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High Intensity Knee Extensor Training Restores Skeletal Muscle Function in COPD Patients.

Brønstad, Eivind M.D.[1, 2*]; Rognmo, Øivind PhD[1*]; Tjonna, Arnt Erik PhD[1]; Dedichen, Hans Henrich M.D.[1,3]; Kirkeby-Garstad, Idar M.D. PhD[1,3]; Håberg, Asta K. M.D. PhD[4,5]; Bjørk Ingul, Charlotte M.D. PhD[1]; Wisløff, Ulrik PhD[1, 6]; Steinshamn, Sigurd M.D. PhD[1,2].

(1) K. G. Jebsen Center of Exercise in Medicine at Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway, (2) Lung Department, St. Olavs Hospital, Trondheim, Norway, (3) Department of Heart and Lung Surgery, St.Olavs University Hospital, Trondheim, Norway, (4) Department of Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway , (5) Department of Medical Imaging, St. Olavs Hospital, Trondheim, Norway, (6) Centre for sports and physical activity research, Norwegian University of Science and Technology, Trondheim, Norway ,(*) shared authorship.

Corresponding author: Eivind Brønstad, Department of Circulation and Medical Imaging, Norwegian University of Science and Technology. Postboks 8905, Medisinsk teknisk forskningssenter 7491 Trondheim, Norway. E-mail: eivind.bronstad@ntnu.no

Fax: +47 728 28 372. Telephone: +47 728 28 022.

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Abstract

Improving reduced skeletal muscle function is important for optimising exercise tolerance and quality of life in COPD patients. By applying high intensity training to a small muscle group, we hypothesised a normalization of muscle function.

Seven patients with COPD performed six weeks (3d/week) of high intensity interval aerobic knee extensor exercise training. Five age-matched healthy individuals served as reference group. Muscle oxygen uptake ($VO_{2\text{-peak}}$) and mitochondrial respiration of the m.vastus lateralis were measured before and after the 6 week training program.

Initial peak work and maximal mitochondrial respiration were reduced in COPD patients and improved significantly after the training program. Peak power and maximal mitochondrial respiration in m.vastus lateralis increased to control subjects' level and were mainly mediated via improved complex I respiration. Furthermore, when normalized to citrate synthase activity, no difference in maximal respiration was found neither after the intervention nor compared to controls, suggesting normal functioning mitochondrial complexes.

The present study shows that high intensity training of a restricted muscle group is highly effective in restoring skeletal muscle function in COPD patients.

Introduction

COPD is now considered to be a multiorgan disease and reduced muscular function is one prominent feature [1]. The principal finding in lower limb skeletal muscle is reduced oxidative capacity with muscle fibre shift, reduced mitochondrial density, reduced mitochondrial biogenesis and impaired mitochondrial respiration [2-5]. Similar changes have been found in sedentary subjects, and it is debated whether these changes are due to the pathogenesis of COPD or a consequence of inactivity [3, 6].

In both sedentary and COPD patients, exercise training is the only intervention shown to partly reverse these changes, mainly by improved muscle oxidative capacity [7, 8] . The effects of exercise on skeletal muscle have mostly been studied with whole body exercise training [8-10] and it has been suggested that the ventilatory limitations prevent the locomotor system from being adequately taxed and thereby reduce the training effects [11]. The existence of a metabolic reserve has been demonstrated in COPD patients when testing in small muscle groups in a model relieved from respiratory constraints [12] and it has also been demonstrated that exercising one-leg improves VO_{2max} more than whole body training [13]. In healthy individuals, training intensity is one of the key factors determining the training response[14]. Due to the COPD patients' ventilatory limitations, high enough exercise intensity is often not possible to attain. By exercising restricted muscle groups, eg choosing an exclusive lower limb exercise model, the central limitation can be avoided [15, 16]. Furthermore, by performing the training in short high intensity intervals, maximum exercise effects would be expected [17].

To further explore whether reduced muscle function in COPD to a large extent is caused specific muscular abnormalities or reversible by exercise training, we studied the muscle

metabolic reserve capacity in a training model unveiled from central constraints to allow the muscle to work at a maximum load. We choose a model of six week high intensity knee-extensor exercise training to bypass the central limitations. We hypothesised that by interval training at high intensity a restricted muscle group, a normal exercise response in the individual mitochondrial respiration complexes would be found.

Methods

Study subjects

Eight patients with stable COPD, a smoking history > 20 pack/years, FEV_1< 60% (post bronchodilator) and age > 50 years and without resting hypoxaemia were included in the study. Two patients were current smokers. Patients with clinical heart disease or a medical condition limiting exercise training were excluded. None of the patients used systemic steroids and none of the patients changed their medications during the study period. None of the patients had participated in a pulmonary rehabilitation program during the last three months. As controls, five healthy non-smoking age matched subjects with normal lung function were included. The control group did not participate in regular sports or leisure activities. These subjects did not participate in the exercise training and served only as controls for the baseline values (knee extensor peak work, muscle biopsy and muscle mass determination by MRI). All subjects underwent pulmonary testing, treadmill aerobic capacity testing and resting echocardiography examination at baseline and after the training period. (see online supplement).

The study was approved by the Norwegian (Regional) Ethics Committee, adhered to the Helsinki Convention and registered at ClinicalTrials.gov (NCT 01079221). All patients gave their written informed consent. Patients' characteristics are shown in table 1.

Knee-extensor peak work testing and exercise training

The muscular work was limited to the quadriceps of one leg only by using a knee-extensor exercise model [15, 16]. To determine their quadriceps peak work capacity, an incremental knee-extensor protocol was performed at baseline and after the training intervention. After a 5 min warm-up, each subject performed a work protocol with 2 watt increments every 3 min until reaching exhaustion with a kicking frequency at 60 kicks per minute. During the session, oxygen consumption, femoral artery flow and arterial and venous blood gasses were sampled. Venous and arterious blood samples were only taken from the COPD group. The femoral artery flow and blood gasses were sampled within the last 30 seconds of each load. All testing was performed on the right leg.

Prior to exercise training each patient adapted to the knee-exercise model by two training sessions. On the last session peak work and oxygen consumption was measured. All COPD patients went through a 6 week exercise program consisting of 3 training sessions pr week. After a 5 min warm up without load, 4 intervals of 4 minutes at 90 % of peak work rate were performed. Each interval was separated by a 2 minutes active period of unloaded kicking. A kicking frequency at 60 kicks per minute was pursued. Both legs were exercised separately and the load was increased each week (see results) to ensure work at 90 % of peak load.

Oxygen uptake in quadriceps muscle

To determinate the oxygen uptake in working quadriceps muscle, the muscle mass, blood flow and the arterio-venous (AV) difference was measured [11]. The O_2 uptake was calculated by multiplying blood flow with arterial–venous oxygen difference (AV-O_2). The AV-O_2 difference was determined by venous and arterial blood gasses sampled from the radial artery and the femoral vein. Samples for blood gas analysis were draw during the last 30 seconds of the working loads and analyzed (ABL 625 blood gas analyzer Radiometer, Copenhagen, Denmark). Venous and arterial access was gained by placing a catheter in the in the right femoral vein and artery catheter in the left radial artery. The femoral blood flow was measured with a ultrasound probe (Wingmed Horten, Norway) placed over the femoral artery [18]. The flow was determined at each load during the last 30 seconds. Flow data were analyzed with EchoPack^{TM}. Muscle mass of the quadriceps was measured by MRI (see online supplement). The quadriceps muscle volume was calculated by multiplying the surface area of each MRI slice by the slice thickness, and then sum up all the slices[19]. To adjust for the gap between each slide, the calculations was done by use of the truncated cone method[20]. To calculate the quadriceps mass, the muscle volume was multiplied by the muscle density[21].

Citrate Synthase activity

Biopsies were obtained from the vastus lateralis and performed at baseline and 72 hours after last training session to avoid the acute training effects[22]. The samples was homogenized in CelLytic buffer (Sigma-Aldriuch, USA) at 6000 shakes/min for 2 x8 seconds in a Precellys24 tissue homogenizer (Bertin,France). The homogenate was then centrifugated at 10000 g for 10 min at 4 °C and the supernatant tested for citrate synthase (CS) activity. The activity was

determined by the method by Srere by the use of Citrate Synthase Assay Kit (Sigma-Aldrich)[23]. Absorbance at 412 nm was measured on a Fluostar Omega spectrometer (BMG Labtech, Germany). Specific activity was calculated by dividing measured activity on the muscle extract protein concentration.

Mitochondrial respiration

Mitochondrial respiration was studied *in situ* in saponin permeabilized fibers as described by Veksler and colleagues [24] and reviewed recently [25]. Briefly, fibers from the biopsies of vastus lateralis were gently separated under binocular microscope using small forceps (Dumont # 5) in separation solution (S) at 4°C, and then permeabilized in the same solution with 50 μg/ml saponin for 30 min at 4°C while shaking. After being rinsed for 10 min in solution S at 4°C and then in respiration solution R at 22°C under shaking, the skinned fibers were transferred to a water-jacketed oxygraphic cell (Strathkelvin Instruments, Glasgow, UK) equipped with a Clark electrode containing 3 ml of solution R. Solutions R and S contained 2.77 mM CaK2EGTA, 7.23 mM K2EGTA (100 nM free Ca2+), 6.56 mM MgCl2 (1 mM free Mg2+), 20 mM taurine, 0.5 mM DTT, 50 mM potassium-methane sulfonate (160 mM ionic strength), and 20 mM imidazole (pH 7.1 at 22°C). Solution S also contained 5.7 mM Na2ATP, 15 mM creatine-phosphate, while solution R contained 10 mM glutamate, 4 mM malate, 3 mM phosphate, and 2 mg/ml bovine serum albumine. Basal respiration rate (V_0) was measured at 22°C under continuous magnet stirring in the oxygraphic cells. Maximal ADP-stimulated respiration above V_0 was measured by addition of 2 mM ADP as phosphate acceptor and the maximal respiration rate (V_{max}) was calculated as (V_0+V_{ADP}). The acceptor control ratio (ACR) was calculated as ratio of V_{max} to V_0. Following ADP additions, functioning of various complexes of the electron transport chain function was assessed [26]. Addition of 10 mM succinate, followed by 1 mM amytal, a specific inhibitor of complex I,

allowed estimation of the maximal respiration involving complexes II, III, and IV ($V_{succinate}$). Thereafter, ascorbate (0.5 mM) and N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD; 0.5 mM) and were added to estimate maximal respiration from complex IV ($V_{asc+TMPD}$). After the measurements the fibers were dried and respiration rates were expressed as micromoles O_2 per minute per gram dry weight.

Statistics

Data are presented as mean ± standard deviation. The changes in physiologic variables were calculated baseline and post training. Control group and COPD baseline differences were analyzed with t-test. Assumptions of normality were assessed by normal probability plots. COPD group baseline and follow-up differences were analysed using paired t-test. The level of significance was set at $p<0.05$

Results

Knee extensor exercise training and testing

Seven patients completed the exercise program with a total of 18 sessions each. The average training intensity was in the first week 12.7±3.8 W and in the sixth week 18.4±5.3 W. The change in training load during the training period is shown in figure 1. The peak power at baseline was significantly lower than in the control subjects, and increased from 14.6±4.9 W to 20.0±5.3 W ($p<0.001$) in the COPD patients. We found no change in $VO_{2\text{-peak}}$ or work economy at the treadmill testing.

Resting echocardiography was performed before and after the exercise training program and no effects on cardiac output, ejection fraction and stroke volume were observed.

No difference in quadriceps muscle mass was observed either at baseline or at post-tests (Table 2).

Oxygen consumption during knee extensor exercise

During the knee-extensor exercise both muscular ($mVO_{2\text{-peak}}$) and pulmonary ($pVO_{2\text{-peak}}$) peak oxygen uptake were measured. After the 6 week exercise program, $mVO_{2\text{-peak}}$ increased from 200±40 ml/min/kg to 248±43 ml/min/kg ($p<0.05$). The $dAV\text{-}O_2$ did not change, but femoral blood flow increased significantly from 2127±655 mL to 2631±348 mL ($p<0.05$). Quadriceps work economy (mWE) was measured in the patients at a load of 6 watts. MWE at baseline was 117±30 ml/min/kg and at follow-up 104 ± 29 ml/min/kg, but failed to reach statistical significance. Likewise the lactate level at work-economy load was 2.16±1.0 mmol/L at baseline and at follow-up 1.67±1.1 ($p=0.17$). The pulmonary oxygen uptake at peak work during the knee-extensor test was not improved (baseline 10.9±1.8 ml/min/kg, follow-up 11.7±1.2 ml/min/kg ($p=0.30$). However, the pulmonary uptake at sub maximal work (pWE) was reduced by 26 %, from 7.8±2.7 ml/min/kg at baseline to 5.8 ±1.3 ($p<0.05$) at follow up (Table 2), suggesting improved work economy. Moreover, minute ventilation (VE) was reduced from 16.7± 1.3 l/min to 13.3 ± 2.0 l/min ($p<0.05$) after the training intervention suggesting reduced ventilatory demands.

Citrate Synthase activity

The CS activity in COPD muscle increased after the training program increased from 0.29±0.07 U/mg to 0.37±0.11 U/mg (p=0.01), indicating increased mitochondrial mass and density. We found no difference in baseline CS activity when compared to the healthy controls

Mitochondria respiration

V_{max} per unit of fiber weight was significantly lower in COPD patients compared to healthy controls (3.68±0.73 vs. 4.52±0.44 μmol $0_2 \cdot min^{-1} \cdot$ g dry $weight^{-1}$, p=0.045). However, six weeks of knee-extensor exercise training improved V_{max} of the COPD patients by 40% to 5.15±1.32 μmol $0_2 \cdot min^{-1} \cdot$ g dry $weight^{-1}$(p=0.013) and was no longer different from controls. The basal respiration was similar between groups and did not change after exercise training (Figure 2). We did not find any differences in the maximal activity of complex II ($V_{succinate}$) and IV ($V_{asc+TMPD}$) of the respiratory chain between groups, or in COPD patients in untrained or trained state (Figure 3). When maximal mitochondrial respiration was normalized to citrate synthase activity, we found no difference between controls and COPD patients at baseline (Figure 4).

Discussion

To our knowledge, this study is the first to specifically assess the effect of high intensity interval exercise training on mitochondrial respiration in COPD in an exercise model relieved

from respiratory constraints, thereby obtaining maximum training loads on the exercising muscles. Compared to healthy age-matched controls, the COPD group had significantly reduced peak aerobic power, peak quadriceps muscle uptake and maximal mitochondrial respiration at baseline. The six week training program resulted in a significant increase in aerobic power, peak quadriceps muscle oxygen uptake and maximal mitochondrial respiration. These results demonstrate an improvement of the quadriceps oxidative capacity in COPD patients by exercising a small muscle group. In addition, all respiration differences were attenuated when adjusted for citrate synthase activity, which suggests reduced mitochondrial mass rather than specific mitochondria respiratory impairment in COPD.

Our findings of a reduced maximal mitochondrial respiration to complex I compared to healthy, age matched controls are consistent with previous studies in COPD patients and interestingly also in sedentary individuals' lower limb muscle [3, 27]. Our data are in line with Picard et al showing a significantly lower maximal respiration per unit of fibres weight involving complex I (V_{max}) in patients with COPD compared to fibres from healthy subjects. Also when comparing sedentary persons to individuals participating in regular high intensity aerobic exercise training, a reduced maximal respiration has been found among sedentary subjects. This difference has been found to be related to a higher complex I activity compared with complex II in more trained subjects [28].

Despite a trend towards lower complex II stimulated respiration ($V_{succinate}$) in our COPD patients compared to the healthy controls, we were not able to show significantly lower values in contrast to two earlier studies [3, 27]. A higher fitness level in our COPD patients may explain why we were not able to detect a significant down-regulation of complex II respiration or supply of $FADH_2$. Despite a similar FEV_1 (%pred), our COPD patients had a

VO_{2max} of 20.4 ml/kg/min which was 40% [3] and 25% [27] higher compared to the two earlier studies. The observation that mitochondrial respiration differences were attenuated after exercise training, suggests absence of specific mitochondria respiratory impairment in COPD. This response to exercise training is also consistent with the hypothesis that physical inactivity may cause peripheral muscle respiratory deficiency in COPD patients.

Whole body exercise in COPD patients has been shown to increase oxidative capacity and reduce lactic acid production during exercise [7-9, 29]. Our findings of an increased maximal mitochondrial respiration and improvement of complex I in COPD are in line with effects found in healthy individuals after endurance training [28]. Compared to athletes, sedentary people have a lower proportion of highly oxidative type I fibres [30], a phenotype also seen in COPD patients [31]. In COPD patients, Picard et al found a reduced mitochondrial respiration that was normalized when correlated for citrate synthase levels [3]. When we normalized our respiration data to citrate synthase activity, we observed that the complex I respiration was not different from the healthy controls. Also, the increase in maximal respiration after training was in the same magnitude as the increased CS activity, suggesting improved respiration due to increased mitochondrial mass. More mitochondria rather than reversal of a dysfunctional respiratory chain, may therefore explain the mechanism behind the increased muscle aerobic capacity. This could point to inactivity, rather than dysfunction, as a possible explanation of the reduced mitochondrial respiration.

Also at the whole muscle level we found increased peak oxygen uptake with an increased femoral blood flow at peak exercise after training. In healthy sedentary individuals endurance training results in both improved cellular bioenergetics and muscle oxygen transport [14]. This therefore adds further support to a normal training response in the COPD patients when

relieved from ventilatory limitation. Surprisingly, we could not show reduced muscle oxygen consumption (mVO_2) at sub maximal load which would reflect an improved work economy. Only the pulmonary O2 uptake (pVO_2) was significantly reduced at sub maximal levels suggestive of an improved work economy in our study. We also observed a reduced VE reflecting the reduced oxygen consumption. This is an important effect of endurance training for COPD patients, as this reflects activity at a moderate level, similar to activities of daily living.

Present data do not suggest that whole-body endurance training is efficient in reversing all aspects of impaired muscle function. Both Vogiatzis and our group (submitted paper) failed to show an improvement in CS activity in thigh muscle even after high intensity aerobic interval training [8]. Dolmage et al has shown that one-legged exercise training is superior to whole body endurance training in improving aerobic capacity in COPD [13]. The present study shows that full reversal of impaired mitochondrial respiration might not be attainable in whole body endurance training, contributes to the advantages of one-legged exercise training in COPD.

There are some limitations to our study. Due to the invasiveness of this study, the number of participants was low. This may have resulted in loss of statistical significance, especially in some of the sub-analysis of the respiratory chain complexes.

We have suggested inactivity as a possible cause of reduced mitochondrial function, but did not measure the physical activity level of the participants. Pitta et al reports that COPD is associated with inactivity which supports our assumption that COPD patients are more inactive than healthy people [32]. Still, although being a possible explanation, we cannot

conclude that inactivity is the aetiology of the reduced muscle function in the COPD patients in our study.

Furthermore, we did not train the control subjects as the effects of exercise training on mitochondrial function in healthy individuals are well documented[28, 33, 34]. The measurements of oxygen uptake in the quadriceps muscle could have been underestimated due potential mixing of venous blood from calf musculature, and thereby leading to underestimated AV-O_2 difference. However placing a cuff distal to the knee to avoid this was not feasible due to the length of our peak work protocol.

Conclusion

High intensity aerobic interval training of a limited muscle group restored work performance and oxidative capacity of the quadriceps muscle in COPD patients. The increased mitochondrial respiration was found mainly to be caused by an improvement of mitochondria complex I. Our results are similar to findings in sedentary individuals, and thereby suggesting inactivity rather than a dysfunction as a possible aetiology.

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Table 1- Study subjects baseline characteristics

	Control group, n=5	***COPD group n=7***	***p-value***
Age (years)	70.0 ± 4.6	67.6 ± 7.2	ns
Height (cm)	172±6	175±7	ns
Weight(kg)	75.2±4.9	75.1±6.4	ns
SpO_2 at rest (%)	Nd	96.4± 1.2	-
BMI (kg/m^2)	25.9 ± 1.3	24.6 ± 2.1	ns
FEV_1 (liters/s)	2.71 ± 0.4	1.50 ± 0.3	0.0027
FEV_1(%pred)	93.3 ± 13.6	45.5 ± 9.8	0.0045
FVC (liters)	3.89 ± 0.31	3.20 ± 0.77	ns
FEV_1%FVC (%)	73.9 ± 2.6	48.2 ± 8.9	0.0045
VO2-peak (ml/kg/min)	38.9± 5.0	20.4 ± 4.0	0.0045
PackYears (pack/year)	0	38 ± 14	0.000

BMI- body mass index, FEV_1- Forced expiratory volume in one second, FVC- forced vital capacity, VO_2-peak- peak oxygen consumption. Nd- no data, Ns- not significant.

Table 2- Exercise testing data

	Control group (n=5)		**COPD group (n=7)**		
	Baseline	p-value	Baseline	Follow-up	p-value
Treadmill testing					
Peak VO2 (ml/kg/min)	38.9±5.0	0.005	20.4±4.0	20.2±3.6†	0.68
WE (ml/kg/min)	nd	-	11.0±0.6	10.6±0.5	0.44
VE at pw (L/min)	87 ±8	0.001	49 ±9	49 ± 11‡	0.87
Knee-extensor testing					
Peak work (W)	23.2±6.7	0.03	14.6±4.9	20.0±5.3	0.001
Quardiceps muscle mass (kg)	1.64±0.20	0.10	1.39±0.27	1.43±0.26	0.45
Femoral bloodflow at 6W (L/min)	1185±236	0.85	1213±242	1195±250	0.87
Femoral blodflow at pw (L/min)	2854±168	0.03	2127±655	2631±348	0.03
mVO_2 at 6W	nd	-	117±30	104±29	0.26

(ml/kg/min)					
mVO_2 at pw (ml/kg/min)	nd	-	200±40	248±43	0.048
pVO_2 at 6W (ml/kg/min)	5.21±1.21	0.07	7.81± 2.67	5.78 ± 1.29	0.026
pVO2 at pw (ml/kg/min)	14.6 ± 4.9	0.036	10.9± 1.8	11.7 ± 1.2	0.30
Lactate at 6W (mmol/L)	nd	-	2.16±1.02	1.67±1.13	0.17
Lactate at pw (mmol/L)	nd	-	4.4±2.2	6.3±2.3	0.06
VE at 6W (L/min)	13.0± 1.2	0.02	16.7±2.7	13.3±2.0	0.007
VE at pw (L/min)	33.9 ± 9.9	0.16	27.4±4.3	29.3±2.8	0.18

Peak VO_2 - peak oxygen consumption, WE- work economy, PW- peak work, pVO_2- pulmonary oxygen uptake during knee extensor testing, mVO_2- quadriceps muscle uptake during knee extensor testing, VE- minute ventilation, Nd-No data.
First column with p-values are significance levels between baseline control and baseline COPD. Last column with p-values are significance levels between COPD baseline and COPD follow-up. Statistical significance between controls and COPD at follow-up: *-$p<0.05$, †-$p<0.01$, ‡ $p<0.001$

Figure 1- Training intensity during the training program

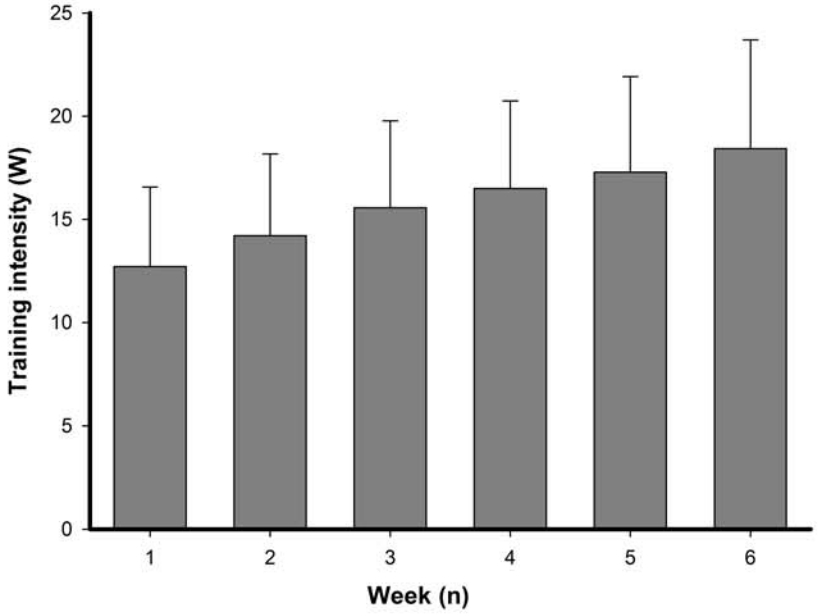

25
20
15
10
5
0
Training intensity (W)
1
2
3
4
5
6
Week (n)

Figure 2- Basal and maximal mitochondrial respiration at baseline and after the training period in quadriceps muscle

V_o- Basal respiration rate with 10mM glutamate and 4 mM malate without ADP as phosphate acceptor. V_{max}- maximal respiration rate with glutamate and malate after addition of 2 mM ADP. Ns –not statistical significant.

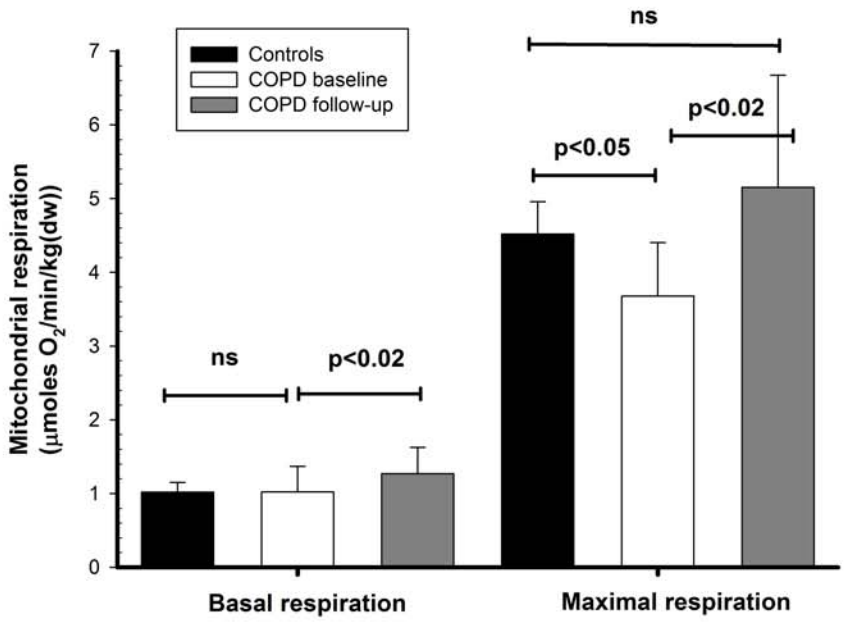

Controls
COPD baseline
COPD follow-up
Mitochondrial respiration
(μmoles O2/min/kg(dw))
0
1
2
3
4
5
6
7
ns
p<0.02
ns
p<0.05
p<0.02
Basal respiration
Maximal respiration

Figure 3-Evaluation of respiration in the individual mitochondrial complexes.
$V_{succinate}$-estimation of the maximal respiration from complexes II, III, and IV, $V_{asc}+_{TMPD}$ - maximal respiration from complex IV, TMPD-N,N,N',N'-tetramethyl-p-phenylenediamine,

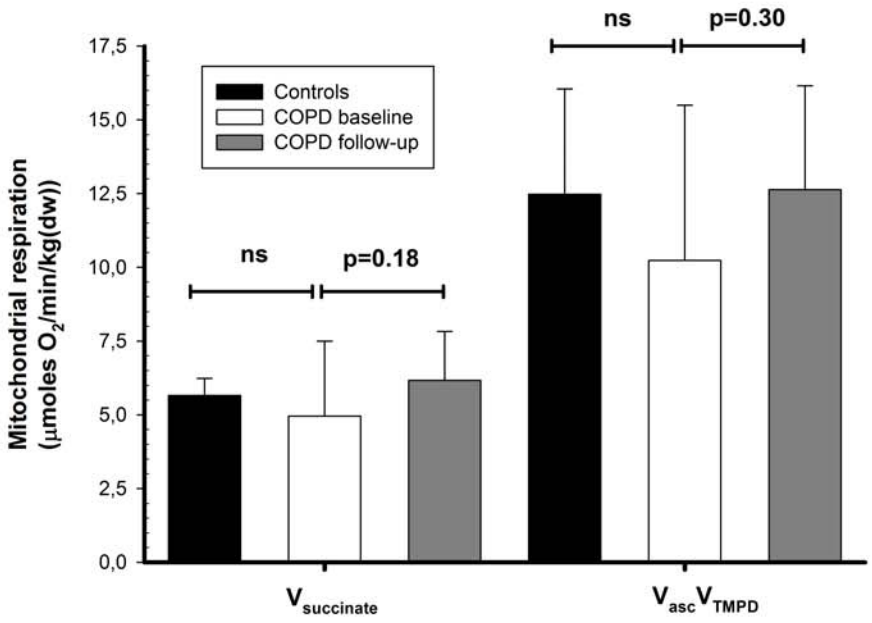
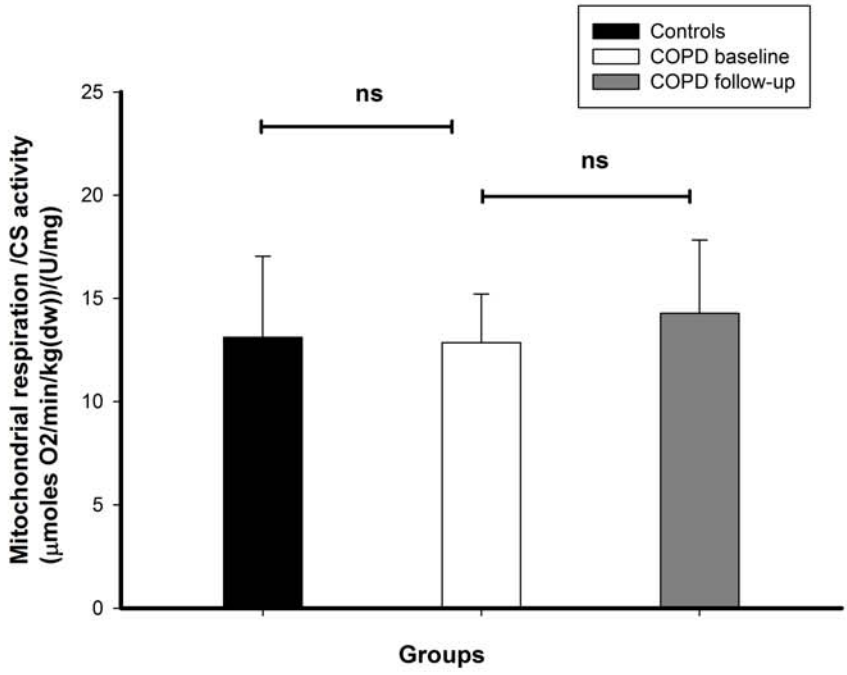


Figure 4- Maximal mitochondrial respiration relative to citrate synthase activity.


Controls
COPD baseline
COPD follow-up
ns
ns
25
20
15
10
5
0
Mitochondrial respiration /CS activity
(µmoles O2/min/kg(dw))/(U/mg)
Groups