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Leg blood flow is impaired during small muscle mass exercise in COPD patients

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Running head: Leg blood flow in COPD

Trial registration: ClinicalTrials.gov (NCT02360865)

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Abstract

Skeletal muscle blood flow is regulated to match the oxygen demand and dysregulation could contribute to exercise intolerance in patients with COPD. We measured leg hemodynamics and metabolites from vasoactive compounds in muscle interstitial fluid and plasma at rest, during one-legged knee-extensor exercise, and during arterial infusions of sodium nitroprusside (SNP) and acetylcholine (ACh), respectively. Ten patients with moderate to severe COPD and eight age- and sex matched healthy controls were studied. During knee-extensor exercise (10 W), leg blood flow was lower in the patients compared with the controls (1.82±0.11 versus 2.36±0.14 L/min, respectively, P<0.05) which compromised leg oxygen delivery (372±26 versus 453±32 mLO₂/min, respectively, P<0.05). At rest, plasma endothelin-1 (vasoconstrictor) was higher in the COPD patients (P<0.05) and also tended to be higher during exercise (p=0.07), while the formation of interstitial prostacyclin (vasodilator) was only increased in the controls. There was no difference between groups in the nitrite/nitrate levels (vasodilator) in plasma or interstitial fluid during exercise. Moreover, patients and controls showed similar vasodilatory capacity in response to both endothelium-independent (SNP) and endothelium-dependent (ACh) stimulation. The results suggests that leg muscle blood flow is impaired during small muscle mass exercise in patients with COPD possibly due to impaired formation of prostacyclin and increased levels of endothelin-1.

New and Noteworthy

This study demonstrates that COPD is associated with a reduced blood flow to skeletal muscle during small muscle mass exercise. In contrast to healthy individuals, interstitial prostacyclin levels did not increase during exercise and plasma endothelin-1 levels were higher in the COPD patients.

Key words: Hemodynamics, Extracellular Fluid, Vasodilator Agents
Introduction

Chronic obstructive pulmonary disease (COPD) is associated with exercise intolerance that may be related to both central and peripheral limitations (19, 29). Patients with COPD often demonstrate leg muscle weakness, atrophy, fiber type shift (from type I to type II), reduced cross sectional area, reduced capillarity, low oxidative capacity and high glycolytic metabolism (4, 7, 9, 19, 21), but it remains unclear whether their regulation of leg muscle blood flow is impaired as a consequence of these structural and functional changes.

In healthy humans, leg blood flow to contracting muscle is tightly regulated to match oxygen (O$_2$) delivery to the metabolic demand (1). Skeletal muscle blood flow is mainly regulated by a balance between circulating vasoconstrictors and locally formed vasoactive substances (11, 23). Local vascular and skeletal muscle cells communicate through the interstitial space by release of vasoactive compounds that are important for distribution of blood to the contracting muscles (11, 23). Commonly studied vasodilatory compounds such as nitric oxide (NO) and prostacyclin are released into the plasma and the interstitial space during exercise (12, 24). Vascular function is likely impaired in COPD (17) as evaluated mainly by vasodilatory function in the forearm (5, 14). Moreover, the potent vasoconstrictor endothelin-1 is released by the endothelial cells and circulating levels are increased in COPD patients (6, 27). Thus, balance between formation of vasodilators and vasoconstrictors might be impaired in patients with COPD which could impair contracting muscle O$_2$ supply.

We tested the hypothesis that regulation of leg blood flow during small muscle mass exercise is impaired in COPD due to inadequate vasodilation. The leg muscle fiber type composition, capillarization and vascular function were characterized as these factors might affect muscle blood flow regulation during exercise. To this end, we measured leg blood flow by Doppler ultrasound.
during one-legged knee-extensor exercise, while sampling vasoactive compounds and arteriovenous variables across the leg in COPD patients and healthy control subjects.

Methods

Participants and ethical approval

The study was approved by the Danish Ethical Committee (Copenhagen Region H-2-2013-150) and conducted in accordance with the Helsinki Declaration. Verbal and written informed consent was obtained from all subjects before enrolment.

We included 10 patients with moderate to severe COPD and 8 healthy age- and sex-matched controls (Table 1). Eligibility criteria for COPD patients: forced expiratory volume in 1 sec (FEV$_1$)/functional volume capacity ratio < 0.7, FEV$_1$ ≤ 70% of predicted, modified Medical Research Council Dyspnea Scale ≤2, resting arterial oxygenation > 90%, and age 40 to 80 years. The COPD patients were all clinically stable and primarily recruited from an outpatient clinic. Eligibility criteria for both groups were use of anticoagulant medications, diabetes, hypertension, claudication, heart failure, unstable ischemic heart disease, or malignant diseases. Sitting spirometry (Model 2120, Vitalograph Ltd., Buckingham, UK) and a general medical examination including blood testing were performed prior to inclusion. Healthy controls were excluded if they engaged in any regular exercise training or formalized physical activity (≥3 times a week) and COPD patients were excluded if they had participated in a rehabilitation program (<6 months). However, we did not quantify the level of daily physical activity with monitors or validated questionnaires.

In parallel with the present study we conducted experiments on chronic heart failure patients and 6 of the control subjects were included in that study (data not published). The COPD assessment test
was used to determine the disease severity of the COPD patients. Six minutes walking distance, peak oxygen uptake (VO$_2$ peak), leg mass and one-legged peak workload (WL$_{peak}$) were determined before the experimental day. The VO$_2$ peak was assessed (CPET system, Cosmed, Rome, Italy) during the cycle ergometer test and included 5 min of unloaded pedaling followed by incremental steps of 10 Watts per minute until exhaustion. The greatest 20-second averaged VO$_2$ value during the exercise test was used as the VO$_2$ peak. WL$_{peak}$ of the dominant leg was assessed by 5 min of one-legged knee extensions at 6 Watts kicking followed by incremental steps of 6 Watts per minute until exhaustion. Prior to the test the participants had been given verbal instruction and were familiarized with the one-leg knee extensor model.

**Experimental protocol**

Participants refrained from caffeine, alcohol and exercise for 24 h before the experimental day. The COPD patients abstained from their medications for 24-48 h depending on the drug profile to avoid potential vasoconstrictor effects of inhaled β2-agonists and muscarinic antagonists. After local anesthesia (lidocaine 20%), catheters were placed in the femoral artery (for infusion) and vein (for blood sampling) of the experimental leg and one in the brachial artery (for blood pressure monitoring and blood sampling). In two of the patients, however, we could not place the femoral arterial catheters. Two microdialysis probes (MDialysis AB, Johanneshov, SWE) were inserted into the vastus lateralis of the quadriceps muscle of the experimental leg and a muscle biopsy was obtained from the vastus lateralis of the contralateral leg using the Bergström-technique. The muscle samples were separated in two; one part for Western blotting was immediately frozen in liquid nitrogen; the second part for immunohistochemistry was mounted in Tissue-Tek (Sakura, The Netherlands) and then frozen in isopentane pre-cooled by liquid nitrogen. All muscle samples were stored at -80°C until analysis.
Sodium Nitroprusside (SNP) at 2 µg/min/kg leg mass was infused into the femoral artery to test endothelium-independent function. Acetylcholine (ACh) was infused into the femoral artery at both 25 and 100 µg/min/kg leg mass to test endothelium-dependent function. Leg hemodynamic responses to the infusions were evaluated and blood collected at baseline and after 3 minutes. Infusions were normalized to leg mass measured by whole body dual-energy X-ray absorptiometry scan (Lunar Prodigy Advance, GE Healthcare, Madison, WI, USA).

Approximately one hour after the infusions the participants performed two bouts of one-legged knee-extensions at 10 W and at 20% of one-legged WL\textsubscript{peak} separated by 30 minutes of rest. Exercise relative to WL\textsubscript{peak} was performed to exclude the possibility that the COPD patients worked at higher workload. Prior to the active exercise the experimental leg was moved passively for 2 minutes at 60 knee extensions/minute and subjects were instructed to keep that pace during exercise. Leg hemodynamics responses to the exercise were evaluated and blood collected at baseline and after 2 minutes of passive leg movement and during active knee-extensions at specific time points until steady state (3.5 minutes of active contractions).

**Leg hemodynamics and vasoactive compounds**

Common femoral artery blood flow was measured using Doppler ultrasound (Logic E9, GE Healthcare, Milwaukee, WI, USA) equipped with a linear probe (9 MHz). The site of the blood flow measurements was below the inguinal ligament but well above the bifurcation of the superior and profound femoral arteries in order to avoid turbulence. All recordings were obtained at the lowest possible insonation angle and always below 60°. The sample volume was maximized according to the width of the vessel and kept clear of the walls. The combined Doppler tracings and B-mode images were recorded continuously. Doppler tracings were averaged over 8 heart cycles at the time as the blood sampling and vessel diameter was determined after each Doppler recording.
Arterial diameter was measured during systole from the arterial B-mode images with the transducer parallel to the vessel (22). Arterial and femoral blood pressures were monitored with transducers (Pressure Monitoring Kit, Baxter) positioned at the level of the femoral artery and sampled continuously (LabChart, ADInstruments Ltd, Oxford, UK). Leg vascular conductance (LVC) was leg blood flow divided by mean femoral arterial pressure (MAP).

Blood samples were drawn simultaneous from the venous and the arterial catheters at the time when leg hemodynamic responses were evaluated. Plasma from the femoral vein was used for content of nitrite/nitrate (NOx), 6-keto PGF₁α (prostacyclin), and endothelin-1. Blood gas variables, hemoglobin and lactate were immediately analyzed (ABL 725, Radiometer, Glostrup, DK). The microdialysis probes were continuously perfused with isotonic saline at 5 µl/min. Interstitial fluid was collected at baseline rest (5 min), during femoral arterial infusions of SNP and ACh, and throughout the one-legged knee-extensor exercise. Interstitial fluid was immediately frozen for later analysis of NOx and prostacyclin. Plasma endothelin-1 was measured using an immunoassay (QuantiGlo, R&D systems, Inc., USA) following manufacturer’s instructions. The stable metabolites of NO, nitrite and nitrate, were measured using fluorometric assay kit (Cayman Chemical, USA) and the stable metabolite of prostacyclin, 6-keto PGF₁α, was measured using an enzyme immunoassay kit (Cayman Chemical, USA) according to the manufacturer’s instructions.

**Muscle immunohistochemistry**

Muscle fiber type proportions (Type I, IIa and IIx) and capillarization were determined by immunohistochemistry using antibodies against myosin heavy chain (MHC) type I, type IIa, endothelial cells and laminin, respectively. Muscle samples were cut in 7-10 µm cross-sections using a cryostat (Microm HM500 M, Heidelberg, Germany) and placed onto microscopy glass slides. The cross-sections were then fixated by applying a formaldehyde solution for 2 min (F1635;
Sigma-Aldrich) and, after rinsing with a pH neutral 1 % phosphate-buffered saline (PBS) solution, blocked with 1 % bovine serum albumin (A7906; Sigma-Aldrich). The primary antibodies MHCI (M2421, Sigma Aldrich) and MHCIIa (SC-71; Developmental Studies Hybridoma Bank) were diluted in an antibody diluent (S0809; DAKO) together with polyclonal rabbit anti-laminin (Z0097; DAKO). The capillaries were identified using Biotinylated Ulex Europaeus Agglutinin I (B-1065; Vector Laboratories). Dilutions of the primary antibodies were: MHCI 1:2000, MHCIIa 1:100, laminin 1:500 and Ulex 1:100. The secondary antibodies (Invitrogen A31570 (red), Life Technologies A21068 (blue), Life Technologies A21202 (green)) were applied in dilution 1:1000 and Streptavidin-FITC (DAKO F0422) in 1:200. Both primary and secondary antibodies were incubated for 50 min at room temperature followed by rinse with PBS. Muscle sections were visualized through a light microscope (Axioplan 2 Imaging; Zeiss) connected to a high-resolution camera (CoolSNAP; Photometrics, Tucson) and analyzed using Microsoft PowerPoint (Microsoft Corporation) and ImageJ 1.47 (National Institutes of Health, USA). Muscle fibers positive for antibodies against both fiber type I and IIa were considered intermediate. Fibers of no coloration were deemed type IIx and we subsequently verified this assumption with antibody staining for type IIx fibers (6H1; Developmental Studies Hybridoma Bank, Iowa).

Muscle protein content

The muscle protein content of endothelial nitric-oxide synthase (eNOS) and cyclooxygenase 1 (COX-1) were evaluated by Western blotting. Muscle samples were dissected free from connective tissue and freeze-dried, then homogenized and lysed. The total protein content was measured (colorimetric assay; Bio-Rad, Hercules, CA, USA) to insure equal sample concentrations. Loading buffer (Thermo Fisher Scientific) and dithiothreitol were applied and samples boiled for 5 min. The lysate was loaded onto the gels (Criterion TGX 4–15% gel; Bio-Rad) where samples from the same participant were loaded side by side on the same gel. Gels were run at 105 V for ~ 120 min. Protein
was then transferred onto a polyvinylidene fluoride membrane (Trans-Blot Turbo, Bio-Rad). Membranes were blocked and incubated with the primary antibodies overnight. Membranes were washed and incubated with secondary horseradish peroxidase-conjugated IgG antibody (Dako, Glostrup, Denmark). Signal was detected (Supersignal West Femto Luminal/Enhancer Solution; Thermo Fisher Scientific) and exposed (Charge couple camera; Bio-Rad). The intensities of the blots were quantified using Image Lab software (Bio-Rad, Hercules, CA, USA) and expressed in arbitrary units (AU). Primary antibodies used were: Endothelial nitric-oxide synthase, mouse monoclonal antibody, BD Transduction Laboratories (cat# 610297), Cyclooxygenase 1, Rabbit polyclonal antibody, abcam (cat# 53766).

Statistics

A two-way repeated measures analysis of variance model was used to detect changes from rest to exercise or infusion of vasoactive drugs and to find differences between groups. If a significant interaction was found, the Tukey’s post hoc test was used to identify the specific sampling points. A Student’s t-test was used to detect differences in baseline characteristics between groups and when assumption of normality failed, Mann-Whitney U test was used. Data were analyzed with SigmaPlot version 13 (Systat Software, San José, CA, USA) and presented as mean and standard error of mean (±SEM) or median with interquartile range (IQR). P-values below 0.05 were considered statistical significant. The outcome assessors were not blinded to interventions or study groups.

Results

Characteristics of the COPD patients and healthy controls are presented in Table 1. There were no differences in age, body mass index, leg mass or one-legged WL_peak between patients and controls,
however, the COPD patients showed reduced whole body exercise capacity (6 minutes walking distance and incremental ergometer cycling test).

Vasodilatory function

There was no difference in leg blood flow (control: 0.22±0.02 versus COPD: 0.20±0.02 L/min) or MAP (control: 87±2 versus COPD: 94±4 mmHg) between groups at baseline. Infusion of SNP reduced MAP, increased leg blood flow, and increased LVC within both groups (p<0.05) with no difference between groups (Figure 1). Infusion of ACh increased both leg blood flow and LVC in a dose dependent manner with no difference between groups, but MAP decreased during the high dose of ACh infusion in the controls (100 µg/min/kg leg mass).

Hemodynamics during exercise

Leg blood flow, MAP and LVC were similar in the COPD patients and in the controls during seated rest. Both groups increased leg blood flow during 2 minutes of passive leg movement with no difference between groups. Leg blood flow and LVC were lower during exercise (10 W) in the COPD group compared with the controls (p<0.05) while MAP was similar (Figure 2).

The workload for the knee extensions at 20% WL_{peak} was not different between groups (control: 8±1 versus COPD: 7±1 W). After the first 30 seconds of knee extensions leg blood flow was higher in the controls (1.73±0.3 L/min) compared with the COPD patients (1.25±0.08 L/min) with significant difference between groups (p<0.05) and at 3.5 minutes leg blood flow reached 1.65±0.10 L/min in the COPD patients versus 2.04±0.15 L/min in the controls (p=0.07).

Blood gas variables and lactate

The arteriovenous O$_2$ difference was higher in the COPD group at rest and remained higher during passive leg movement and 10 W exercise (p<0.05) (Figure 3). The leg O$_2$ delivery increased in both
groups (p<0.05), but was lower in the patients during exercise (p<0.05). Leg oxygen uptake increased in both groups during exercise (p<0.05), with no difference between groups. Blood gas variables, hemoglobin and lactate are shown in Table 2.

Vasoactive compounds

Interstitial prostacyclin increased in the control group during exercise, but no change was observed in the patients (Figure 4). There was no between-group difference in plasma NOx formation at rest, during ACh infusion (COPD 12.4±1.3 pg/mL; controls median 11.4 pg/mL (IQR 10.6-19.0)), during passive leg movements (COPD 15.2±2.1 pg/mL; controls 15.0±1.0 pg/mL) or during 10 W knee extensor exercise (COPD 15.1±2.6 pg/mL; controls 15.7±2.3 pg/mL). The muscle interstitial concentration of NOx did not change significantly in response to ACh infusion in controls (from 4964±451 µM to 5503±504 µM) or in patients (from 5208±726 µM to 5700±800 µM) but in both groups interstitial NOx increased during exercise, with no difference between groups. At rest plasma endothelin-1 was higher in the COPD patients compared with controls (Figure 5) and also tended to be higher during exercise (p=0.07).

Muscle morphology and protein content

There was no difference in muscle fiber type proportions, mean fiber size or capillarization between the two groups. The proportion of type I fibers was 34±3% in patients versus 42±4% in controls (P=0.131); type IIa 47±3% in patients versus 44±6% in controls (P=0.609); and type IIx 7±2% in patients versus 6±2% in controls (P=0.821). The intermediate fiber type proportion was 10% (IQR 8-14) in the patients versus 8% (IQR 3-12) in the controls (P=0.397). Fiber size was 6183±604 µm² in the patients versus 6072±305 µm² in the controls, and the number of capillaries per fiber was 1.75±0.22 in the patients versus 1.84±0.15 in the controls. The muscle protein content of eNOS and COX-1 were evaluated in healthy controls and COPD patients. We found no difference between
groups in COX-1 content (COPD: median 0.79 AU (IQR 0.50-1.54) versus controls: median 0.66 AU (IQR 0.54-2.04), P=0.92) or in eNOS content (COPD: 1.05±0.06 versus controls: 0.94±0.14 AU, P=0.46).

Discussion

The leg blood flow response to small muscle mass exercise is impaired in COPD. In the COPD patients, the vasoconstrictor endothelin-1 was elevated in plasma and the interstitial formation of the vasodilator prostacyclin was blunted during exercise, whereas the formation of NOx in plasma and in the muscle interstitium was intact. Thus, imbalance between vasoconstrictors and locally formed vasodilators is likely to explain the observed lower leg blood flow in COPD patients.

Muscle blood flow regulation

The complex regulation of muscle blood flow during exercise involves an interaction between intra- and extra-vascular formed vasodilators and vasoconstrictors (12). Vasodilators in the muscle interstitium during exercise have, to our knowledge, not been evaluated in COPD patients. Here we report that during exercise interstitial NOx levels increased to the same extent in both groups whereas prostacyclin only increased in the controls, indicating an impaired ability to form prostacyclin in response to exercise in the COPD patients, although muscle content of COX-1 is not affected. The data suggest a vasoconstrictor/vasodilator imbalance during exercise in patients with COPD, as endothelin-1 levels were higher and interstitial prostacyclin secretion blunted during exercise. This finding could, at least in part, explain the low leg blood flow response to exercise. Exercise-induced interstitial prostacyclin formation and leg blood flow has also been reported to be impaired in individuals with essential hypertension, but is normalized after 8 weeks of exercise.
training (10). Thus, exercise training may be also an effective strategy to improve the balance between vasodilators and vasoconstrictors and thereby muscle function in COPD patients.

The COPD patients and controls showed similar NO-mediated vasodilation to either endothelium-independent (SNP) or endothelium-dependent (ACh) stimulation, and there was no between-group difference in muscle eNOS content. Likewise, there were no differences in interstitial and plasma levels of NOx between groups during infusion of ACh. The findings of intact formation and sensitivity of NO in the COPD patients, was supported by an increase in leg blood flow during passive leg movement similar to the controls, as this test is almost entirely NO-dependent (22). Our findings are in agreement with those of Maclay et al, who also used SNP and ACh to assess vasodilatory function in the forearm vasculature of COPD patients (18), whereas studies using flow mediated dilation in a larger population of COPD patients and healthy controls have found that the vasodilatory function related to the NO-system was impaired in COPD (2, 5). The conflicting results might reflect the different techniques applied or may be related to statistical power issues in the invasive vascular assessments.

Implications for exercise capacity

The low leg blood flow during small muscle mass exercise in the COPD group suggests that the reduced exercise capacity experienced by patients with COPD may not only explained by pulmonary limitations, but also due to inadequate limb muscle perfusion. Since we studied a small muscle group during submaximal exercise, the pulmonary function is unlikely to limit performance in the COPD patients, and hence, the arterial O\textsubscript{2} content was not reduced during exercise. The large difference in leg blood flow (~ 500 mL blood per minute) between the groups was compensated for by O\textsubscript{2} extraction in the COPD group resulting in similar O\textsubscript{2} uptake. During maximal whole body exercise, leg blood flow is also severely impaired in COPD (20), and although impaired vascular
function is likely to contribute, this could also reflect “stealing” of the blood by the respiratory muscles (3, 16). However, redistribution of blood volume from leg- to respiratory muscles is not likely to explain our results during small muscle mass exercise. In contrast to our results, others have found no difference in leg blood flow and O₂ delivery during small muscle mass exercise between COPD and healthy individuals (28) or even higher leg blood flow and O₂ delivery in patients with COPD (26). In the investigation by Richardson et al, however, COPD patients with more severe airflow limitation (FEV₁ ~ 36 of predicted) were studied, which was reflected by reduced proportion of type I fibers in the quadriceps muscle and lower one-legged WL_peak compared with the healthy controls included (7, 26). Improved training status is likely to increase leg blood flow during small muscle mass exercise and induce a fiber type shift in COPD patients (13). The present study showed no differences in fiber type distribution or one-legged WL_peak between COPD and control subjects. The lack of difference in fiber type composition between groups is somewhat surprising given that the majority of studies report a shift from oxidative type I fibers to glycolytic type II fibers in COPD patients (7). Our control group showed lower type I fiber proportions than seen in previous studies (8) and we suggest factors such as age and especially inactivity might have influenced fiber type composition negatively in healthy subjects (15).

The higher O₂ extraction in the COPD group is likely to have been the result of the low leg blood flow rather than improved capacity for O₂ extraction, as muscle capillarization was not different between groups. The consequence of lower leg muscle O₂-delivery/VO₂- ratio in the COPD patients would be lower microvascular O₂ partial pressure and if the driving pressure for blood-to-myocyte O₂ transport is diminished even at small muscle mass exercise, the muscle cells are closer to the anaerobic tipping-point when muscle mass or intensity is increased (25).
Limitations

The level of physical activity is often reduced in patients with COPD, and therefore we included sedentary controls. Although we did not quantify the daily physical activity level, there were no differences between groups in fiber type composition, leg mass or one-legged WL\textsubscript{peak}. Thus, evaluations of leg blood flow regulation during one-legged knee-extensor exercise were not confounded by intrinsic or extrinsic differences in muscle characteristics. The mechanisms underlying the exercise intolerance are complex and often multifactorial in COPD. The pathophysiological factors that contribute are ventilatory limitations, gas exchange deficits, reduced cardiac function and limb muscle dysfunction. COPD is a heterogeneous disease and any combination of the factors above might contribute or dominate the phenotype. Our sample size was relatively small because of the invasive nature of this experiment and results may not be extrapolated to all patients with COPD. Determination of interstitial metabolites is limited to small muscle mass exercise and exercise has to be performed at a relative light workload due to the long sampling time. The present findings suggest that an impaired blood flow to contracting muscles could be a contributing factor to whole body exercise intolerance in COPD, but it remains uncertain whether the findings also apply during more intense exercise involving a larger muscle mass.

In conclusion, regulation of leg blood flow was impaired in COPD patients causing a higher resistance in the muscle vasculature during small muscle mass exercise. We found that interstitial formation of prostacyclin was blunted during exercise and circulating endothelin-1 levels were elevated in the COPD patients, suggesting an imbalance between the formation of vasoconstrictors and vasodilators.
Acknowledgements

Funding information

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Disclosures

None
References


<table>
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<tr>
<th>Table 1. Baseline characteristics</th>
<th>COPD (n=10)</th>
<th>Controls (n=8)</th>
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<tbody>
<tr>
<td>Age, years</td>
<td>64 (3)</td>
<td>64 (3)</td>
</tr>
<tr>
<td>Men/women</td>
<td>8/2</td>
<td>7/1</td>
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<tr>
<td>MRC grade</td>
<td>2 (2-3)</td>
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<tr>
<td>CAT score</td>
<td>12 (9-16)</td>
<td>4 (3-6)*</td>
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<tr>
<td>Pack years</td>
<td>32 (3)</td>
<td>6 (3)*</td>
</tr>
<tr>
<td>Current smokers, n</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26 (1)</td>
<td>25 (1)</td>
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<tr>
<td>FVC, liters</td>
<td>3.7 (0.4)</td>
<td>4.7 (0.4)*</td>
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<tr>
<td>FVC, %</td>
<td>117 (111-124)</td>
<td>102 (75-120)</td>
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<tr>
<td>FEV₁, liters</td>
<td>1.6 (0.2)</td>
<td>3.6 (0.2)*</td>
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<td>FEV₁, % of predicted</td>
<td>50.6 (4.5)</td>
<td>109.8 (3.1)*</td>
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<td>FEV₁/FVC</td>
<td>0.43 (0.02)</td>
<td>0.76 (0.02)*</td>
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<tr>
<td>Leg mass, kg</td>
<td>11.9 (0.7)</td>
<td>12.1 (0.7)</td>
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<td>One-legged WL peak, Watt</td>
<td>36 (4)</td>
<td>41 (4)</td>
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<td>6MWD, meter</td>
<td>621 (568-648)</td>
<td>673 (664-692)*</td>
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<tr>
<td>Incremental ergometer cycling</td>
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<tr>
<td>WL peak, Watt</td>
<td>118 (12)</td>
<td>170 (14)*</td>
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<tr>
<td>VO₂ peak, mlO₂/min/kg</td>
<td>22 (19-27)</td>
<td>35 (27-39)*</td>
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<td>VO₂ peak, mlO₂/min</td>
<td>1820 (113)</td>
<td>2520 (198)*</td>
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<td>VT peak, liters</td>
<td>1.8 (0.2)</td>
<td>2.9 (0.1)*</td>
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<tr>
<td>RF peak, breaths/min</td>
<td>37 (2)</td>
<td>35 (2)</td>
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</table>

Data in mean (standard error of means) or median (interquartile range), * different from COPD group (p<0.05); p-value was calculated using a student’s t-test or Mann-Whitney U

Abbreviations: CAT: COPD Assessment test, BMI: body mass index, FEV₁: forced expiratory volume in 1 sec, FVC: functional vital capacity, MRC: medical research council, RF: respiration frequency, VT: tidal volume, 6MWD: six min walking distance, WL: workload
Table 2 Blood gas variables, lactate and hemoglobin

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise 10 Watt</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>COPD</td>
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<tr>
<td>PaO₂, mmHg</td>
<td>90(3)</td>
<td>77(3)*</td>
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<tr>
<td>PvO₂, mmHg</td>
<td>31(2)</td>
<td>25(1)*</td>
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<tr>
<td>Hbₐ, g/dl</td>
<td>13.9(0.4)</td>
<td>14.6(0.4)</td>
</tr>
<tr>
<td>Hbᵥ, g/dl</td>
<td>14.1(0.4)</td>
<td>15.0(0.3)</td>
</tr>
<tr>
<td>SaO₂, %</td>
<td>97(0.3)</td>
<td>96(0.6)</td>
</tr>
<tr>
<td>SvO₂, %</td>
<td>55(4)</td>
<td>43(3)*</td>
</tr>
<tr>
<td>CaO₂, ml/l</td>
<td>187(5)</td>
<td>191(5)</td>
</tr>
<tr>
<td>CvO₂, ml/l</td>
<td>106(8)</td>
<td>88(8)</td>
</tr>
<tr>
<td>Venous lactate, mmol</td>
<td>0.69(0.08)</td>
<td>0.91(0.05)*</td>
</tr>
</tbody>
</table>

Data in means (standard error of means), *difference between groups (p<0.05), † difference from rest within group (p<0.05). p-values were calculated using a student’s t-test (between groups) and a one-way analysis of variance with Tukey’s post hoc test (within groups), respectively.

**Abbreviations:** CaO₂: arterial oxygen content, CvO₂: femoral venous oxygen content, Hbₐ: arterial hemoglobin concentration, Hbᵥ: femoral venous hemoglobin concentration, PaO₂: arterial oxygen partial pressure, PvO₂: femoral venous oxygen partial pressure, SaO₂: arterial oxygen saturation, SvO₂: femoral venous oxygen saturation
Figure legends

Figure 1. Vasodilatory function. Hemodynamic responses to Sodium Nitroprusside (SNP) and Acetylcholine (ACh). Leg blood flow (A), mean arterial pressure (B) and leg vascular conductance (C) in chronic obstructive pulmonary disease (COPD) patients (open bars) compared with healthy age- and sex matched controls (solid bars). A two-way repeated measures analysis of variance model with Tukey’s post-hoc test was used to detect differences between groups. Data presented as mean change from baseline and error bars represent standard errors of means. * Difference between groups (p<0.05).

Figure 2. Hemodynamics during exercise. Leg blood flow (A), mean arterial pressure (B), and leg vascular conductance (C) during passive leg movements (shaded area) and 10 W one-legged knee-extensor exercise in chronic obstructive pulmonary disease (COPD) patients (open circles) and controls (solid circles). A two-way repeated measures analysis of variance model with Tukey’s post-hoc test was used to detect differences between groups. Data presented as means and error bars represent standard errors of means. * Difference between groups (p<0.05).

Figure 3. Oxygen delivery during exercise. Leg oxygen (O2) delivery (A), leg arteriovenous O2 difference (B), and leg O2 uptake (VO2) (C) during passive leg movements (shaded area) and 10 W one-legged knee-extensor exercise in chronic obstructive pulmonary disease (COPD) patients (open circles) and controls (solid circles). Data presented as means and error bars represent standard errors of means. A two-way repeated measures analysis of variance model with Tukey’s post-hoc test was used to detect differences between groups. * Difference between groups (p<0.05).

Figure 4. Vasodilators in the interstitial fluid. The stable metabolites of (A) prostacyclin (6-keto PGF1α) and (B) nitric oxide (NOx) in the interstitial fluid during knee-extensor exercise at 10 Watt and at 20% of peak workload (20% of max) in chronic obstructive pulmonary disease (COPD)
patients (open bars) and controls (solid bars). Data presented as means and error bars represent standard errors of means. A two-way repeated measures analysis of variance model with Tukey’s post-hoc test was used to detect differences within and between groups. * Different from rest (p<0.05).

**Figure 5.** Endothelin-1 in venous plasma. Scatter plots of endothelin-1 concentrations in femoral venous plasma at rest and during knee-extensor exercise at 10 W in chronic obstructive pulmonary disease (COPD) patients and controls. Horizontal dashed lines indicate mean values. A two-way repeated measures analysis of variance model with Tukey’s post-hoc test was used to detect differences between groups. Difference between groups; * p<0.05, # p=0.07.
### Figure A

- **Leg O₂ delivery (mLO₂/min)**
- *CONTROL* and *COPD*
- Passive leg movement

### Figure B

- **Leg arteriovenous O₂ difference (mLO₂/L)**
- *CONTROL* and *COPD*
- Passive leg movement

### Figure C

- **Leg VO₂ (mLO₂/min)**
- *CONTROL* and *COPD*
- Passive leg movement

**Legend:**
- CONTROL
- COPD
- Passive leg movement

**Data Points:**
- *Rest*
- 120 seconds
- 240 seconds
- 360 seconds

**Notes:**
- Data points marked with asterisks (*).