillarization and oxidative enzymes (Jakobsson et al., 1995; Jobin et al., 1998; Whittom et al., 1998; Maltais et al., 2000; Allaire et al., 2004; Richardson et al., 2004). Studies are equivocal as to whether these muscular changes are linked to an exercise-induced skeletal muscle dysfunction or to muscle atrophy from lack of use in this population (Maltais et al., 1998; Richardson, 1999).

In normal healthy people, studies during exercise with a small muscle mass (using a single-leg knee extensor (1-KE) ergometer) it has been recognized that skeletal muscle has a capacity for blood flow and oxygen uptake that far exceeds the capacity of the heart during whole body exercise (Andersen & Saltin, 1985). Despite speculation of muscular dysfunction in COPD patients, they demonstrate an unused exercise potential when freed from central limitations (Richardson et al., 1999b), and it has even been demonstrated that their skeletal muscular metabolic capacity is comparable to healthy inactive subjects, although their mechanical efficiency is reduced (Richardson et al., 2004). This suggests that COPD patients are not limited by the muscular metabolic capacity of their skeletal muscles during whole body exercise. However, the level of muscle mass recruitment that is necessary to yield central limitations in COPD patients is currently unknown.

This study investigated the relationship between peripheral and central limitations in COPD patients during exercise with a small, medium, and large muscle mass using the knee-extensor model and bicycling. Our first objective was to quantify the muscular metabolic capacity using the established 1-KE exercise model. The second objective was to evaluate how much of the muscular metabolic capacity the patients could utilize during exercise with double the muscle mass of 1-KE and during whole body exercise. New knowledge in this area may be useful to better balance muscular metabolic challenge with oxygen supply capacity when designing effective exercise rehabilitation programs for COPD patients.

Materials and methods

Subjects

Eight patients with well-established and long-standing COPD and eight healthy controls participated in this study. The patients were included based on the level of reduced lung function during spirometry tests (FEV_1 <50%), ventilation (VE >85% of maximal voluntary ventilation [MVV]), and arterial hemoglobin desaturation (SpO_2 <86%) while walking

Table 1. Subjects' characteristics including estimated muscle masses during knee extension and ordinary bicycling exercise as well as spirometry data and blood gases at rest

Group/gender (female/male)	Age (year)	Height (cm)	Weight (kg)	Knee extensors muscle mass (kg)	Muscle mass during bicycling (kg)	FEV_1 (L/s)	FEV_1 (% Predicted)	FEV_1/FVC (%)	pH	$PaCO_2$ (kPa)	PaO_2 (kPa)
COPD											
M	67	172	78	1.6	20	0.83	28	29	7.44	4.9	8.4
M	63	174	80	2.2	21	1.10	35	28	7.41	5.5	7.6
F	59	152	53	1.7	13	0.91	48	71	7.42	4.9	8.8
F	64	160	65	1.9	16	0.89	41	38	7.40	4.9	8.3
M	54	180	83	1.9	22	0.83	23	30	7.35	7.0	8.0
M	60	169	104	2.0	28	0.95	31	45	7.34	7.5	7.2
F	49	163	66	1.3	17	1.04	40	40	7.46	4.0	9.6
F	58	160	57	1.2	15	0.80	35	39	7.42	4.9	8.3
Average	59#	166	73	1.7	19	0.92*	35*	40*	7.41	5.5	8.3*
SD	5	9	15	0.3	5	0.11	8	14	0.04	1.3	0.8
Controls											
F	42	160	64	1.3	16	2.80	105	82	7.41	4.9	12.7
F	61	174	68	2.0	17	2.72	99	85	7.42	4.9	11.9
F	61	180	72	2.0	18	3.59	115	89	7.41	4.6	11.1
F	57	169	64	1.8	16	2.39	111	86	7.41	4.6	12.3
M	51	176	110	2.8	30	3.59	101	87	7.44	4.4	12.2
F	48	167	72	1.6	18	3.66	116	91	7.40	5.2	13.2
M	50	174	74	1.9	20	2.88	81	78	7.42	4.3	10.4
F	40	178	86	2.6	20	3.04	93	67	7.42	5.6	11.4
Average	51#	172	76	2.0	20	3.09*	103*	83*	7.42	4.8	11.9*
SD	7	6	14	0.5	4.6	0.47	13	8	0.01	0.4	0.9

Statistical differences are indicated by numbers sign (#) for P-value <0.1 and $^*P<0.05$.

an incremental treadmill test to exhaustion (COPD GOLD stage III–IV, accorded to the GOLD criteria (GOLD, 2006)). Exclusion criteria for the COPD patients were arrhythmia during the treadmill inclusion test, left-sided heart disease (increased heart-evaluated by X-ray), previous heart infarction or high blood pressure ($>180/110$). The COPD patients were all receiving optimal medication, including inhalation corticosteroids; none were using supplemental oxygen and all were without cardiac pathology. All patients had a smoking history of at least 10 pack-years (daily cigarettes/20 $\times$ years of smoking) and had a clinically documented diagnosis of COPD for more than 5 years.

Control subjects were included by announcements in a local newspaper. Inclusion criteria were sedentary lifestyle with normal lung function, as evaluated by spirometry. All but one smoked. Subject characteristics including estimated muscle masses during knee extension and ordinary bicycling exercises, are recorded in Table 1. Subjects gave informed consent to participate in the study, which was approved by the Regional Ethical Committee and performed according to the Declaration of Helsinki.

Exercise protocols

Subjects performed three different exercise tests, each lasting 9–15 min with 3-min stepwise increments in intensity until exhaustion, defined as inability to maintain contraction frequency (60 rpm). The exercises modalities were one - leg knee extension (1-KE), two-leg knee extension (2-KE) and bicycling. Intensity increments for COPD patients were 2 W/leg during knee extensions and 10 W during bicycling; increments for the control subjects were double those of the COPD patients. The peak power output corresponds to the final increment lasting more than 60 s. All tests were performed in 1 day with at least 1 h rest between tests. The order of the knee extensor exercises (1-KE, 2-KE) was counterbalanced to avoid potential ordering effects, while all subjects performed bicycling last. The 1-KE test was counterbalanced between right and left leg. Pulmonary oxygen uptake ($\dot{V}O_2$), heart rate (HR), and arterial blood pressure (BP) were measured continuously before (10 min) and during exercise. Arterial blood samples were drawn immediately before exercise and at exhaustion for analysis of arterial oxygen saturation of hemoglobin (SaO_2), partial pressure of O_2 (PaO_2) and CO_2 ($PaCO_2$), pH, and lactate concentration. Subjects were familiarized with the tests on four separate days before experiment day.

Ergometers

Knee extension exercises were performed on a custom-built electromagnetically braked knee extensor ergometer as previously described by Andersen and Saltin (1985) and modified by Hallén et al. (1996). This ergometer ensures that only the quadriceps muscle group is used during extension of the lower legs (Richardson et al., 1998). Additionally a four points safety belt was employed to minimize body movements and the subjects were instructed to relax their non-exercising extremities. Bicycling was performed on a mechanically braked cycle ergometer (Ergoline 90 Jaeger Toennies, Würzburg, Germany).

Muscle mass calculations

The knee extensor muscle mass was estimated anthropometrically (Jones & Pearson, 1969). The thigh volume (V),

excluding subcutaneous fat, was calculated from the formula:

$$V = L(12\pi)^{-1}(O_1^2 + O_2^2 + O_3^2) - (S - 0.4)2^{-1}L(O_1^2 + O_2^2 + O_3^2)3^{-1}$$

where L is the thigh length estimated from the top of patella to spina iliaca anterior superior, O_1, O_2, and O_3 are the circumferences at the points 1/4, 1/2, and 3/4 between the patella and the spina iliaca anterior superior and S is the mean skinfold thickness posterior and anterior at the middle measuring point. Skinfold thickness was determined by a Harpenden skin-fold calliper. The knee extensor muscle mass was then calculated as: mass = $0.307V + 0.353$ and then corrected as suggested by Radegran et al. (1999): $\text{mass}_{\text{corr}} = 0.792\text{mass} - 0.382$. The active muscle mass during bicycling was estimated from gender and weight and corrected for age in accordance with Gallagher et al. (1997): women: active muscle mass = $(\text{Mb} \cdot 0.26 - (\text{age} - 20) \cdot 0.03_{\text{mr}})$; men: active muscle mass = $(\text{Mb} \cdot 0.29 - ((\text{age} - 20) \cdot 0.05_{\text{mr}}))$ where Mb is the total body mass and "mr" is the factor of annual muscle mass reduction.

Gas exchange and ventilation

Pulmonary gas exchange was measured breath by breath. Subjects breathed through a mouthpiece while the nose was sealed with a nose clip. The average of the last 2 min during rest and 60 s before exhaustion were used in further analyses. Gas exchange was measured with either an Oxycon Pro ($n = 10$), Oxycon Alpha ($n = 1$) (both; Eric Jaeger, Hoechberg, Germany) or Vmax29 ($n = 5$) (SensorMedics, Yorba Linda, Calif., USA) oxygen analyzer. The use of different exercise spirometry systems during the test period was due to system breakdown and is considered insignificant because each subject performed all three exercise tests with the same system and served as their own control. The gas analyzers were routinely calibrated against certified calibration gases of known concentrations, and the flow turbines were calibrated with a 3 L calibration syringe (5530 series, Hans Rudolph, Kansas City, Missouri, USA).

Arterial blood pressure and blood samples

Approximately 1 h before the first test, subjects were catheterized in arteria radialis, from where blood samples were drawn and blood pressure was recorded. Blood samples were analyzed for SaO_2, PaO_2, $PaCO_2$, pH, and lactate (ABL 725 Radiometer, Copenhagen, Denmark). Arterial blood pressure was measured with a pressure gauge (Baxter B.V., Uden, Holland) via a transducer amplifier (GOULD instrument systems, Valley View, Ohio, USA). Blood pressure was calibrated before each test using a column of water equal to 6.67 kPa. Blood pressure signals were AD converted and collected on a PC with a sampling rate of 100 Hz. Average of the last 60 s before exhaustion was used for further analyses.

Data and statistical analyses

The reported resting values for each variable are the average of the measured values before 1-KE, 2-KE and bicycling. Maximal oxygen uptake (VO_{2max}), HR and blood pressure from each exercise test are presented as mean values from the highest workload the subjects could perform for at least 60 s, maximal values for blood gases and pH were analyzed from the blood sample drawn at exhaustion. Differences between the groups were tested by homoscedastic Student's t-tests. Significance level was set at $P < 0.05$ (two-tailed), while $P < 0.1$ was considered a tendency. Results are presented as mean standard error of mean.

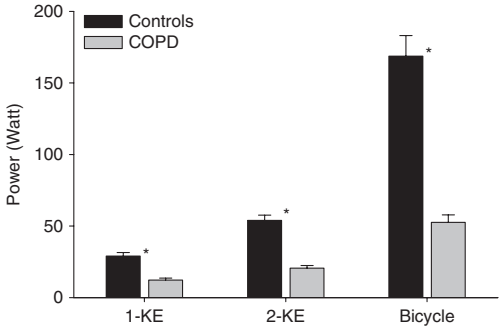

Fig. 1. Maximal power output during single-leg knee extensor exercise (1-KE, muscle mass ~ 2 kg) two-leg knee extensor (2-KE, mass ~ 4 kg) and bicycling (mass ~ 20 kg). *Indicates $P < 0.05$.

Results

Power output

Maximal power achieved by the controls was 29 (2), 54 (4), and 169 (14) W during 1-KE, 2-KE, and bicycling, respectively. The corresponding values for COPD patients were 42%, 38%, and 31% of the controls ($P < 0.05$ for all modes) (Fig. 1).

Oxygen uptake

Resting $\dot{V}O_2$ was not different between controls and COPD patients. Maximal oxygen uptake in controls was 976 (76), 1468 (124), and 2453 (210) mL/min during 1-KE, 2-KE, and bicycling, respectively. The COPD patients achieved 81% ($P = 0.10$), 62% ($P < 0.05$), and 48% ($P < 0.05$) of the controls' values (Fig. 2(a)).

Maximal mass-specific $\dot{V}O_2$ ($VO_{2max} - \dot{V}O_2$ at rest divided by exercising muscle mass) in controls was 345 (25), 295 (24), and 104 (6) mL/kg/min during 1-KE, 2-KE, and bicycling, respectively. COPD patients achieved 77% ($P = 0.06$), 59% ($P < 0.05$), and 42% ($P < 0.05$) of the controls' values, respectively (Fig. 3(a)).

Ventilation values for COPD patients were 132%, 73%, 53%, and 37% of the controls' values at rest and during 1-KE, 2-KE, and bicycling, respectively ($P < 0.05$ for all modes) (Fig. 2(b)).

Blood gases and pH

SaO_2 was higher in controls than COPD patients both at rest and at exhaustion in all three exercise tests ($P < 0.05$). No change in SaO_2 was seen in controls from rest to exhaustion during 1-KE and

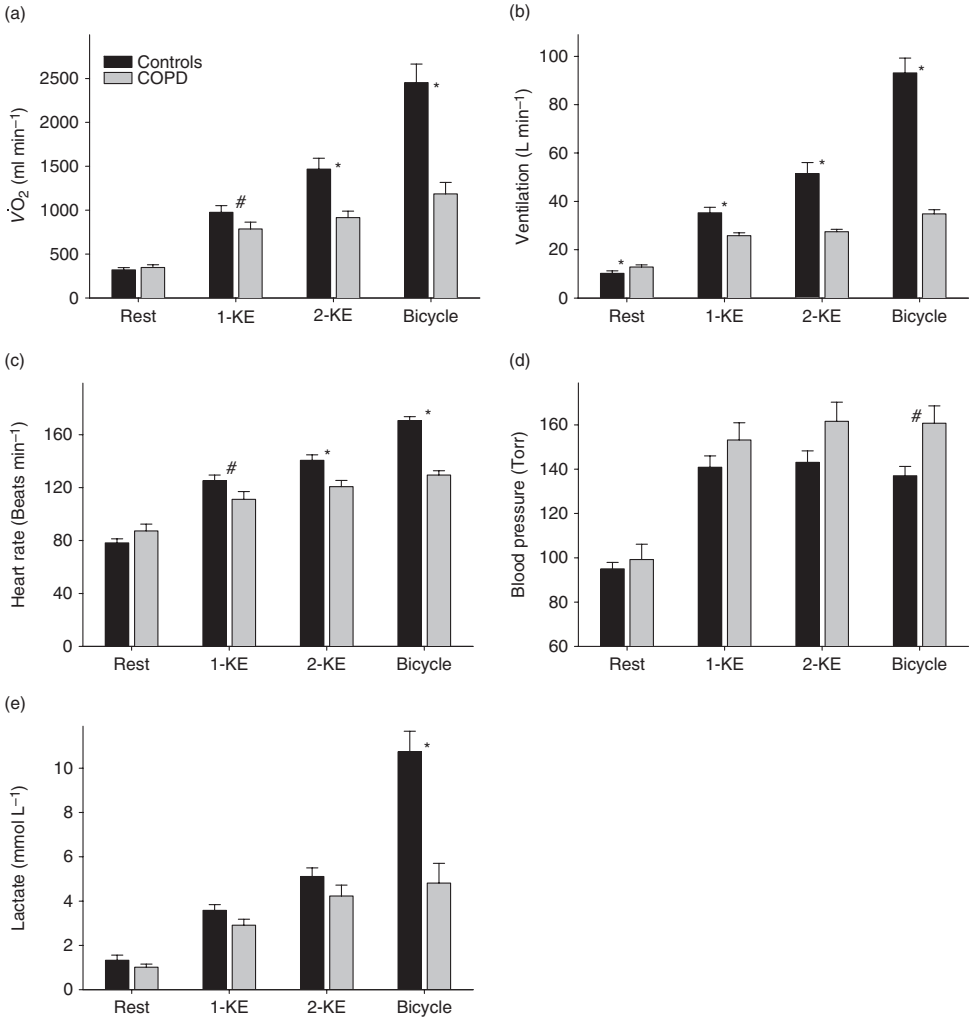

Fig. 2. VO_{2max} (a), ventilation (b), heart rate (c), (d), blood pressure (e), and lactate (f) at rest and during single-leg knee extensor exercises (1-KE, muscle mass ~2 kg) two-leg knee extensor (2-KE, mass ~4 kg) and bicycling (mass ~20 kg). Numbers sign (#) indicates $P<0.1$ and $*P<0.05$.

2-KE, while a small decrease was seen during bicycling ($1.8 \pm 1.2\%$) ($P<0.05$). The SaO_2 in COPD patients was 90% (2%), 88% (2%), and 84% (3%) during 1-KE, 2-KE, and bicycling, respectively (Fig. 4(a)). PaO_2 in controls was 11.9 (0.3), 13.4 (1.5), 13.0 (1.25), and 12.2 (0.5) kPa at rest, 1-KE, 2-KE, and bicycling, respectively. The respective values for the COPD patients were 8.3 (0.3), 8.1 (0.4), 7.8 (0.5), and 7.0 (0.4) kPa ($P<0.05$ for all modes) (Fig. 4(b)). $PaCO_2$ was significantly higher in the COPD patients compared with controls only during 1-KE (134%, $P<0.05$), but the P-value at rest and for the other exercises ranged from 0.11 to 0.07 (Fig. 4(c)). No differences in pH between the groups was observed (Fig. 4(d)).

Heart rate and arterial blood pressure

There was no difference in HR between controls and COPD patients at rest. During 1-KE the HR tended to be lower in COPD patients ($P = 0.08$) while it was 86% and 76% of the controls' HR during 2-KE and bicycling, respectively ($P<0.05$) (Fig. 2(d)). Blood pressure was not statistically different between groups during knee extension, but tended to be

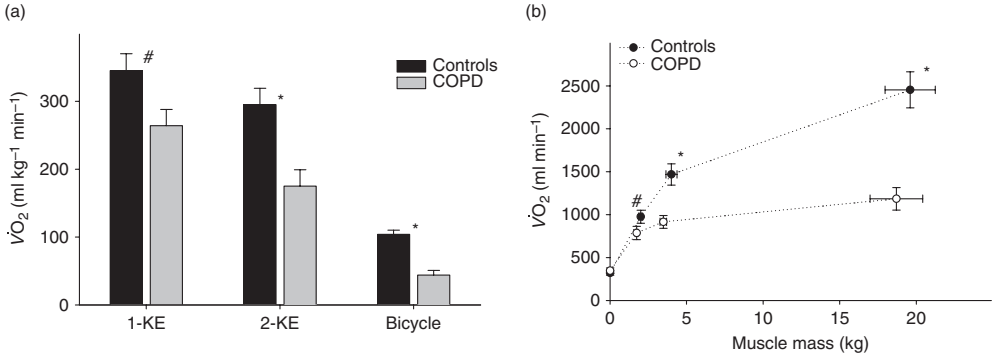

Fig. 3. Mass-specific $\dot{V}O_2$ (a) and oxygen uptake vs muscle mass (b) during single-leg knee extensor exercises (1-KE, muscle mass ~ 2 kg) two-leg knee extensor (2-KE, mass ~4 kg) and bicycling (mass ~20 kg). Numbers sign (#) indicates $P<0.1$ and $*P<0.05$.

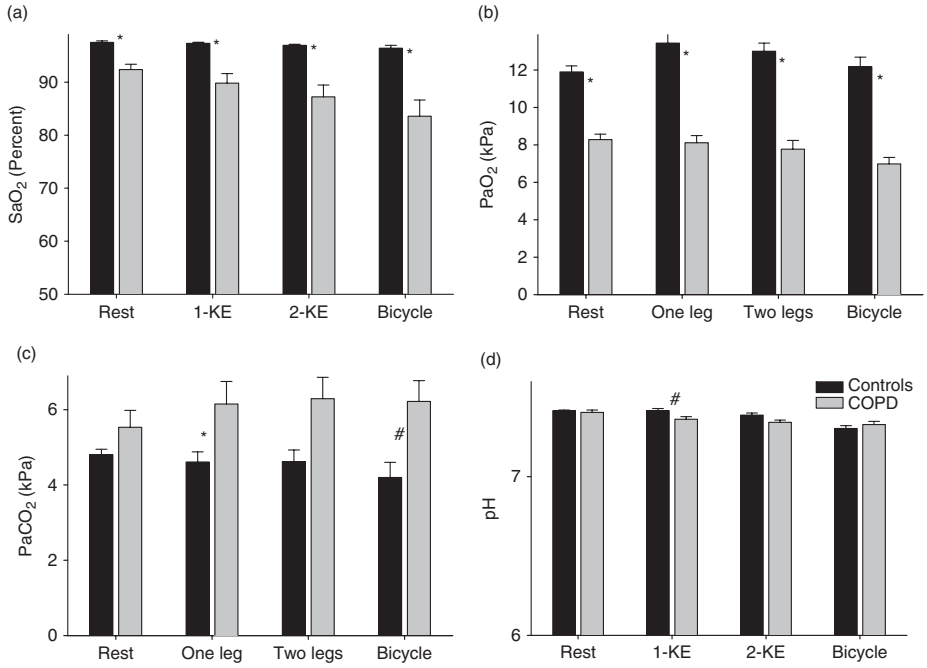

Fig. 4. SaO_2 (a), PaO_2 (b), $PaCO_2$ (c), and pH rest and during single-leg knee extensor exercises (1-KE, muscle mass ~ 2 kg) two-leg knee extensor (2-KE, mass ~ 4 kg) and bicycling (mass ~ 20 kg). Numbers sign (#) indicates $P<0.1$ and $*P<0.05$.

higher (14%) in COPD patients than controls during bicycling ($P=0.05$ for both modes) (Fig. 2(c) and (d)).

Lactate

There was no difference in arterial lactate concentration ($[La^-]art$) between controls and COPD patients at rest, 1-KE or 2-KE. During bicycling the $[La^-]art$ in COPD patients at exhaustion was 45% of that of the controls ($P<0.05$) (Fig. 2(e)).

Discussion

The present study supplements previous studies on COPD patients by showing that exercise capacity is limited by central factors already when exercising

with a relatively small muscle mass. This is demonstrated by a progressive fall in oxygen uptake in the COPD patients compared with controls as the exercising muscle mass increases. During 1-KE the oxygen uptake in the COPD was 81% ($P = 0.10$) of the controls while it was 62% ($P < 0.05$) and 48% ($P < 0.05$) during 2-KE and bicycling, respectively.

Muscular metabolic capacity

In COPD patients, VO_{2max} during 1-KE was 81% ($P = 0.10$) and mass-specific capacity ($\dot{V}O_2 \cdot kg\ muscle^{-1} min^{-1}$) was 77% ($P = 0.06$) of controls. This difference in mass-specific capacity agrees with the previously reported muscular changes in this patient group (Jakobsson et al., 1995; Jobin et al., 1998; Whittom et al., 1998; Maltais et al., 2000; Allaire et al., 2004). However, Richardson et al. (2004) found identical leg $\dot{V}O_2$ in COPD patients and controls during 1-KE (as well as similar citrate synthase activity and mitochondrial volume density), but the controls in their study were probably more unfit than the controls in the present study, as indicated by 30% lower VO_{2max} during bicycling. It is, therefore, likely that the group difference in muscular metabolic capacity in the present study mirrors differences in physical activity level.

It is assumed that the mass-specific oxygen uptake achieved during maximal 1-KE exercise is engaging the muscular metabolic capacity in both healthy subjects (Andersen & Saltin, 1985) and COPD patients (Richardson et al., 1999b). The work performed during 1-KE in the present study was probably too small to challenge the ventilatory capacity of the COPD patients because they had 25% lower ventilation during 1-KE than bicycling. Furthermore, another study found that breathing a mixture of helium and oxygen, which unloads the ventilatory muscles and reduces their energy cost, did not increase peak workload during 1-KE in COPD patients (Richardson et al., 1999b). This suggests that there is no significant blood flow compromise between ventilatory and locomotor muscles during exercise when the muscle mass is sufficiently small, as in 1-KE. This is supported by the previously recognized overperfusion of the exercising muscles during 1-KE and short mean transit time (Rowell et al., 1986; Richardson et al., 1999a, b). Thus, the muscular metabolic capacity is probably the main limiting factor during 1-KE in the COPD patients, meaning that the 23% lower muscular metabolic capacity found in the COPD patients compared with controls during 1-KE may not be attributed to insufficient oxygen supply.

$\dot{V}O_2$ during 2-KE and bicycling

During bicycling, VO_{2max} in COPD patients was 48% of that of the controls. From the present and other studies (Richardson et al., 1999b, 2004) it can be concluded that this low value cannot be solely attributed to lower muscular metabolic capacity in the muscles, because when the engaged muscle mass is small and O_2 delivery sufficient, the energy turnover is higher and closer to that of the controls. Therefore, the lung disease reduces maximal whole body $\dot{V}O_2$ more than the muscle mass-specific VO_{2max}.

The present study supplements previous studies by including 2-KE, which engages double the muscle mass of 1-KE, but still no more than about 25% of the muscle mass used during bicycling. The increase in $\dot{V}O_2$ for the controls from 1-KE to 2-KE was 73% (9%) of the increase from rest to 1-KE (Fig. 3(b)). For COPD patients, the corresponding relative increase in $\dot{V}O_2$ was 33% (8%), less than half the increase seen in the controls ($P < 0.05$). This indicates that the muscles in COPD patients are oxygen supply limited already during 2-KE.

From the low $\dot{V}O_2$ and a 10–20% lower a-v O_2 difference (Maltais et al., 1998; Richardson et al., 2004), the CO can be estimated to be 30% and 40% lower in the COPD patients than controls during 2-KE and bicycling, respectively. Considering that the COPD patients lacked any cardiac pathology and were not taking medication, this difference seems to be of significance. Maximal HR was also reduced in COPD patients during 2-KE and bicycling, and neither deconditioning nor physical training affects maximal HR in healthy subjects. The reduced oxygen uptake during bicycling and 2-KE in COPD patients may therefore be an indication of a flow down-regulation mechanism, perhaps supported by the ventilatory and $\dot{V}O_2$ reserve at exhaustion of 2-KE (21% and 23%, respectively, $P < 0.05$).

Muscular metabolic reserve capacity

During 2-KE and bicycling, the mass-specific $\dot{V}O_2$ in controls was approximately 85% and 31% of the mass-specific $\dot{V}O_2$ during 1-KE, respectively. The corresponding values in COPD patients were 67 ($P = 0.06$) and 17% ($P < 0.05$). A lower utilization of the muscular metabolic capacity demonstrates that COPD patients have a relatively higher muscular metabolic reserve capacity compared with controls when exercising with a muscle mass similar to or higher than 2-KE (Fig. 3(a)).

The calculations of the muscular metabolic reserve capacities are based on estimates of muscle masses. The active muscle mass during bicycling was estimated based on gender and weight according to Gallagher et al. (1997) and gave muscle masses in controls and COPD patients of 20 and 19 kg, respectively. These values seem reasonable when comparing with direct measurements using dual-energy X-ray absorptiometry scanning of leg muscle mass in healthy

young adults (Weber & Schneider, 2000; Mortensen et al., 2005). The equality of the muscle mass in the groups is also indicated by the equality in body mass index (BMI), which was 26.3 for controls and 25.6 for COPD patients (NS). But even if the COPD patients in the present study had had a lower muscle mass to BMI ratio than healthy subjects, as in the findings of Allaire et al. (2004), it would probably not jeopardize the study model or the comprehension of the data. Firstly, a lower muscle mass during 1-KE would mean that the muscular metabolic capacity in the COPD patients is underestimated. Secondly, it would not influence the 2-KE/1-KE ratio because 2-KE includes a doubling of the 1-KE muscle mass. Finally, the active muscle mass during bicycling has to be more than 35% smaller in the COPD patients than controls in order to not achieve a statistically significant difference in muscular metabolic reserve capacity.

Mechanical efficiency and energy cost of breathing

In the present study, gross efficiency (the ratio of energy output to energy input as calculated from pulmonary $\dot{V}O_2$) during 1-KE, 2-KE, and bicycling at maximal workloads was 5% better in controls than COPD patients. The pulmonary $\dot{V}O_2$ ($\dot{V}O_2$ pul) can be separated into the oxygen cost of the exercising muscles ($\dot{V}O_2$ leg), the oxygen cost of breathing ($\dot{V}O_2$ vent) and the oxygen cost of the rest of the body ($\dot{V}O_2$ rest) and is described by the equation: $\dot{V}O_2$ pul = $\dot{V}O_2$ rest + $\dot{V}O_2$ vent + $\dot{V}O_2$ leg. In COPD patients, both oxygen cost of breathing and exercising are shown to be increased (Levison & Cherniack, 1968; Sala et al., 1999; Richardson et al., 2004) and this is thought to be the reason for the reduced gross efficiency (Baarends et al., 1997; Sala et al., 1999).

$\dot{V}O_2$ rest can be assumed to be close to $\dot{V}O_2$ while sitting at the ergometer. Aaron et al. (1992) calculated the oxygen cost of breathing at maximal exercise to be 2.9 mL/min/liter VE or 10% of total $\dot{V}O_2$ in healthy subjects. Applying this value for the controls in the present study, leg efficiency as calculated from $\dot{V}O_2$ leg is about 26% during bicycling. If we assume that the mechanical efficiency is the same for COPD patients and controls, $\dot{V}O_2$ vent for COPD patients would be about 23% of the total $\dot{V}O_2$. Levison and Cherniack (1968) reported that the $\dot{V}O_2$ vent in COPD patients accounted for 35–40% of pulmonary $\dot{V}O_2$. Such a high value is not possible in the present study unless mechanical efficiency were to exceed that of the healthy subjects, which is rather controversial. This indicates that the cost of ventilation in the present study is lower than previously reported by Levison and Cherniack (1968).

Conclusions

The COPD patients have a reduced skeletal muscular metabolic capacity. However, the central limitations are much greater, causing the muscles suffer from oxygen supply limitations during strenuous exercise already when exercising with a relative small muscle mass, such as during 2-KE. Consequently, the COPD patients have a relatively higher skeletal muscular metabolic reserve capacity than controls during whole body exercise.

Perspectives

COPD patients are centrally limited during maximal exercise, but it has also been suggested that, due to changes in the muscles comparable to what happens during deconditioning, they may be peripherally limited as well. This field has been frequently studied during the last two decades because of the implications for rehabilitation in this patient group. The present study suggests that because of the severe limitations in oxygen supply in COPD patients, training with a rather small muscle mass may be optimal and have the potential to exercise the heart and ventilatory muscles as well as the skeletal muscles themselves. Therefore, if during training of patients with COPD the active muscle mass was individually titrated according to their capacity for oxygen supply, exercise training could be optimized relative to disease severity. This may facilitate improved peripheral exercise adaptation that would be of benefit to patients with stable COPD and those who could be specifically targeted both pre- and post-lung transplantation to more efficiently aid recovery.

Key words: lung disease, muscular metabolic capacity, central limitations, muscular efficiency.

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Paper II

Rud B & Hallen J (2009). Is the balance between skeletal muscular metabolic capacity and oxygen supply capacity the same in endurance trained and untrained subjects? *Eur J Appl Physiol* **105**, 679-685.

Paper III

B. Rud, Ø. Foss, P. Krustrup, N.H. Secher and J. Hallén. One-legged endurance training: leg blood flow and oxygen extraction during cycling exercise (Paper in revision).

One-legged endurance training: leg blood flow and oxygen extraction during cycling exercise

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Running head: Training-effects on leg oxygen uptake during exercise

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Abstract

As a consequence of enhanced local vascular conductance, perfusion of muscles increases with exercise intensity to suffice the oxygen demand. However, when maximal oxygen uptake (VO_2max) and cardiac output are approached, the increase in conductance is blunted. Endurance training increases muscle metabolic capacity, but to what extend that affects regulation of muscle vascular conductance during exercise is unknown. **Methods and results:** Seven weeks of one-legged endurance training was carried out by twelve subjects without affecting their VO_2max for cycling although their one-legged VO_2max became 6.7 (2.3)% (mean±SE) larger with the trained (TL) than with the control leg (CL). Also the TL citrate synthase activity increased (by 30 (12)%; $P<0.05$) and 3-hydroxyacyl-CoA dehydrogenase activity tended to increase ($P<0.12$). With the two legs working at precisely the same intensity during cycling in eight of the subjects, leg oxygen uptake was 21 (8)% ($P<0.05$) larger for the TL than for the CL (oxygen extraction increased by 3.5 (1.1)%; $P<0.05$) supported by an increment in leg blood flow by 16.0 (7.0)%; $P<0.06$). **Conclusion:** That enhanced TL VO_2max had no implication for cycling VO_2max supports that there is a central limitation to VO_2max during whole-body exercise. However, the metabolic balance between the legs was changed during high intensity exercise since blood flow and oxygen extraction were higher in the trained leg, suggesting that endurance training ameliorates blunting of leg blood flow and oxygen uptake during whole-body exercise.

Keywords: Leg oxygen uptake, lactate, enzyme activity, peripheral and central adaptations.

Introduction

During one-legged knee extension exercise, Andersen & Saltin (1985) quantified muscle flow capacity to 2.5 litres per kilo per minute. During whole body exercise, however, limited venous return to the heart and hence cardiac output provokes peripheral vasoconstriction that implicates muscle blood flow and oxygen uptake (Mortensen *et al.*, 2005;Secher *et al.*, 1977;Volianitis & Secher, 2002). For example, both in endurance trained and untrained subjects, cycling exercise allows for a leg oxygen uptake that is smaller than half of the leg capacity as revealed during one-legged knee extension exercise (Rud & Hallén, 2009). That is the case since the arterial baroreceptors secure arterial blood pressure at the expense of flow to working skeletal muscles (Collins *et al.*, 2001;Ogoh *et al.*, 2003).

Despite the apparent muscle vascular conductance and metabolic reserve during whole body exercise, endurance training not only enhances central cardiovascular variables but also metabolic capacity of skeletal muscles (Saltin *et al.*, 1976). In sedentary subjects, leg oxygen extraction reaches only 70%-75% of the arterial oxygen content (CaO_2) (Richardson *et al.*, 2004;Roca *et al.*, 1992) while in endurance trained subjects, leg oxygen extraction may be larger than 90% (Calbet *et al.*, 2005;Knight *et al.*, 1992). Therefore, both oxygen delivery and oxygen extraction may limit maximal oxygen uptake (VO_2max) and their relative importance may depend on the training status of the subjects.

To evaluate the respective contribution of oxygen delivery vs. oxygen extraction to VO_2max is challenging because the two variables are interdependent. For example, during one-legged knee extension exercise, oxygen extraction is much lower than during cycling probably due to the higher muscle blood flow during one-legged exercise (Richardson *et al.*, 1999a). In the

isolated rat hind limb the oxidative capacity is important for oxygen extraction (Hepple *et al.*, 2002;McAllister & Terjung, 1990;Robinson *et al.*, 1994) but, on the other hand, reduced oxidative capacity of skeletal muscles after bed rest in humans does not reduce their oxygen extraction (Saltin *et al.*, 1968). During hypoxic maximal exercise intramuscular PO_2 does not reach zero, maybe indicating that potentially more oxygen may be extracted if there is induced an increase in their oxidative capacity by training (Richardson *et al.*, 1999b).

The aim of this study was to determine the effect of a training-induced increased muscular oxidative capacity on oxygen extraction and delivery during high intensity whole body exercise. Following one-legged training, the oxygen delivery and oxygen extraction over the trained and untrained leg were determined during cycling exercise. Previously such an evaluation of one-legged exercise on oxygen extraction by the trained and untrained leg has been carried out during low intensity cycling exercise (Saltin *et al.*, 1976). For the present study, care was taken to secure that the trained and untrained leg carried out precisely the same workload during cycling exercise. We hypothesized that training would increase leg VO_2 during high intensity exercise by increasing leg oxygen extraction without any change in blood flow.

Methods

Twelve healthy subjects provided informed consent to participate in the study (Table 1) that was approved by the regional ethics committee (http://helseforskning.etikkom.no) according to the Declaration of Helsinki. To make it unlikely that one-legged training affected the central circulation, endurance trained subjects were preferred and the subjects were asked to maintain their usual level of physical activity during the study as secured by a weekly report (not shown). The subjects were familiarized with the study procedures over four days and were instructed to abstain from strenuous exercise for one day prior to experimental procedures.

Insert table 1 here

Initial evaluation

VO_2max was determined on a cycling ergometer (Excalibur Sport, Lode B.V., Groningen, The Netherlands) with a step increment in intensity each minute (20 W for women; 25 W for men) and continued until exhaustion defined as when the subject was unable to maintain a pedalling frequency of 80 revolutions per minute (RPM). During the final increment(s) in work intensity VO_2 was measured by a Douglas bag system (Foss & Hallen, 2005), heart rate (HR) was registered by short distance telemetry (Polar Electro Oy, Kempele, Finland) and at exhaustion, a fingertip blood sample was analyzed for lactate (YSI 1500 Sport, Yellow Springs Instruments, Ohio, USA) (Fig.1).

Training

The subjects conducted supervised one-legged training four times per week, for seven weeks, on a mechanical braked ergometer with a fixed flywheel (Monark 818E, Monark Exercise

AB, Sweden). One-legged training was counterbalanced among subjects for the right and left leg and the cadence was 80 RPM while the control leg rested on a chair. The average training volume was 1159 (range 791-1578) kJ per week at a workload of 108 (76-160) W corresponding to 72 (70-77)% of the one-legged maximal HR. Training bouts included a 10 min warm-up period followed by continuous exercise at a constant workload. The four weekly training bouts varied in intensity (59% to 90% of HR) and progressed in duration (40 to 100 min). To familiarize the subject to one-legged exercise also with the control leg, the subjects performed several short sessions of one-legged exercise with that leg throughout the training period (Fig.1)..

After training

The cycling exercise VO_2max was re-evaluated after the seven weeks of one-legged training and also the one-legged VO_2max was determined both with the trained and untrained leg. These evaluations were carried out as described, except that for one-legged exercise the increments were by 10 (women) or 15 W (men) and the order of the three evaluations was counterbalanced with a ~1½ h recovery between trials. While HR was monitored, VO_2 was obtained during the last ~2 min of each type of exercise and a fingertip blood sample was obtained within the final 30 s for analyses of lactate (Fig.1)..

Invasive experiment

Eight of the subjects volunteered to participate in an invasive study. These subjects had a light breakfast and reported to the laboratory 2 h prior to the experiment. In the supine position a catheter was placed in the brachial artery and following local anaesthesia (2% lidocaine), Seldinger technique was used to place catheters (with side holes for infusion of ice cold physiological saline solution, Model Radiopack TFE; Cook, Bjæverskov, Denmark) in both

femoral veins approximately two centimetres below the inguinal ligament and advanced 7 cm in anterograd direction. Thereafter, the subjects rested supine for ~30 min prior to exercise. Three bouts of cycling exercise were performed: at 80 and 120 W for women and at 100 and 150 W for men, each bout lasting 8 min followed by high-intensity exercise at 210±22 (♀) or 303±24 W (♂) and continued until exhaustion (6-9 min) (Fig.1).

To ensure equal involvement of the two legs in cycling exercise, balance between the power developed by the two legs was displayed in front of the subjects using signals from an crank arm strain gauge while the angel of the arm was recorded. Difference in power output between the two legs was 1.8%, 2.2%, and 0.6% for the 80/100, 120/150, and 210/303W exercises, respectively, and never reached a statistically significant level.

Femoral venous blood flow was measured by constant infusion thermodilution technique (Andersen & Saltin, 1985). Ice-cold saline was infused in the femoral vein at a rate of 20-120 $ml \cdot min^{-1}$, depending on blood flow, for 15-25 s to obtain a 0.4–0.9° C drop in blood temperature. During submaximal exercise measurements were carried out in the 3rd and 6th min and during maximal exercise also immediately prior to exhaustion. Infusate temperature was determined at the entry of the catheter with a thermistor set in a flow–through chamber (model 93.505 Edwards Lifesciences LLC, Irvine CA) and venous blood temperature was measured with the thermistor placed 8 cm into the femoral veins (Edslab 94-0.30-2.5F T.D. Edslab probe; Baxter Irvine, CA). The thermal data were acquisitioned on a PC with a sampling frequency of 100 Hz (Maclab 16/s AD Instruments, Sydney, Australia or by Labview, National instruments, Texas, USA).

Immediately prior to flow measurements, blood samples were drawn anaerobically from each catheter into 2 ml syringes that were placed immediately in ice cold water until analyzed for blood gas variables and lactate (ABL 700, Radiometer, Copenhagen, Denmark). Arterial pressure was recorded with a pressure gauge (Baxter B.V. Uden, Holland) and amplifier (GOULD instrument systems, Valley View, USA) and acquisitioned on a PC with a sampling rate of 100 Hz. The system was calibrated with a water column corresponding to 50 mmHg.

Calculations

Arterial to venous oxygen differences (a-v O_2diff) were calculated from the respective ctO_2. Leg blood flow was the average of two to three measurements at each bout of exercise and its oxygen uptake was flow times the mean leg a-v O_2diff. Net leg lactate release was the product of leg plasma flow (1-haematocrit) and the venous-arterial plasma lactate difference. Leg vascular conductance was expressed as leg blood flow divided by mean arterial pressure minus the femoral venous pressure (4 mmHg; Calbet et al. (2004) and delivery of oxygen was the arterial ctO_2 times the leg blood flow.

Biopsies

Under local anaesthesia (2% lidocaine) and with manual suction, a percutaneous needle (6 mm Pelomi needle, Albertslund, Denmark) m. vasus lateralis biopsy, 50-200 mg, was obtained from of both legs within one week after training and rinsed in saline before fat and connective tissue were carefully removed. Samples were frozen in isopentane on dry ice and stored at -80º C until analysis. Citrate synthase and 3-hydroxyacyl-CoA dehydrogenase activity were determined by fluourometric methods with NAD-NADH coupled reactions (Krustrup *et al.*, 2009).

Statistics

Variables were tested for differences between the control and the trained leg and for the effect of training by homoscedastic Student's t-tests. The level of significance was set at $P<0.05$ (2-tailed) and results are presented as mean ± SE.

Results

During maximal one-legged exercise with the trained leg, the subjects reached a 9.5 (2.0)% higher power output, a 30.0 (6.6)% longer time to exhaustion, a 6.7 (2.3)% higher VO_2max, a 9.7 (4.0)% higher ventilation, a 3.2 (0.7)% higher HR, and a 13.2 (3.3)% higher blood lactate compared to when exercise was carried out with the untrained leg (for all variables; $p<0.05$) (Table 2).

During maximal cycling exercise, there were no statistical significant differences in power output, time to exhaustion, VO_2max, ventilation, or HR before and after the training, but blood lactate was 11.3 (1.6)% lower after the one-legged training ($p<0.05$) (Table 2).

Invasive study

At rest the a-v O_2diff (by 23.9 (11.5)%; $p=0.06$), leg blood flow (by 31.5 (13.1)%; $p<0.05$), and thus VO_2 (by 61 (18)%; $p<0.05$) were larger for the trained than for the control leg. In contrast, there were no statistically significant differences in these variables during the low to moderate workloads. Only at the moderate workload, the a-v O_2diff tended to be (1.3 (0.7)%; $p=0.10$) higher in the trained leg (Fig. 2).

During high intensity exercise, however, the a-v O_2diff was 4.2 (1.2)% ($p<0.05$) higher (with no change in P50; the PO_2 value that causes haemoglobin to be 50% saturated; Table 3) and also blood flow tended to be higher (by 16.0 (7.0)%; $p=0.06$) in the trained compared to the untrained leg. Accordingly, the combined effect of a higher extraction (by 3.5%) and blood flow resulted in 21.1 (8.1)% higher VO_2 in the trained than in the control leg ($p<0.05$) (Fig. 2).

There were no lactate releases for the legs at rest (n=8). For the control leg the release was 0.4 (0.2), 0.7 (0.2) and 1.4 (0.4) $mmol \cdot min^{-1}$ during 80/100 W (n=7), 120/150 W (n=7) and 210/303 W (n=6) exercise, respectively (all; $p<0.05$), while there were no significant release of lactate from the trained leg, but with no statistical differences between the legs.

Citrate synthase activity was 30.4 (12.2)% higher in the trained than in the untrained leg ($p<0.05$), while 3-hydroxyacyl-CoA dehydrogenase activity only tended to increase ($p=0.12$; Fig. 3).

Discussion

This study evaluated the effect of increased oxidative capacity on skeletal muscle oxygen extraction and blood flow regulation during whole body exercise. Both muscle oxidative capacity and one-legged VO_2max were increased by one-legged endurance training in endurance trained subjects, without affecting their VO_2max for cycling. While the two legs exercised with the same power output at close to maximal oxygen uptake, both blood flow and oxygen extraction were highest in the trained leg resulting in a 21% higher oxygen uptake. Hence, the data indicate that training-induced increase in muscular oxidative capacity affects both oxygen delivery and extraction and thereby ameliorates blunting of leg VO_2 during high intensity whole body exercise.

During exercise, muscle perfusion increases with intensity to suffice the oxygen demand. This increase in muscle blood flow is a consequence of an increase in local vascular conductance, albeit muscle blood flow is balanced by sympathetic vasoconstrictor activity during whole-body exercise (Mortensen *et al.*, 2005;Rud & Hallén, 2009;Secher *et al.*, 1977;Volianitis & Secher, 2002). Accordingly, at high exercise intensities, when maximal cardiac output is approached, the increase in blood flow to the exercising muscles is restricted and the increase in oxygen uptake per unit of developed power was attenuated. In the present study, this attenuation of the increase in oxygen uptake with power is larger in the untrained as compared to the trained leg (Fig. 1f). Yet, the oxidative capacity of the muscles is likely a determinant of blood flow during high exercise intensities when maximal cardiac output is challenged and accordingly, sympathetic vasoconstrictor activity dominates over local vasodilator influence on muscle blood flow. It can be hypothesized that the training-induced increase in blood flow at a given power took place at the expense of the untrained leg. This is supported by the fact

that pulmonary VO_2max during cycling did not change in response to one-legged training and, therefore probably also cardiac output was the same.

With the same perfusion pressure and arterial O_2 content, oxygen delivery is determined by peripheral conductance. Both oxygen extraction (by 3.5%) and blood flow (by 16%) were higher in the trained compared to the control leg during the most intense exercise. Red cell deoxygenation appears to control of local tissue perfusion by overriding an increase in sympathetic vasoconstrictor activity during exercise (Dufour *et al.*, 2010;Mortensen *et al.*, 2009). Accordingly, the tendency for an increase in the trained leg's blood flow may have been influenced by the low oxygen saturation developed in response to its enhanced enzymatic activity.

Roca et al. (1992) found oxygen extraction to increase from 71% to 82% as cycling VO_2max increased 38% (from 37 to 51 $ml \cdot kg^{-1} \cdot min^{-1}$) after 9 weeks of intense endurance training. In competitive cyclists with a VO_2max of 65 $ml \cdot kg^{-1} \cdot min^{-1}$, oxygen extraction was 92% (Knight *et al.*, 1992) and in cross country skiers with a VO_2max of 72 $ml \cdot kg^{-1} \cdot min^{-1}$, leg oxygen extraction was 93% (Calbet *et al.*, 2005). For the present subjects, VO_2max was 58 $ml \cdot kg^{-1} \cdot min^{-1}$ and oxygen extraction 85% and 89% over the untrained and trained leg, respectively (Fig. 4). If we relate oxygen extraction to leg VO_2 in these studies, including the trained and untrained state of Roca et al. (1992), it suggests that maximal values for leg VO_2 depends on oxygen extraction. The VO_2max in the cross country skiers of Calbet et al. (2005) was approximately two-fold higher than that of the untrained subjects of Roca et al. (1992). Taken together, these studies indicate that 80% of the training-induced increase in VO_2max during whole body exercise is due to increased oxygen delivery, whereas 20% is due to increases in oxygen extraction.

(Insert Fig.4 here)

The interrelated relationship between leg VO_2 and oxygen extraction is challenged in some situations. After bed rest and inactivity in previously active subjects, VO_2max declines faster than oxygen extraction. Ferretti et al. (1997) found that oxygen extraction was maintained after 42 days of inactivity even though the activity of the oxidative enzymes was reduced by 11%. However, they also reported a 31% reduction in cardiac output after bed rest although muscle capillary density was maintained, suggesting that oxygen extraction was affected by a prolonged capillary mean transit time. Also in heart failure patients oxygen extraction is high despite a low oxidative capacity of the exercising muscles. In these patients a low cardiac output is likely to maintain mean muscle capillary blood transit time.

In addition to the increased muscle enzyme activity, several mechanisms may account for the higher oxygen extraction in the trained leg. The kinetics of oxygen off-loading from haemoglobin may be different in the trained an untrained leg. However, P50 (the PO_2 value that causes haemoglobin to be 50% saturated) was the same during exercise with the two legs (Table 3), indicating that following one-legged training, endurance trained subjects may be an exception to the close relationship found between P50 and oxygen extraction established both in dogs and humans (Calbet *et al.*, 2005;Richardson *et al.*, 1998). Capillary density was not evaluated in the present study, but blood flow was increased by 16% which is identical to the increase found by Klausen et al. (1982) after 8 weeks of one-legged training. Further, Klausen et al. (1982) reported an increased a-v O_2diff of 4% paralleled by a 20-30% increase of oxidative enzyme capacity. However, (Klausen *et al.*, 1981;Klausen *et al.*, 1982) reported a 20% increase in muscle capillarization following one-legged training indicating an almost unchanged mean transit time. Together, these data indicate that the higher oxidative capacity of the mitochondria is as important as an increased mean transit time for oxygen extraction,

although interpretation of classical enzyme markers for mitochondrial oxidative capacity remains debated (Tweedie *et al.*, 2011).

One-legged training did not change pulmonary VO_2max during cycling exercise, although during one-legged cycling exercise work capacity was higher for the trained leg as indicated by a 9.5% higher power output and a 6.7% higher pulmonary VO_2. Because of the fixed wheel configuration, it was straight forward for the subjects to perform one-legged cycling and they had regularly been practicing one-legged exercise with the untrained leg during the training period. Hence, the difference in one-legged work capacity is thought to reflect training-induced changes in the exercising muscles rather than a motor learning effect due to practicing one-legged exercise. The robustness of the model is supported by the linearity of the VO_2/power relationship for the two legs (Fig. 2f). Yet, the training-induced change in one-legged VO_2max (6.7%) was less than previously reported (12-24%) (Davies & Sargeant, 1975;Gleser, 1973;Klausen *et al.*, 1982;Saltin *et al.*, 1976), probably reflecting that the present subjects were endurance trained (present study range; 49-72 $ml \cdot kg^{-1} \cdot min^{-1}$ vs. other studies (41-46 $ml \cdot kg^{-1} \cdot min^{-1}$).

In conclusion, one-legged endurance training increased muscle enzyme capacity, one-legged exercise capacity, and peak oxygen uptake without affecting cycling exercise VO_2max. That was the case although during high intensity cycling exercise, the endurance trained leg demonstrated a higher oxygen extraction, blood flow, and VO_2 than the untrained leg. These findings indicate that blood flow distribution and thereby oxygen delivery between exercising muscles is affected by the muscular metabolic capacity without affecting maximal oxygen uptake during cycling.

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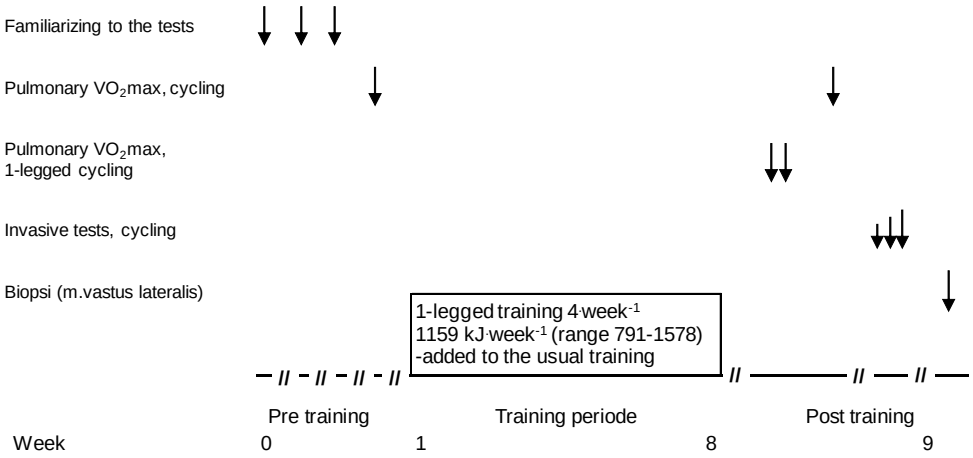

Fig. 1. Experimental protocol. The double slash (//) separation of days.

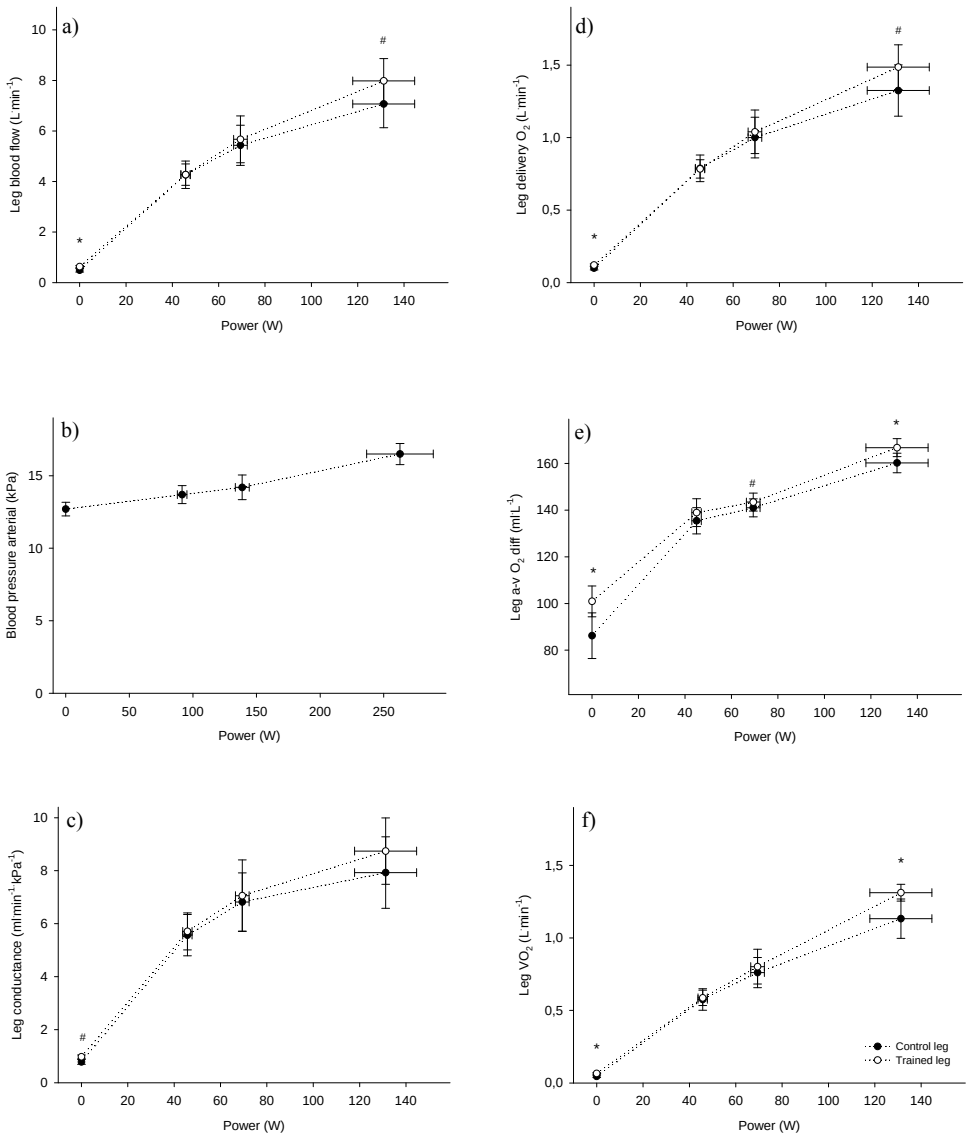

Fig. 2. Leg blood flow (a); blood pressure (b); leg conductance(c); leg delivery O_2 (d); leg arterial-venous O_2 diff (e), and leg oxygen uptake (f) during submaximal cycling exercises at different power outputs after 7 weeks of one-legged endurance training. Data are mean±SE (n=7-8). Asterisks and numbers p-value < 0.05 and 0.1 between exercise with the trained and control leg, respectively.

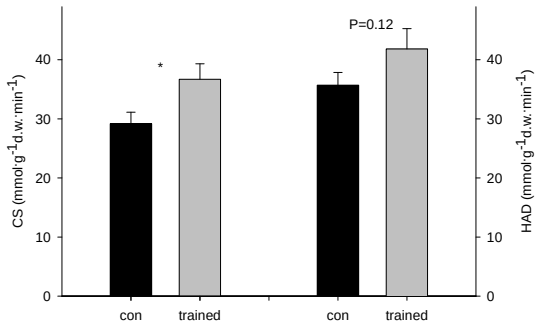

Fig. 3. Citrate synthase (CS) and 3-hydroxyacyl-CoA dehydrogenase (HAD) activity in the trained and control leg after 7 weeks of one-legged endurance training. Data are mean±SE (n=12). Asterisk. p-value <0.05 between the trained and control leg.

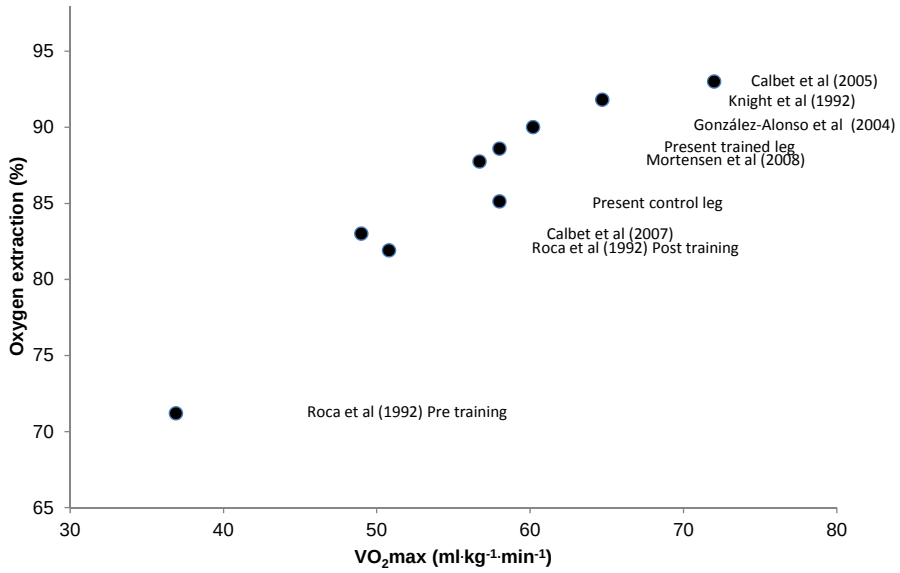

Fig. 4. Relation between oxygen extraction and VO_2max, maximal oxygen uptake. Data adapted from previous studies and the present study. Exercise mode is cycling except for Calbet et al (2004) who studied skiing.

Table 1: Subjects characteristics (mean ± S.D.)

	All subjects			Invasive study		
	All	Women	Men	All	Women	Men
n	12	6	6	8	4	4
Age (years)	24 (3.1)	22 (1.9)	26 (3.3)	23 (1.8)	23 (1.7)	24 (2.0)
Height (cm)	174 (8.8)	167 (3.4)	182 (4.0)	174 (8.7)	166 (4.0)	182 (5.0)
Mass (kg)	68 (7.6)	63 (3.6)	74 (6.6)	67 (7.2)	62 (3.3)	72 (7.3)
VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	57 (7.6)	52 (2.4)	61 (8.1)	58 (8.8)	51 (2.0)	65 (6.9)
VEmax ($l \cdot min^{-1}$)	165 (32.9)	136 (11.0)	188 (22.0)	164 (33.3)	135 (11.2)	198 (8.7)
HRmax ($beats \cdot min^{-1}$)	185 (3.3)	192 (3.8)	180 (9.0)	186 (8.2)	193 (3.9)	178 (5.3)

VO_2max, maximal oxygen uptake; VEmax, maximal ventilation; HRmax, maximal heart rate.

Table 2: Values achieved during maximal 1-legged exercise and cycling exercise (mean ± S.E).

Exercise	VO_2max ($ml \cdot min^{-1}$)	VE max ($L \cdot min^{-1}$)	HR max ($Beats \cdot min^{-1}$)	Lactate ($Mmol \cdot L^{-1}$)	Power (Watt)	TTE (mm:ss)
1-legged cycling						
Control leg	3.06 (0.18)	145 (8.8)	176 (3.5)	6.9 (0.29)	195 (11.5)	05:13 (00:12)
Trained leg	3.25 (0.17)*	158 (9.4)*	182 (3.3)*	7.5 (0.21)*	212 (9.6)*	06:42 (00:20)*
Cycling						
Pre training	3.92 (0.23)	162 (9.3)	186 (2.6)	9.5 (0.36)	343 (19.4)	05:40 (00:08)
Post training	3.91 (0.22)	163 (9.5)	187 (2.4)	8.4 (0.28)*	346 (18.8)	05:45 (00:10)

VO_2max, maximal oxygen uptake (pulmonary); VEmax, maximal ventilation; HRmax, maximal heart rate; TTE time to exhaustion (n=12). Asterisk p-value < 0.05 between exercise with the trained and control leg (1-legged cycling) and pre and post training period (cycling).

Table 3: Blood variables during the invasive cycling exercise tests of different intensities (mean ± S.E).

Variable	Location	Rest	Easy	Moderate	Hard
Hb ($g \cdot dl^{-1}$)	a	13.3 (0.41)	13.5 (0.48)	13.5 (0.40)	14.0 (0.45)
n=7-8	fvc	13.4 (0.46)	13.5 (0.47)	13.5 (0.43)	13.9 (0.45)
	fvt	13.4 (0.42)	13.6 (0.45)	13.6 (0.43)	14.0 (0.45)
sO_2 (%)	a	98.7 (0.12)	98.6 (0.23)	98.4 (0.15)	96.3 (0.53)
n=7-8	fvc	54.4 (4.03)	29.0 (2.16)	25.3 (1.84)	15.7 (1.92)
	fvt	46.6 (1.41)	27.0 (1.35)*	23.6 (1.38)#	12.8 (1.56)*
PO_2 (kPa)	a	13.9 (0.25)	13.4 (0.21)	13.3 (0.17)	12.5 (0.45)
n=7-8	fvc	3.8 (0.35)	2.7 (0.07)	2.5 (0.07)	2.2 (0.10)
	fvt	3.4 (0.12)	2.5 (0.05)	2.4 (0.06)*	2.0 (0.12)*
ctO_2 (Vol%)	a	18.5 (0.60)	18.6 (0.65)	18.6 (0.54)	18.8 (0.56)
n=7-8	fvc	9.9 (0.65)	5.0 (0.36)	4.5 (0.33)	2.8 (0.34)
	fvt	8.4 (0.32)*	4.7 (0.25)	4.2 (0.26)#	2.3 (0.30)*
PCO_2 (kPa)	a	4.6 (0.10)	4.9 (0.14)	4.6 (0.19)	3.9 (0.12)
n=7-8	fvc	5.8 (0.14)	7.1 (0.22)	7.4 (0.17)	8.5 (0.42)
	fvt	6.3 (0.21)#	7.5 (0.25)	7.5 (0.21)	8.7 (0.41)
pH (-lgM)	a	7.41 (0.01)	7.39 (0.01)	7.39 (0.01)	7.28 (0.01)
n=7-8	fvc	7.36 (0.01)	7.30 (0.01)	7.29 (0.01)	7.12 (0.02)
	fvt	7.35 (0.01)	7.30 (0.01)	7.23 (0.01)*	7.12 (0.02)
Lactate ($mmol \cdot L^{-1}$)	a	0.93 (0.09)	1.46 (0.28)	1.90 (0.34)	9.57 (0.49)
n=6-8	fvc	0.96 (0.07)	1.61 (0.33)	2.13 (0.39)	9.88 (0.49)
	fvt	0.98 (0.08)	1.46 (0.25)	1.86 (0.33)#	9.78 (0.52)
$p50O_2$ (kPa)	a	-	-	-	-
n=6-7	fvc	3,51 (0.11)	3,63 (0.16)	3,66 (0.17)	4,24 (0.28)
	fvt	3,50 (0.11)	3,61 (0.17)	3,80 (0.21)*	4,40 (0.26)

Hb, haemoglobin; sO_2, oxygenation of haemoglobin; PO_2, partial pressure of oxygen; ctO2, concentration of oxygen; PCO_2, partial pressure of carbon dioxide; $p50O_2$, partial pressure of oxygen required to achieve 50% haemoglobin saturation; a, arterial; fvc, femoral vein control leg; fvt, femoral vein trained leg. Asterisks and numbers indicate a p-value < 0.05 and 0.1 between fvc and fvt, respectively.

Paper IV

B. Rud, N. H. Secher, J. Nilsson, G. Smith, and J. Hallén. Metabolic balance between the arms and the legs during simulated skiing (Paper in preparation).

Metabolic balance between arms and legs during simulated skiing

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Running head: Metabolic balance during arm and leg exercise

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Abstract

During combined arm and leg exercise it is unknown how the workload is shared between the limbs. **Methods:** To express the relative involvement of the arms and the legs during combined exercise, arm and leg metabolism was evaluated during two workload levels of simulated skiing using the "double poling" technique to emphasize the role of the arms in the work performed. Arm and leg blood flow (by thermodilution) were evaluated together with their respective arterial to venous differences for oxygen and lactate in eight recreational skiers (24±7 yr.; mean±SD). **Results:** At a workload of mean 82 (SE 5) W arm oxygen uptake was 0.65 (0.07) $L \cdot min^{-1}$ and leg oxygen uptake 0.82 (0.06) $L \cdot min^{-1}$ (ns) reflecting an arm blood flow of 5.4 (0.6) $L \cdot min^{-1}$ and oxygen extraction of 59 (2.3)%, while the values for the legs were 5.8 (0.5) $L \cdot min^{-1}$ and 67 (1.3)%, respectively. With the workload increased to 117 (8) W, arm oxygen uptake increased only 20 (5)% (to 0.78 [0.08] $L \cdot min^{-1}$) because of an increase in blood flow (to 6.3 [0.7] $L \cdot min^{-1}$), while both leg blood flow (to 8.0 [0.5] $L \cdot min^{-1}$) and oxygen extraction (to 75 [1.5]%) increased ($P<0.05$) resulting in a 53 (8)% increase in leg oxygen uptake (to 1.24 [0.07] $L \cdot min^{-1}$). At both workloads, the arms released lactate, while the legs took up lactate. **Conclusion:** The results confirm that during combined arm and leg exercise, the arms release lactate while the legs consume lactate. The new finding was that during combined arm and leg exercise, the arms play the largest role at low exercise intensity. When exercise intensity increases, the arm muscles may be overperfused due lack of sufficient sympoatoadrenergic vasoconstriction and this may be the reason for the low oxygen extraction.

Keywords: arm oxygen uptake, leg oxygen uptake, lactate.

Introduction

Comparison of the muscle blood flow achieved during exercise with a "single" or a small muscle group versus with that established during, e.g. combined arm and leg exercise is used to demonstrate a "central" limitation in the control of muscle blood flow during exercise (Volianitis & Secher, 2002). However, it may be that adding arm to leg exercise is not simply increasing the muscle mass involved in an exercise but may also reflect the total amount of work carried out by various muscle groups. In addition, it is likely that these are differently trained and may use cardiovascular regulation which is fundamentally different from that involved in leg exercise. Arm exercise is in general associated with a larger blood pressure response than leg exercise – a response which is lost when leg exercise is added to arm exercise (Volianitis & Secher, 2002). Also the metabolic response to exercise seems to be different between the arms and the legs. Thus during progressive exercise, the increase in leg oxygen uptake is established by a combined increase in leg blood flow and a widening of the legs' arterial to venous difference (a-v diff) for oxygen (Andersen & Saltin, 1985). In contrast, there seems to be little if any increase in the a-v diff for oxygen over the arms during progressive arm cranking (Volianitis *et al.*, 2004). Only for trained rowers, the a-v diff for oxygen seems to increase with workload during arm cranking and then only to approximately 160 ml L^{-1} (Volianitis *et al.*, 2004).

Accordingly, there are to be several reasons for that the arms are in an unfavorable metabolic situation during combined arm and leg exercise and, yet, their metabolic contribution to progressive arm and leg exercise remains unknown. When combined arm and leg exercise is conducted in the laboratory, the relative workload for the arms, typically during arm cranking, is determined by the investigator. Similarly, the workload for the legs (typically during

cycling) is determined by the investigator (Secher *et al.*, 1977;Mortensen *et al.*, 2005). Unique for double poling, is that the subject determines the distribution between arms and legs, not the investigator. In the present study we used the opportunity offered by simulated double poling cross country skiing to evaluate whether the apparent disadvantage of the arms to perform work limits their involvement when exercise intensity increases during combined arm and leg exercise.

Methods

Subjects

Eight healthy recreational cross-country skiers with an mean (SD) age, weight and height of 24 (7) yrs, 178 (6) cm, 74 (6) kg, respectively participated in the study. Their maximum oxygen uptake during running (VO_{2max}) was 4.9 (0.4) l·min^{-1} or 66 (4.7) ml kg^{-1} min^{-1}). The subjects provided informed consent to participate in the study as approved by the Regional Ethics Committee (http://helseforskning.etikkom.no) according to the Declaration of Helsinki. Before the experimental days, the subjects were instructed not to carry out any strenuous exercise for 24 h.

Protocol

On the experimental day the subjects performed simulated double poling skiing at two submaximal workloads on a modified (Holmberg & Nilsson, 2008;Nilsson *et al.*, 2004) rowing ergometer (Concept II C, Concept Inc., Morrisville, Vt., USA). The two submaximal workloads, each lasting 8 min, were repeated after 10 min for duplication of all measurements, while there was a 30 min pause between the two workloads (Fig. 1). The first workload was at 82 (range 66-97) W and the second at 117 (79-143) W as determined by the computer of the ergometer.

Initial evaluation

Before the experimental day, the subjects reported to the laboratory on three days to be familiarized with the ergometer and to have their VO_2max determined during treadmill running and double poling on the ergometer. Both for the initial evaluations and for the

experimental study, pulmonary VO_2 was measured by a computerized metabolic system (OxyconPro, Eric Jaeger, Hoechberg, Germany) and heart rate was registered by short distance telemetry (Polar Electro Oy, Kempele, Finland).

Invasive procedures

On the experimental day, the subjects had a light breakfast and reported to the laboratory 2 h prior to the experiment. In the supine position catheters were placed in the brachial artery and in the subclavian and femoral veins using Seldinger technique under local anaesthesia (2% lidocaine). For placement of the catheter in the subclavian vein, a triple-lumen catheter (132F5, Baxter Healthcare, Irvine, CA) was used, inserted through the left antecubital vein, and advanced to approximately 5 cm before the subclavian vein mergers with the jugular vein (Volianitis & Secher, 2002). One lumen of the catheter was used for determination of blood flow and another lumen was used for blood sampling. The femoral venous catheters were provided with side holes (Radiopack TFE, Cook, Bjaerverskov, Denmark), inserted 2 cm below the inguinal ligament, and advanced ~7 cm in anterograd direction. To determine leg blood flow, a thermistor (Edslab 94-0.30-2.5F, Edwards Edslab Baxter) was inserted through the catheter and placed 8 cm beyond its tip. The catheter was connected to a three-way stopcock for blood sampling and flow measurements. Following these preparations, the subjects rested supine for ~30 min prior to exercise.

In the 3rd and 6th min of the exercise femoral and subclavian venous blood flow were measured by the constant infusion thermodilution technique (Andersen & Saltin, 1985). Ice-cold saline was infused simultaneously into the two veins at a constant rate of 20-120 ml·min^{-1} for 15-25 s (Harvard pump, Harvard Apparatus, Millis, MA USA) depending on blood flow,

to achieve a drop in blood temperature of 0.4-0.9° C, while the infusate temperature was determined (model 93.505 Edwards Lifesciences LLC). The thermal data were acquired on a PC with a samplings frequency of 100 Hz by a custom build program in Labview (National Instruments, Texas, USA).

Immediately prior to the flow measurements, blood samples were drawn anaerobically from each catheter into 2 ml syringes and immediately placed in ice cold water until analyzed for blood gas variables and plasma potassium and lactate (ABL 700, Radiometer, Copenhagen, Denmark). Arterial pressure was recorded with a pressure gauge (Baxter B.V. Uden, Holland) by a transducer amplifier (GOULD instrument systems, Valley View, USA) A/D converted and acquired on a PC with a sampling rate of 100 Hz. Accuracy of the blood pressure measurement was assured by calibration using a column of water equal to 50 mmHg.

Kinetic and kinematic measurements

When the subjects performed double poling continuous measurements of pole, trunk and knee angles, vertical displacement of the pelvis, horizontally applied poling force, horizontal displacement of the pole trolley and the force in the z-direction (G) were performed. The force in the z-direction was measured by a force plate (Biomechanics type SG-9, AMTI, Massachusetts, USA) placed beneath the ergometer´s standing platform. The vertical reaction force data was amplified and low pass filtered (1050Hz). The horizontal applied poling force was measured by a strain gauge load cell arranged at the back of the horizontal slide of the trolley (U2A 200 Hottinger Baldwin Mestechnik, Darmstadt, Germany) and by integrating this force to measurements of the horizontal displacement (custom device) of the "pole-

trolley" we confirmed the power output of the ergometer. All data were simultaneously and continuously sampled on a computer at 100 Hz.

A video camera (Handycam Vision DCR TRV900E, Sony Inc., Japan) for continuous recordings of elbow, trunk and knee angles was arranged at the right side of the subjects. Analyses of the recording was done by digitizing the video frames in Adobe Premiere Pro (Adobe Systems Incorporated, Version 2.0, Stingray) and thereafter analyzed frame by frame in the HU-M-AN (Version 5.0 2D-3D) software (HMA Technology Inc.). Averages from the recordings of the 1^{th}, 4^{th} and 7^{th} min were used in the final kinematic analyzes.

Calculations

The a-v O_2diff was calculated from the arm arterial to the arm and femoral venous oxygen content, averaged for the four blood samples obtained at each workload. Similarly, arm and leg blood flow are expressed as the average of four measurements. Accordingly, arm and leg oxygen uptake were the product of these flow measurements and their respective mean leg a-v O_2diff, respectivly Also the net blood lactate and potassium balances for the arms and legs were calculated based on the respective plasma values with plasma flow being blood flow corrected for haematocrit [1-haematocrit]). Arm and leg vascular conductance (blood flow divided by the difference in mean arterial minus the estimated venous pressure assumed to be 4 mmHg for the femoral vein and 7 mmHg for the subclavian vein) was calculated (Calbet *et al.*, 2004). Oxygen delivery was calculated as the arterial O_2 content times blood flow.

Statistics

Variables were evaluated for differences between the arms and legs and for differences between workloads by homoscedastic Student's t-tests. The level of significance was set at $P<0.05$ (2-tailed) and results are presented as mean ± SE.

Results

During double poling on the ergometer, VO_2max was 60.5 (SE 3.6) $ml \cdot kg^{-1} \cdot min^{-1}$ or 91.5 (1.5)% of the value obtained during treadmill running ($p<0.05$). At the two chosen workloads, VO_2 was 54 (1.6)% and 76 (2.3)% of double poling VO_2max or 49 (1.4)% and 69 (1.9)% of the running VO_2max. At these workloads, heart rate was 142 (16) and 165 (15) $beats \cdot min^{-1}$ and arterial lactate concentration 4.3 (0.2) and 6.4 (0.3) $mmol \cdot L^{-1}$, respectively.

At rest, mean arterial pressure was 12.2 (0.3) kPa and increased slightly to 13.7 (0.5) and 13.3 (0.5) kPa ($p<0.05$ and $p=0.10$) during the two levels of exercise. Yet at the low workload, arm blood flow was 5.4 (0.6) $L \cdot min^{-1}$ and leg blood flow 5.8 (0.5) $L \cdot min^{-1}$ (ns) while for the higher workload the values were 6.3 (0.7) and 8.0 (0.5) $L \cdot min^{-1}$ ($p=0.14$), respectively. Accordingly, vascular conductance increased with workload (by 21 (5.8)% and 43 (14)% for the arms and legs, respectively; $p<0.05$) (Fig. 2).

At the low workload arm oxygen uptake was 0.65 (0.07) $L \cdot min^{-1}$ and leg oxygen uptake 0.82 (0.06) $L \cdot min^{-1}$ (ns) reflecting an arm oxygen extraction (O_2 extraction) of 59 (2.3)%, while the value for the legs was and 67 (1.3)%. With the workload increased, arm oxygen uptake increased only 20 (5)% (to 0.78 [0.08] $L \cdot min^{-1}$), without any increase in O_2 extraction, while leg O_2 extraction increased to 75 (1.5)% ($P<0.05$) resulting in a 53 (8)% increase in leg oxygen uptake (to 1.24 [0.07] $L \cdot min^{-1}$). Accordingly, the contribution of the arms and the legs to the pulmonary VO_2 was 28 (2.9)% and 35 (3.0)% at the low workload and 24 (3.1)% and 37 (2.0)% at the higher workload.

At both workloads, the arms released lactate, while the legs took up lactate and similarly, the arms released potassium while there was no significant arterial-venous difference for potassium over the legs (Table 1, fig. 3 and 4).

When the workload increased from low to moderate the work by the legs increased to counteract gravity from 70 (6.5) to 100 (9.1) J per stroke ($p<0.05$). Kinematic changes associated with increased workload by the legs included increased knee angle range of motion from 51 (8.9)° to 58 (9.9)° and vertical displacement of the hip from 10 (1.0) to 14 (1.0) cm ($p<0.05$). Trunk angle however, did not change from low to moderate intensity. Further, the elbow angle range of motion tended to increase from 71 (11.3)° to 75 (10.9)° ($p=0.07$) and the net horizontal poling displacement increased from 1.41 to 1.47 m, while the poling cycle time was reduced from 1.41 to 1.34 seconds as sub-maximal workload increased ($p<0.05$).

Discussion

During double poling the workload is shared between the arms, trunk and legs and relative distribution between the upper- and lower body is determined by the skier's technique. This study showed that during low intensity exercise, the arms and legs make an equal aerobic metabolic contribution. This was demonstrated by similar oxygen uptake in arms and legs despite the fact that muscle mass in the legs is more than double that of the arms. However, when exercise intensity was increased, the double poling technique changed with more emphasis on leg exercise shown both from kinematic data, as well as the changes in limb oxygen uptake. The increase in leg VO_2 is a consequence of both increased leg blood flow as well as O_2 extraction while arm VO_2 increases as a result of increase blood flow only.

In cross country skiing (classical style) two main techniques are used; diagonal stride and double poling. Both techniques involve legs and arms, but in the diagonal stride technique most of the work is performed by the legs while during double poling, arms contribute more to the work performed. This conclusion is based on simultaneously oxygen uptake measurements from arms and legs during skiing with these two techniques (Calbet *et al.*, 2004). These two skiing techniques are used differently. Diagonal stride is used uphill when the speed is rather low, while double poling is used on flat terrain or when the terrain is slightly uphill or is downhill. In the double poling technique a higher speed can be maintained at a relatively low oxygen cost compared to the diagonal technique (Bjorklund *et al.*, 2010). However, during uphill skiing the speed is reduced and the load is increased. In the present study we simulated this change in load by increasing the power performed on the double poling ergometer. While still using the double poling technique, the skiers changed technique putting more emphasis on the leg muscles based on significant increase of hip/pelvis vertical displacement and leg work against gravity, while the range of trunk and pole angle did not

changed. This change in double poling technique to more work performed by the leg muscles was confirmed by the fact that when power was increased, legs oxygen uptake increased more than arms oxygen uptake. One may question why this is happening, since the poling technique with the force from the arms applied as horizontally as possible still would have been beneficial biomechanically.

One obvious answer is that the upper body muscle mass that can be engaged in double poling is limited and hence poling power production is limited. However, there are also metabolic differences in the upper body and the legs in this context. The arms extract less of the supplied O_2 during double poling as well as during other types of arm exercise (Secher *et al.*, 1977;Volianitis & Secher, 2002;Calbet *et al.*, 2004;Clausen *et al.*, 1973). The present study adds to the previous studies by showing that when double poling power increases, oxygen uptake increases in the legs by increasing both blood flow and O_2 extraction, while only blood flow increases in the arms. During arm exercise only (arm cranking), oxygen uptake increases by increase in blood flow with a constant O_2 extraction in normally fit individuals (Ahlborg & Jensen-Urstad, 1991;Volianitis *et al.*, 2004), while Volianitis et al, (2004) showed that in very fit individuals (rowers), also O_2 extraction in the arms increased with workload. However, the present study is the first to show that during combined leg and arm exercise, O_2 extraction do not increases in the arms with increasing oxygen uptake even in relatively fit individuals. However, our subjects were not elite skiers, and we cannot refute that elite skiers are able to increase their O_2 extraction with increasing workload. The O_2 extraction in the present study is about 5% lower than in the elite skiers (Calbet *et al.*, 2005).

Interestingly, O_2 extraction is higher during diagonal skiing both at submaximal and maximal intensities (Calbet *et al.*, 2005;Bjorklund *et al.*, 2010). However, arm blood flow and oxygen

uptake is lower during maximal diagonal striding than during submaximal double poling (Calbet *et al.*, 2005). The moderate intensity during submaximal double poling in the present study was at 69% of running VO_2max and similar to the intensity used by Calbet et al. (2005). They concluded that their skiers were close to the maximal intensity during double poling, even though pulmonary oxygen uptake was 72% of VO_2max, supported by a high arterial lactate concentration which was close to the lactate concentration in our study (van Hall *et al.*, 2003). It is therefore likely that if our skiers used diagonal stride to perform the moderate workload in the present study, the demand on the arms would be reduced to a more submaximal workload. This is supported by recordings of reaction force data during double poling and in the diagonal stride, showing lower force amplitude in the diagonal stride compared to double poling (Nilsson *et al.* 2004b). Therefore, in the field the diagonal stride would probably be the skiers' choice.

In contrast to arm cranking, associated with a marked blood pressure response making the perfusion pressure to the arm similar that for the legs during cycling, there was confirmed that combined arm and leg exercise during double poling elicits only a small blood pressure response (Secher *et al.*, 1977). Furthermore, when exercise intensity increases, blood pressure does not increase and vascular conductance increases more in the legs than in the arms. The relatively small increase in conductance in the arms despite that the metabolic stress is higher in the arms than in the legs, supports the hypothesis that red cell deoxygenation, which is relatively small in the arms, is important in blood flow regulation (Gonzalez-Alonso *et al.*, 2001). Accordingly, blood is directed towards the muscles with the highest metabolic capacity. This was also supported in a study where one leg was trained to increase the metabolic capacity of that leg. Blood flow as well as O_2 extraction were higher in the trained leg even though the legs exercised exactly at the same power (Rud et al., in revision).

Our data confirm that arms demonstrate a net release of lactate while the legs have a net uptake during combined arm and leg exercise. The present study adds to previous studies by showing that even if the oxygen uptake in the legs increased by more than 50%, net lactate uptake doubled. At the same time lactate release from the arms increased by 84% as oxygen uptake increased by 20%. This support the hypothesis that the lactate taken up by the exercising muscles in the legs is oxidized and that the capacity for uptake depends on the exercise intensity (van Hall *et al.*, 2003). We accept that we did not quantify the unidirectional uptake of lactate in the present study and therefore cannot quantify how much lactate that was oxidized. However, van Hall et al. (2003) showed that the unidirectional release of lactate is only 25% of the unidirectional uptake during double poling at a similar intensity as the moderate intensity in the present study. Hence, it is likely that the oxidation of lactate doubles from low to high intensity.

A new finding was that the arms also released a significant amount of potassium during combined arm and leg exercise (~0.5 $mmol \cdot min^{-1}$), while there was no net potassium release (or uptake) from the legs. Potassium is part of the signaling system and its efflux over the sarcolemma is connected to the depolarization of the membrane potential while its reuptake in the cells is accomplished by the sodium-potassium pump (Na^+/K^+-ATPase). Yet, regulation of potassium over the sarcolemma may be coupled to the metabolism, for example through ATP sensitive ion channels and the metabolic cost of reuptake via the Na^+/K^+-ATPase. More importantly, the Na^+/K^+-ATPase is partly stimulated by the sympathoadrenergic system (Hallen, 1996), and during both the low and the moderate exercise intensity the arm muscles worked at high intensity and probably close to their maximal capacity (Holmberg et el. 2005). The central cardio respiratory stress was however moderate and hence the

sympathoadrenergic activation is probably also likely to be moderate. Low sympoatoadrenergic stimulation may cause an “understimulation” of the sodium-potassium pump and explain the large net potassium release from the arms. The relatively low sympoatoadrenergic stimulation may also cause the exercising arm muscles to be overperfused, due to lack of a sympathoadrenergic vasoconstriction.

In conclusion, during double poling a large contribution from the arms is advantageous from a biomechanical point of view, but at high workloads the arm muscle mass might be too small and therefore the work carried out by the legs. In this situation, local vasodilatation might dominate over central sympoatoadrenergic vasoconstriction in the arms, which make the muscles overperfused. This may results in an oxygen delivery that is larger than the capacity of the muscles to take up oxygen. Hence, O_2 extraction becomes low.

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Table 2. Blood gas variables, blood lactate and potassium during simulated skiing.

Variable	Location	Rest	Low	Moderate
Hb ($g \cdot dl^{-1}$)	arterial	14.9 (0.3)	15.3 (0.3)	15.2 (0.2)
	sv arm	15.0 (0.2)	15.3 (0.3)	15.1 (0.4)
	fv leg	14.7 (0.4)	15.0 (0.3)	15.0 (0.2)
PO_2 (kPa)	arterial	13.2 (0.2)	13.7 (0.2)	13.5 (0.2)
	sv arm	4.1 (0.3)	3.0 (0.1)	3.0 (0.1)
	fv leg	3.2 (0.1)*	2.4 (0.1)*	2.0 (0.1)*†
pH	arterial	7.4 (0.0)	7.4 (0.0)	7.4 (0.0)†
	sv arm	7.4 (0.0)	7.3 (0.0)	7.2 (0.0)†
	fv leg	7.4 (0.0)	7.3 (0.0)	7.3 (0.0)†
sO_2 (%)	arterial	99.0 (0.1)	99.0 (0.1)	98.8 (0.1)
	sv arm	60.9 (3.9)	40.3 (2.1)	39.3 (1.6)
	fv leg	48.0 (2.2)*	32.7 (1.1)*	24.5 (1.4)*†
ctO_2 (Vol%)	arterial	20.3 (0.4)	20.8 (0.4)	20.7 (0.3)
	sv arm	12.5 (0.8)	8.5 (0.6)	8.2 (0.5)
	fv leg	9.7 (0.7)*	6.8 (0.3)*	5.1 (0.4)*†
PCO_2 (kPa)	arterial	5.0 (0.1)	4.8 (0.1)	4.3 (0.1)†
	sv arm	6.8 (0.2)	8.2 (0.2)	7.8 (0.3)†
	fv leg	6.7 (0.2)	7.7 (0.1)*	7.5 (0.2)¤
Lactate ($mmol \cdot L^{-1}$)	arterial	1.8 (0.2)	4.3 (0.2)	6.4 (0.3)†
	sv arm	2.0 (0.3)	5.7 (0.4)	8.1 (0.4)†
	fv leg	1.7 (0.2)	3.5 (0.3)*	5.4 (0.3)*†
K^+ ($mmol \cdot L^{-1}$)	arterial	4.18 (0.05)	4.99 (0.08)	5.42 (0.05)†
	sv arm	4.14 (0.10)	5.33 (0.10)	5.75 (0.09)†
	fv leg	4.17 (0.09)	4.95 (0.08)*	5.36 (0.03)*†

Hb, hemoglobin; PO_2, partial pressure of oxygen; sO_2, oxygenation of hemoglobin; ctO2, concentration of oxygen; PCO_2, partial pressure of carbon dioxide; K^+, potassium; a, arterial; sv arm, subclavian vein arm; fv leg, femoral vein leg, Asterisks and numbers indicate a P-value < 0.05 and 0.1 between fv leg and sv arm between arms and legs, respectively, Cross and currency (¤) indicate a p-value < 0.05 and 0.1 between low and mod exercise, respectively.

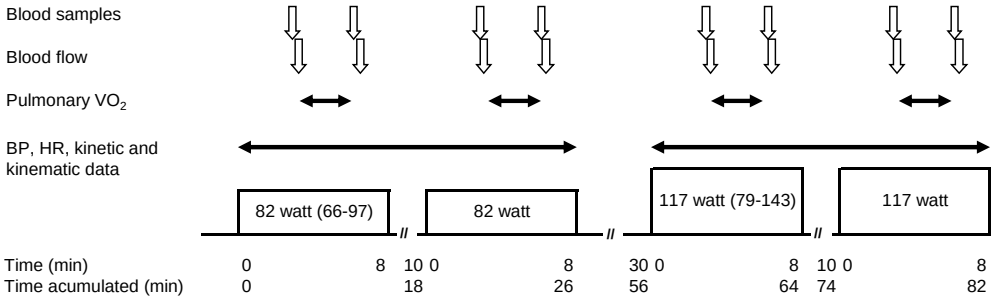

Figure 1. Schematic protocol of the experiment.

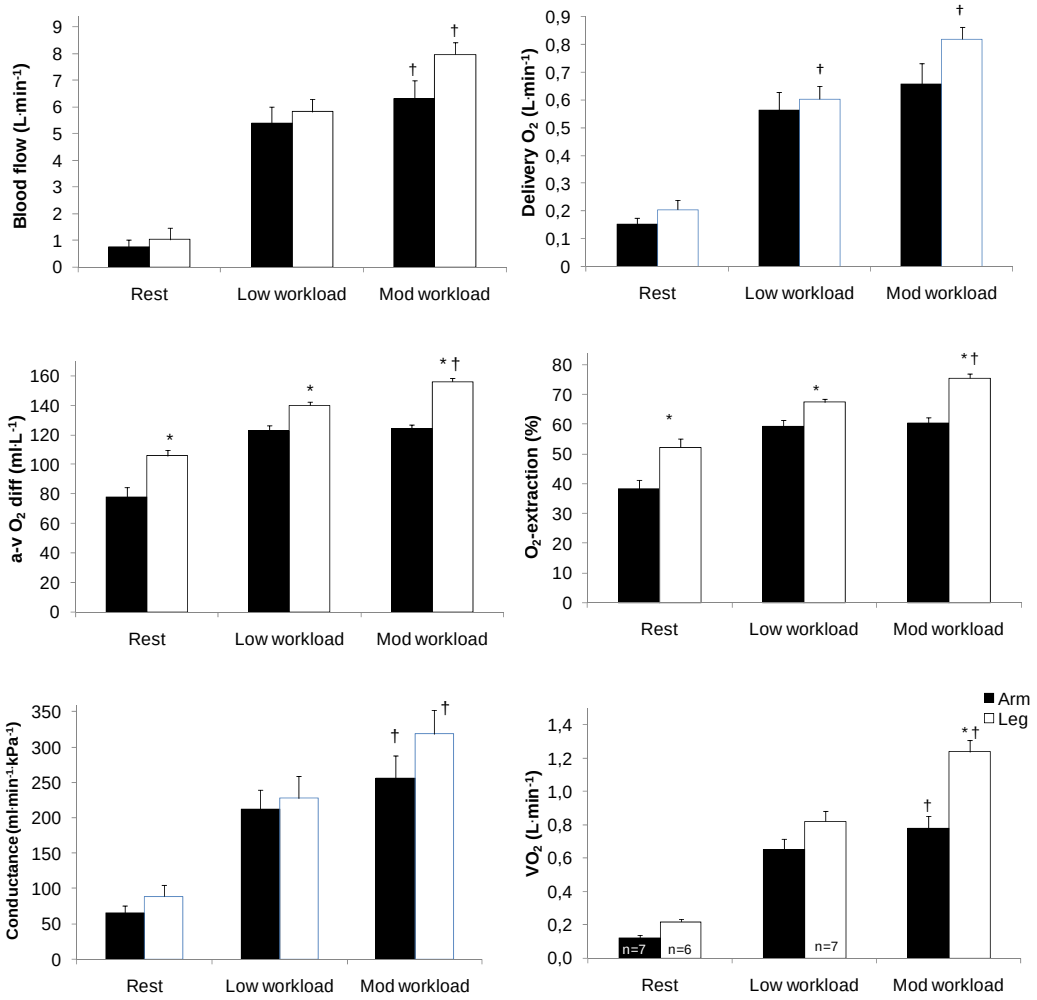

Figure 2. Blood flow, arm and leg a-v O_2diff; arterial – venous oxygen difference, Conductance; vascular conductance, Delivery O_2; delivery oxygen, O_2-extraction; oxygen extraction and VO_2; oxygen uptake plotted at different workloads. Asterisks indicates statistical significant difference between arm and leg ($P<0.05$) and cross sign statistical significant difference between workloads ($P<0.05$). N is as indicated, otherwise and for a-v O_2diff and oxygen extraction n=8.

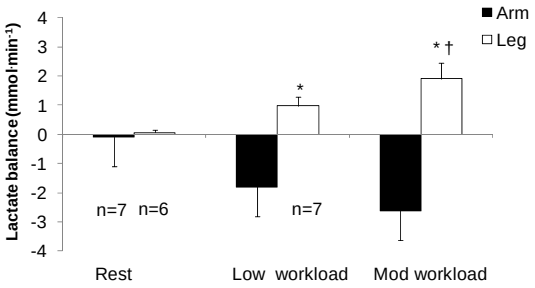

Figure 3. Lactate balance in arm and leg at rest and during easy and moderate workloads. Negative values indicate net release, and vice versa. Both the arms and legs had a significant release or uptake during both workloads ($p<0.05$). Asterisks indicate a p value less than 0.05 between arm and leg, and cross sign between workloads. N is as indicated, otherwise n=8.

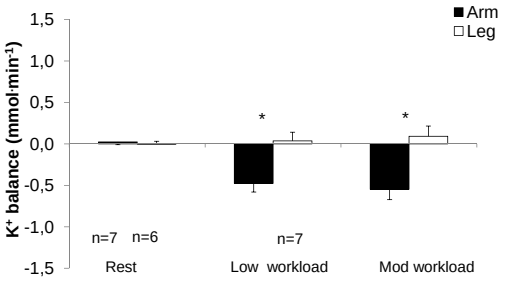

Figure 4. Potassium (K^+) balance in arm and leg at rest and during easy and moderate workloads. Negative values indicate net release, and vice versa. Arms had a significant release during both workloads ($P<0.05$), while potassium was in balance over the legs. Asterisks indicate a P value less than 0.05 between arm and leg. N is as indicated, otherwise n=8.