MS CAMILLA KOCH RYRSØ (Orcid ID : 0000-0002-8059-7331)
PROFESSOR CARSTEN LUNDBY (Orcid ID : 0000-0002-1684-0026)

Article type : Original Article

Effect of endurance versus resistance training on local muscle and systemic inflammation and oxidative stress in COPD

Ryrsø CK[1], Thaning P[1,2], Siebenmann C[1], Lundby C[1], Lange P[1,2,3], Pedersen BK[1], Hellsten Y[4], Iepsen UW[1]

[1] The Centre of Inflammation and Metabolism and the Centre for Physical Activity Research, Rigshospitalet, University of Copenhagen, Denmark

[2] Department of Respiratory Medicine, University Hospital Hvidovre, Denmark

[3] Department of Public Health, Section of Social Medicine, University of Copenhagen, Denmark

[4] Department of Nutrition, Exercise, and Sports, University of Copenhagen, Denmark

Running head: Inflammation and exercise in COPD

Trial registration: ClinicalTrials.gov (NCT02050945).

Corresponding author

Camilla Koch Ryrsø
Rigshospitalet, University of Copenhagen
Centre of Inflammation and Metabolism (CIM) and
The Centre for Physical Activity Research (CFAS)

Blegdamsvej 9, 2100 Copenhagen, Denmark
E-mail: camilla.koch.ryrsoe@regionh.dk

Abstract

Limb muscle dysfunction in patients with COPD may be associated with local muscle and/or systemic inflammation, and therefore, we investigated whether exercise training altered markers of inflammation and oxidative stress. We obtained vastus lateralis muscle biopsies and venous blood samples from patients with COPD (n=30) before and after 8 weeks of resistance training (RT) (n=15) or endurance training (ET) (n=15). Healthy age-matched subjects were included as baseline controls (n=8). Inflammatory markers in muscle and systemically were determined by interleukins (IL), tumour necrosis factor alfa (TNF-α), leukocyte concentration together with immunohistochemical staining for macrophages. Muscle oxidative stress and antioxidant capacity were determined by NADPH oxidase (NOX) and superoxide dismutase 2 (SOD2), respectively. Before exercise training, COPD patients had a higher muscular NOX protein content and circulating IL-8, IL-18, CRP and leukocyte levels but a similar number of muscle-infiltrating macrophages compared with controls. Eight weeks of ET or RT increased muscle SOD2 content with no difference between groups. Plasma TNF-α, increased (P<0.05) after ET and tended to (P=0.06) increase after RT, but had no effect on muscular NOX protein content, number of muscle-infiltrating macrophages or systemic levels of other pro-inflammatory cytokines or leukocytes.
In patients with COPD, we found no evidence for muscular inflammation and no effect of exercise training. However, systemic inflammation was elevated in COPD and both training modalities induced an upregulation of muscle antioxidant capacity.

Key words: muscle dysfunction, inflammation, oxidative stress, rehabilitation.

Introduction

Skeletal muscle dysfunction, characterised by muscle wasting, reduced muscle strength and endurance is an important manifestation of chronic obstructive pulmonary disease (COPD) . The impaired muscle function leads to reduced exercise tolerance, health-related quality of life, and survival . The aetiology is not completely understood but systemic inflammation, oxidative stress, and deconditioning have been suggested to be causal factors in the progression of the COPD-related muscle dysfunction (3, 4).

This elevated inflammatory status in COPD is probably accompanied by systemic oxidative stress and an impaired antioxidant system capacity (5, 6). The enzymes xanthine oxidase and NADPH oxidase (NOX) may be responsible for the elevated production of reactive oxygen species (ROS) in COPD, whereas the mitochondrial antioxidant enzyme superoxide dismutase 2 (SOD2) counteracts the ROS production (7, 8). Patients with moderate-to-severe COPD show higher ROS production than age-matched healthy subjects, both at rest and during exercise (4, 9). Elevated ROS may acutely increase muscle fatigue, and chronically contribute to the development of muscle dysfunction in COPD (4, 5, 10, 11).

Exercise training improves symptom burden, exercise capacity and peripheral muscle function at all stages of COPD (12-14). The anti-inflammatory effect of exercise training is well established in healthy and patients with cardiovascular diseases. Physical activity suppresses systemic inflammation through local muscle release of myokines, however this effect remain unclear in COPD patients (6, 15, 16). Endurance training (ET) has long been preferred as an exercise modality in the pulmonary rehabilitation. However, specific muscle stimulation with resistance training (RT) has been thought to target the muscle dysfunction, while inducing the same positive effects on disease burden and exercise capacity (12, 17).

To our knowledge, a comparison between the effect of resistance training and endurance training on local muscle and systemic inflammation has not been studied in COPD. Hence, it remains unclear whether the effects of resistance training are similar to those of endurance training. Therefore, the primary aim of the present study was to evaluate the effect of ET compared with RT on cytokines and leukocytes in circulation and in quadriceps muscle of COPD patients. Secondly, we evaluated the influence of the two training regimes on quadriceps muscle protein levels of the pro-oxidant enzyme NOX and the antioxidant enzyme SOD2. We hypothesized that the degree of inflammation at the systemic and muscular level would differ between the two training modalities in parallel with reductions in oxidative stress. Specifically we expected RT to induce local muscle damage with subsequently immune cell-infiltration in muscle. In contrast, we anticipated that ET would induce a more systemic anti-inflammatory effect with less immune cell-infiltration in muscle.

Materials and methods

Participants and ethical approval

The trial was approved by the Ethics Committee of Copenhagen Region (H-2-2013-150), registered on ClinicalTrials.gov (NCT02050945), and conducted in accordance with the Declaration of Helsinki. Oral and written informed consent was obtained from all subjects before enrolment.

This study was part of a larger project involving thirty patients with moderate to severe COPD . Eight age-matched, sedentary subjects previously studied in our laboratory were included as baseline controls. COPD patients (age 63±2 yrs.; body mass index (BMI) 27±1 $kg \cdot m^{-2}$; fat free mass index (FFMI) 17.3±0.5 $kg \cdot m^{-2}$; systolic blood pressure (BP) 138±4 mmHg and diastolic BP 89±3 mmHg) encompassed a broad spectrum of disease severity. Patients were characterized by moderate to severe airflow limitations (FEV_1 56±3% of predicted value (range 26-79% predicted)), with extensive respiratory symptoms (COPD Assessment test (CAT) score 13±1) and limited exercise tolerance (VO_2peak 21±1 $ml \cdot kg^{-1} \cdot min^{-1}$) (Table 1) . Comorbidities of patients included diabetes (n=3) hypertension (n=12) and ischemic heart disease (n=3). Patients received individually tailored medication consisting of β_2-agoinsts, corticosteroids, anticholinergics, antiplatelet drug, and diuretics. Medical therapy did not change during the study period.

Healthy sedentary control subjects (age 64±3 yrs.; BMI 25±1 $kg \cdot m^{-2}$; FFMI 18.7±0.7 $kg \cdot m^{-2}$) demonstrated normal pulmonary function (FEV_1 110±3% predicted), respiratory symptoms (CAT score 4±1), blood pressure (systolic BP 136±3 mmHg, diastolic BP 84±2 mmHg), and exercise tolerance (33±3 $ml \cdot kg^{-1} \cdot min^{-1}$) (Table 1).

Intervention

Patients were randomised into an ET (n=15) and an RT (n=15) group. Two patients in RT group were lost to follow-up after the intervention do to an exacerbation (n=1) and neck pain (n=1) during training. Both groups performed 35 minutes of supervised exercise, 3 times a week for 8 weeks. ET consisted of either ergometer cycling or treadmill walking at moderate intensity individually adjusted to level 14-15 on the Borg scale of perceived exertion (20). RT consisted of 4 strength exercises of the major upper and lower body muscle groups (chest press, rowing, leg press, and leg extension), and were performed on resistance machines (Technogym, Cesena, Italy). Each exercise included 4 sets with duration of 30 seconds with a 20-second break between sets and a 60-second break between exercises. Load was 30-40% of

one repetition maximum (1 RM). 1-RM was determined by the greatest amount of weight that the subject could move in a single repetition. Prior to performing the 1-RM test, all participants performed one set of each exercise without any load for familiarisation

Exercise capacity

Exercise capacity was determined by peak oxygen uptake (VO_2peak) and a 6-minute walking test. Subjects completed an incremental cycling test on a cycle ergometer (Monark 839E, Monark) with continuous measurement of oxygen uptake (CPET; Cosmed, Rome, Italy). The test consisted of 5 minute of unloaded pedalling followed by incremental steps of 10 W per minute until volitional exhaustion (W_{max}). VO_2peak is reported as the highest value averaged more than 20 seconds during the incremental test. The 6-minute walking test was performed between 2 cones that were 30 m apart. The subjects were instructed to walk the greatest distance possible in 6 minutes, at a self-determined walking speed and resting as needed .

Blood samples

Blood samples were collected 48 hours before and after the last exercise training of the training intervention. The samples were analysed for immune cells and CRP with standard laboratory assays (Department of Clinical Biochemistry, Rigshospitalet, Denmark). For analysis of pro-inflammatory cytokines, samples were immediately centrifuged at 3500 *g* for 10 min at 4°C and the plasma was stored at -80°C until analysis. Plasma concentration of interleukin (IL)-1β, IL-6, IL-8, IL-18, and tumour necrosis factor-α (TNF-α) were measured using cytokine immunoassay kits (Meso Scale Discovery, MD, USA). All were analysed in duplicates, the average is presented.

Muscle biopsies

On the same experimental day, after the blood samples were collected, a biopsy was obtained from the m. vastus lateralis of the non-dominant leg with percutaneous needle biopsy technique after local anaesthesia (lidocaine 2%) of the skin and muscle fascia. Biopsies were separated; one part for protein analysis was immediately frozen in liquid nitrogen, and the second part for immunohistochemistry was mounted in Tissue-Tek® O.C.T.^{TM}Compound (Sakura Finetek USA, Inc., Torrance, CA, USA) and frozen in isopentane precooled in liquid nitrogen.

Immunohistochemistry

The distribution of macrophages per muscle tissue area within the skeletal muscle was determined immunohistochemically using a monoclonal antibody specific to the macrophage (M1 and M2) CD163 receptor (Cat#: MA5-17716, Thermo Fisher Scientific Inc., MA, USA). Frozen muscle biopsies were cut at 8 µm and fixed in -20°C acetone followed by 2% formaldehyde (F1635; Sigma-Aldrich Co., Sigma, MO, USA). The sections were blocked with 1% bovine serum albumin (A7906; Sigma-Aldrich Co.) and incubated with primary antibody diluted 1:500 in antibody diluent (Dako, code no. S0809) and secondary antibody, biotinylated rabbit-anti mouse immunoglobulin (Dako, code no. E0354). The staining was visualized by addition of Alexa Flour® 488 conjugated avidin (A21370, Life Technologies, Thermo Fisher Scientific Inc., MA, USA) The sections were mounted using Mounting Medium for Fluorescence with Dapi (Cat no. H-1200, Vector Laboratories Inc., Burligame, CA) to detect cell nuclei and stored at -20°C until visualized through a light microscope (Axioplan 2 Imaging; Zeiss) connected to a high-resolution camera (CoolSNAP; Photometrics, Tucson) and analysed using ImageJ 1.51 (National Institutes of Health, USA). Results corresponding to inflammatory cells count were expressed as the ratio of macrophages to total muscle section area in square millimetres. A total area of 282 mm^2 was counted.

Quantification of protein expression by Western blot

Biopsies were freeze-dried and dissected free from fat, blood and connective tissue. Approximately 5 mg dry weight of muscle tissue was homogenized in lysis buffer. The protein concentration of the lysate samples was measured (colorimetric assay; Bio-Rad Laboratories Inc., Hercules, CA, USA) to ensure equal sample concentrations. Lysate was diluted in 6 x sample buffer to a final concentration of 1.35 µg/µl. Equal amount of total protein was loaded from each sample onto the gels in accordance to the antibody optimization (detailed antibody description is available in the supplementary material). Samples from one subject (before and after training) were loaded on the same gel in duplicates. Proteins were transferred onto the polyvinylidene fluoride (PVDF) membrane (Trans-Blot; Bio-Rad Laboratories Inc.). Membranes were then blocked with blocking buffer (Bovine Serum Albumin (BSA) or milk powder) and incubated overnight at 4°C with the primary antibody followed by 1 hour of incubation with secondary antibodies (HRP AP IgG; Jackson ImmunoResearch Laboratories Inc., USA). The bands were visualized in a Bio-Rad

molecular Imager, ChemiDoc ^{TM}Xrs^{+} Imaging System (Bio-Rad Laboratories Inc., USA) and quantified by Image Lab version 4.0 build 16 (Bio-Rad Laboratories, USA), the protein content was expressed in arbitrary units relative to standard samples run on each gel. The membranes were analysed for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and actin as a loading control after inactivation of peroxidase activity and re-probing with anti GAPDH and actin antibody.

Quantification of cytokines in muscle

Concentrations of cytokines in the muscle lysate were detected using human pro-inflammatory kits (Meso Scale Discovery, MD, USA). Muscle lysates were added onto the plates and assays were analysed according to the protocol of the manufacturer. Samples from one subject (before and after training) were loaded on the same kit in duplicates. Concentration of IL-1β, IL-6, IL-8, and TNF-α were measured by Proinflammatory Panel II immunoassays (V-PLEX Human Proinflammatory Panel II, (4-plex), catalogue no. K15053D-1, Meso Scale Discovery, MD, USA) whereas IL-18 was measured by IL-18 immunoassays (Human IL-18 Assay, catalogue no. K151MCD-2, Meso Scale Discovery, MD, USA). Cytokine concentrations in muscle lysate were subsequently normalized to the protein concentration in the lysate due to different protein concentration of the samples. Due to methodological problems, we could not quantify muscle cytokines in the healthy control group.

Statistics

A one-way analysis of variance test was used to detect differences between groups (ET group vs. RT group vs. controls) at baseline. Kruskal-Wallis H test was used for data that were not normally distributed. A paired Student's t-test was used to detect differences within training groups (pre vs. post training) and if assumptions of normality failed Wilcoxon signed-rank test was used. A Student's t-test was used to detect differences in delta values between training groups (Δ-ET vs. Δ-RT) and if assumptions of normality failed Mann-Whitney U test was used. Data were analysed using SigmaPlot (v 11.0; Systat Software, San Jose, CA, USA). The significance level was set at $P<0.05$. Results are presented in mean and standard error of mean (SEM) or median with interquartile range (IQR).

Results

Baseline characteristics

Age, BMI, and blood pressure were not significantly different between COPD and control subjects ($P>0.05$). At baseline COPD patients had higher symptom burden measured by the mean COPD assessment test score (CAT score), lower 6-minutes walking distance, and lower maximal workload compared with control subjects ($P<0.05$). A total of 7 out of 30 COPD patients showed low fat free mass index (FFMI) ($\leq$16 kg·m^{-2} in men and $\leq$15 kg·m^{-2} in women) but normal BMI ($\leq$21 kg·m^{-2}) indicating some degree of muscle atrophy . In the control group one subject had a low FFMI and low BMI (Table 1). At baseline COPD patients with low FFMI had higher CAT score, lower 6MWD, lower absolute VO_2peak, and lower maximal workload compared with COPD patients with normal FFMI ($P<0.05$).
As previously reported, both training modalities improved the symptom burden as well as six-minute walking distance (7% and 5% in ET and RT) and maximal workload (12% and 9% in ET and RT) ($P<0.05$) to a similar extend with no differences between COPD patients with low FFMI and normal FFMI. The improvements in absolute or relative VO_2peak within training groups did not reach statistical significance (absolute VO_2peak 6% in both ET and RT; relative VO_2peak: 5% and 10% in ET and RT) (Table 1) .

Cytokines in plasma and in muscle

Baseline plasma concentrations of IL-8, IL-18, and CRP were higher ($P<0.05$) in COPD patients than in control subjects (Fig. 1). Muscle content of IL-1β, IL-6, IL-8, IL-18, and TNF-α were similar between the ET and the RT group at baseline with no effect of exercise training (Fig. 2). Plasma TNF-α increased after ET ($P<0.05$) and tended to increase after RT ($P=0.06$), with no difference between groups. All other cytokines in plasma remained unaffected by both training interventions (Fig. 1). We found no differences between patients with low and normal FFMI in cytokine levels at baseline, with no effects of exercise within the two subgroups.

Inflammatory cells in circulation and in muscle

A higher concentration of circulating leukocytes (due to elevated neutrophil number) were found in COPD patients compared to control subjects and the levels were not altered by either exercise training regime (Table 2). Macrophages were counted in a total area of 282 mm^2 muscle tissue with a mean counted area of 67±0.6 mm^2 per biopsy. The number of

macrophages per area were similar in COPD patients and the control subjects at baseline (ET: 30±7; RT: 22±5; control: 20±3 macrophages/mm^2) (Fig. 3F). This number of macrophages per area increased with RT, but not with ET (RT: 45±10, P<0.05; ET: 30±6) (Fig. 3F). At baseline there were no difference in muscle or circulating inflammatory cells between patients with low and normal FFMI and the effect of exercise training were also similar in the subgroup analysis.

Oxidative stress in muscle

Muscle protein content of NOX was higher in the COPD patients compared with control subjects at baseline (86% and 75% higher in ET and RT group, respectively) (Fig. 4C). Muscle protein content of SOD2 did not differ between COPD patients and control subjects. The NOX content was unaffected by RT and ET. However, exercise training increased the SOD2 protein levels to a similar extend in the two training groups (by 53% and 32% in the ET and RT group, respectively) (Fig. 4A-C). There were no differences at baseline between patients with low and normal FFMI, and the adaptations seen after exercise training were not influenced by the FFMI.

Discussion

Moderate to severe COPD patients had a higher muscular NOX protein content, a similar number of muscle-infiltrating macrophage and a higher level of circulating IL-8, IL-18, CRP as well as leukocytes. Eight weeks of ET or RT increased muscle SOD2 content and plasma TNF-α, but had no effect on systemic levels of other pro-inflammatory cytokines or leukocytes. Moreover, exercise training did not influence muscular NOX protein. Our results do not support the idea of a highly inflammatory environment in the quadriceps muscle of patients with COPD. In consistency with our hypothesis, RT induced macrophage infiltration in the skeletal muscle although local cytokine level remained unaltered. However, the expected systemic anti-inflammatory effect of ET was undetectable.

Systemic and muscle cytokines

Pro-inflammatory cytokines have been related to the loss of muscle mass in patients with COPD (11, 22, 23). In support, the present study and others (3, 10, 23) have shown higher circulating levels of cytokines (e.g. IL-6, IL-8, IL-18, TNF-α) and CRP in COPD patients compared with healthy subjects. This is in accordance with the low-grade inflammation that

accompanies many chronic diseases associated with physical inactivity . Nevertheless, two of the plasma cytokines, which specifically have been proposed to be associated with muscle wasting in COPD, TNF-α and IL-6, were not found to be elevated in the current COPD subjects. This finding is in accordance with one previous study but not with a study by Vogiatzis and colleagues .

Several studies have investigated cytokines in skeletal muscle of COPD patients. But, whether the muscular cytokine levels of especially TNF-α is elevated in COPD remains controversial. While some studies show elevated muscle TNF-α (24, 25) and IL-18 in the locomotor muscles of COPD, others report similar muscle TNF-α and IL-6 between COPD and healthy subjects (11, 23). The marked differences between the reported plasma and muscle cytokine might be explained by the heterogeneity in patient population, by differences in study design, and/or in the sensitivity of the assays that are used to measure the cytokines. We freeze-dried and carefully dissected all muscle tissue free of blood and connective tissue, a procedure not so commonly used by others, but which we find essential because cytokine levels otherwise might be falsely elevated by contamination from blood or other tissues.

Interestingly, in the abovementioned study by Vogiatzis et al. the elevated plasma IL-6 and TNF-α concentrations in the COPD group were not affected by exercise training . In accordance, the elevated plasma IL-8, IL-18, or CRP levels in the COPD group at baseline were unaffected by the training intervention. Thus, we add to current knowledge (3, 25) that regardless of an endurance or strength based training modality circulating cytokines are unaltered by exercise in COPD.

Despite training for 8 weeks, which is the recommended duration for pulmonary rehabilitation in COPD, the patients did not experience changes in muscle cytokines levels . This finding is in accordance with previous studies involving endurance training in COPD (3, 25). Exercise training has repeatedly been shown to reverse key features of muscle dysfunction in COPD and concomitantly induce higher exercise capacity and increased quality of life. However, our results do not support the idea that intra-muscular inflammation plays a key role in these adaptations.

Circulating leukocytes and infiltration in muscle

The increased concentration of circulating leukocytes commonly seen in COPD patients has been proposed to infiltrate into the skeletal muscle, and similarly to the pathogenesis in the lungs, break down the muscle tissue . However, in the present study we found equal amounts of macrophages between individual muscle fibres in COPD patients and healthy subjects. These results are in line with observations from Gosker *et al.* who found normal amounts of macrophages in the skeletal muscle of COPD patients. Nevertheless, in other studies higher immune cell infiltration of the skeletal muscle has been documented in COPD (24, 28, 29). These conflicting results could be the result of different stages of COPD studied or that we did not specifically select patients with the muscle depleted phenotype for the present trial. However, the normal levels of macrophage muscle infiltration and high amounts of circulating immune cells supports the idea of low-muscle/high-plasma cytokine concentrations in COPD . Likewise, the lack of effect of exercise training on systemic and muscular inflammation is consistent with previous observations (3, 25, 30). RT increased macrophage infiltration into the muscle, a response that might reflect a degree of muscle damage and repair . Whether this response is beneficial is unclear since the control group did not exercise. However, exercise-induced macrophage filtration might stimulate muscle growth although this was not observed immediately after the RT .

Oxidative stress and detoxification

Muscular protein content of NOX was elevated in COPD at baseline (Fig. 4). This may reflect a greater ROS formation but with a similar antioxidative capacity as in healthy subjects (Fig. 4C). Physiological levels of ROS are required for cell signalling and normal skeletal muscle force production, but high ROS accumulation promotes contractile dysfunction and muscular fatigue . Therefore, exercise capacity in COPD might be affected by a higher oxidative stress burden (10, 33). In the present study muscular NOX content was unaffected by exercise training but protein content of SOD2 increased with both ET and RT. The increased SOD2 content could be the result of an overall increase in mitochondrial content in the skeletal muscle of COPD after exercise training. Previously, we found a tendency for increased muscle protein content of the enzyme citrate synthase, a marker of mitochondrial volume density , after ET in the same COPD patients . Likewise, mitochondrial volume density increases with endurance training in healthy humans . Exercise training can reduce NOX activity and expression , which together with up-regulations of antioxidant systems in blood (erythrocytes) and skeletal muscle (15, 34, 36, 37), results in

lower ROS generation and accumulation . Thus, a possibly greater removal of ROS may contribute to improvements in quadriceps endurance in COPD patients (5, 9), but it remains unknown whether the absolute level of oxidative stress remains unchanged since other sources of ROS formation, e.g. mitochondria and xanthine oxidase, were not assessed in this study. Likewise, upregulation of other important antioxidants could have occurred.

Limitations

COPD is a heterogeneous disease and some patients experience a high degree of respiratory symptoms whereas in others muscle wasting dominates the phenotype. Therefore, these results may not be extrapolated to all patients with COPD. We did not obtain sufficient muscle specimens from all subjects. The number of samples was smaller in RT than ET. The duration of the training intervention was relatively short and we cannot exclude that a longer intervention would have had a more anti-inflammatory effect. Indeed, a decrease in plasma and muscular pro-inflammatory cytokines concentrations as well as an increase in plasma level of anti-inflammatory cytokines has been observed after longer periods of exercise training (38, 39). We did not include an inactive control group of COPD patients, since previous research have shown large beneficial effects of exercise training compared with standard care. In addition, after three months of no intervention (except standard medical treatment) COPD patients showed no change in exercise capacity, muscle strength or health related quality of life . However, we recognize that inactivity might alter markers of inflammation and oxidative stress in patients with COPD.

Conclusions

In patients with moderate to severe COPD, we found no evidence for muscular inflammation. Eight weeks of either RT or ET did not alter the inflammatory status. However, both training modalities increased local muscle antioxidant content which might improve muscle function and prevent local inflammation and oxidative stress.

Perspectives

The aetiology of limb muscle dysfunction in COPD is debated and one of the prevailing hypotheses is that a local inflammatory process drives the breakdown of the muscle. Our results do not support the idea of a highly inflammatory environment in the locomotor

muscles in COPD patients despite elevated levels of the pro-oxidant NOX. Although the current exercise training had limited effect on inflammation, the possible long-term anti-inflammatory effects of adherence to exercise are unexplored, and the inflammation in the lung tissue has not been examined in response to exercise training.

Acknowledgements

Maria Gemma Martinez Rubio Kroos, Karina Olsen, Lene Foged, and Anne-Kristine Meinild-Lundby are acknowledged for their technical assistance.

Funding information

The Centre for Physical Activity Research (CFAS) is supported by a grant from TrygFonden. During the study period, The Centre of Inflammation and Metabolism (CIM) was supported by a grant from the Danish National Research Foundation (DNRF55). The study was further supported by grants from Axel Muusfeldts Foundation, the Capital Region of Denmark, and the Novo Nordisk Foundation. CIM/CFAS is a member of DD2 - the Danish Center for Strategic Research in Type 2 Diabetes (the Danish Council for Strategic Research, grant no. 09-067009 and 09-075724).

Disclosure

No conflicts of interest are declared by the authors.

References

1. Iepsen UW, Munch GDW, Rugbjerg M, Rinnov AR, Zacho M, Mortensen SP, et al. Effect of endurance versusu resistance training on quadriceps muscle dysfunction in COPD: a pilot study. *Int J Chron Obstruct Pulmon Dis.* 2016; 11:2659-2669.

2. Mostert R, Goris A, Weling-Scheepers C, Wouters EF, Schols AM. Tissue depletion and health related quality of life in patients with chronic obstructive pulmonary disease. *Respir Med.* 2000; 94(9):859-867.

3. Vogiatzis I, Stratakos G, Simoes DCM, Terzis G, Georgiadou O, Roussos C, et al. Effects of rehabilitative exercise on peripheral muscle TNFα, IL-6, IGF-1 and MyoD expression in patients with COPD. *Thorax.* 2007; 62(11):950-956.

4. Couillard A, Maltais F, Saey D, Debigáre R, Michaud A, Koechlin C, et al. Exercise-induced quadriceps oxidative stress and peripheral muscle dysfunction in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2003; 167(12):1664-1669.

5. Koechlin C, Couillard A, Simar D, Cristol JP, Bellet H, Hayot M, Préfaut C. Does oxidative stress alter quadriceps endurance in chronic obstructive pulmonary disease? *Am J Respir Crit Care Med.* 2004; 169(9): 1022-1027.

6. Toledo-Arruda AC, Vieira RP, Guarnier FA, Suehiro CL, Caleman-Neto A, Olivo CR, et al. Time-course effects of erobic physical training in the prevention of cigarette smoke-induced COPD. *J Appl Physiol (1985).* 2017; 123(3): 674-683.

7. Couillard A, Préfaut C. From muscle disuse to myopathy in COPD: potential contribution of oxidative stress. *Eur Respir J.* 2005; 26(4): 703-719.

8. Gliemann L, Nyberg M, Hellsten Y. Nitric oxide and reactive oxygen species in limb vascular function: what is the effect of physical activity? *Free Radic Res.* 2014; 48(1): 71-83.

9. Mercken EM, Hageman GJ, Schols AM, Akkermans MA, Bast A, Wouters EF. Rehabilitation decreases exercise-induced oxidative stress in chronic obstructive Pulmonary disease. *Am J Respir Crit Care Med.* 2005; 172(8): 994-1001.

10. Van Helvoort HA, Heijdra YF, Thijs HM Vina J, Wanten GJ, Dekuijzen PN. Exercise-induced systemic effects in muscle-wasted patients with COPD. *Med Sci Sports Exerc.* 2006; 38(9): 1543-1552.

11. Barreiro E, Schols AM, Polkey MI, Galdiz HR, Swallow EB, Coronell C, et al. Cytokine profile in quadriceps muscles of patients with servere COPD. *Thorax.* 2008; 63(2): 100-107.

12. Iepsen UW, Jørgensen KJ, Ringbæk T, Hansen H, Skrubbeltrang C, Lange P. A Systematic Review of Resistance Training Versus Endurance Training in COPD. *J Cardiopulm Rehabil Prev.* 2015; 35(3): 163-172.

13. McCarthy B, Casey D, Devane D, Murphy K, Murphy E, Lacasse Y. Pulmonary rehabilitation for chronic obstructive pulmonary. *Cochrane Database Syst Rev.* 2015; 23(2): CD003793.

14. Rugbjerg M, Iepsen UW, Jørgensen KJ, Lange P. Effectiveness of pulmonary rehabilitation in COPD with mild symptoms: a systematic review with meta-analyses. *Int J Chron Obstruct Pulmon Dis.* 2015; 10: 791-801.

15. Miyazaki H, Oh-ishi S, Ookawara T, Kizaki T, Toshinai K, Ha S, et al. Strenous endurance training in humans reduces oxidative stress following exhausting exercise. *Eur J Appl Physiol.* 2001; 84(1-2): 1-6.

16. Pedersen BK. Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease. *Eur J Clin Invest.* 2017;47(8): 600-611.

17. Spruit MA, Gosselink R, Troosters T, De Paepe K, Decramer M. Resistance versus endurance training in patients with COPD and peripheral muscle weakness. *Eur Respir J.* 2002; 19(6): 1072-1078.

18. Iepsen UW, Munch GW, Rugbjerg M, Ryrsø CK, Secher NH, Hellsten Y, et al. Leg blood flow is impaired during small muscle mass exercise in patients with COPD. *J Appl Physiol (1985).* 2017; 123(3): 624-631.

19. American Thoracic Society; American College of Chest Physicians. ATS/ACCP Statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med.* 2003; 167(2):211-77.

20. Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc.* 1982; 14(5):377-81.

21. Bergström J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest.* 1975; 35(7): 609-616.

22. Maltais F, Decramer M, Casaburi R, Barreiro E, Burelle Y, Debigáre R, et al. An official American Thoracic Society/European Respiratory Society statement: update on limb muscle dysfunction in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2014; 189(9): e15-62.

23. Petersen AM, Penkowa M, Iversen M, Frydelund-Larsen L, Andersen JL, Mortensen J, et al. Elevated levels of IL-18 in plasma and skeletal muscle in chronic obstructive pulmonary disease. *Lung.* 2007; 185(3): 161-171.

24. Montes de Oca M, Torres SH, De Sanctis J, Mata M, Hernández N, Tálamo C. Skeletal muscle inflammation and nitric oxide in patients with COPD. *Eur Respir J.* 2005; 26(3): 390-397.

25. Rabinovich RA, Figueras M, Ardite E, Carbó N, Troosters T, Filella X, et al. Increased tumor necrosis factor-α plasma levels during moderate-intensity exercise in COPD patients. *Eur Respir J.* 2003; 21(5): 798-794.

26. Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructve Lung Disease (GOLD) 2017. Available from: http://goldcopd.org.

27. Gosker HR, Kubat B, Schaart G, van der Vusse GJ, Wouters EF, Schols AM Myopathological features in skeletal muscle of patients with chronic obstructive pulmonary disease. *Eur Respir J.* 2003; 22(2): 280-285.

28. Barreiro E, Ferrer D, Sanchez F, Minguella J, Marin-Corral J, Martinez-Llorens J, et al. Inflammatory cells and apoptosis in respiratory and limb muscles of patients with COPD. *J Appl Physiol (1985).* 2011; 111(3): 808-817.

29. Menon MK, Houchen L, Singh SJ, Morgan MD, Bradding P, Steiner MC. Inflammatory and staelitte cells in the quadriceps of patients with COPD and response to resistance training. *Chest.* 2012; 142(5): 1134-1142.

30. Vogiatzis I, Simoes DC, Dtratakos G, Kourepini E, Terzis G, Manta P, et al. Effect of pulmonary rehabilitation on muscle remodelling in cachectic patients with COPD. *Eur Respir J.* 2010; 36(2): 201-310.

31. Jones DA, Newham DJ, Round JM, Tolfree SE. Experimental human muscle damage: morphological changes in relation to other indices of damage. *J Physiol.* 1986; 375: 435-448.

32. Tidball JG,Villalta SA. Regulatory interactions between muscle and the immune system during muscle regeneration. *Am J Physiol Regul Integr Comp Physiol.* 2010; 298(5): R1173-87.

33. Powers SK, Ji LL, Kavazis AN, Jackson MJ. Reactive oxygen species: impact on skeletal muscle. *Compr Physiol.* 2011; 1(2): 941-969.

34. Meinild Lundby A-K, Jacobs RA, Gehrig S, de Leur J, Hauser M, Bonne TC, et al. Exercise training increases skeletal muscle mitochondrial volume density by enlargement of existing mitochondria and not de novo biogenesis. *Acta Physiol (Oxf).* 2018; 222(1).

35. Adams V, Linke A, Kränkel N, Erbs S, Gielen S, Möbius-Winkler S, et al. Impact of regular physical activity on the NAD(P)H Oxidase and angiotensin receptor system in patients with coronary artery disease. *Circulation.* 2005; 111(5): 555-562.

36. Criswell D, Powers S, Dodd S, Lawler J, Edwards W, Renshler K, et al. High intensity training-induced changes in skeletal muscle antioxidant enzyme activity. *Med Sci Sports Exerc.* 1993; 25(10): 1135-1140.

37. Gliemann L, Schmidt JF, Olesen J, Biensø RS, Peronard SL, Grandjean SU, et al. Resveratrol blunts the positive effects of exercise training on cardiovascular health in aged men. *J Physiol.* 2013; 591(20): 5047-5059.

38. Gielen S, Adams V, Möbius-Winkler S, Linke A, Erbs S, Yu J, et al. Anti-inflammatory effects of exercise training in the skeletal muscle of patients with chronic heart failure. *J Am Coll Cardiol.* 2003; 42(5): 861-868.

39. Goldhammer E, Tanchilevitch A, Maor I, Beniamini Y, Rosenschein U, Sagiv M. Exercise training modulates cytokines activity in coronary heart disease patients. *Int J Cardiol.* 2005; 100(1): 93-99.

40. Troosters T, Gosselink R, Decramer M. Short- and long-term effects of outpatient rehabilitation in patients with chronic obstructive pulmonary disease: a randomized trial. *Am J Med.* 2000; 109(3):207-12.

Figure legends

Figure 1. Cytokine concentration in plasma. Data are in mean (SEM or median (IQR). Hatched bars indicate after training intervention. *Different from control: $P<0.05$, $^{\#}$Difference within group: $P<0.05$.Abbreviations: N.d., not detectable.

Figure 2. Cytokine concentration in muscle. Data are in mean (SEM or median (IQR). Hatched bars indicate after training intervention.

Figure 3. A-E: Representative skeletal muscle cross-sectional from the vastus lateralis. Immunohistochemistry was performed using antibody against macrophages (anti-CD163, green indicated by arrows) using a light microscope x10 objective. White scale bars = 0.1 mm. Muscle sections from two patients with COPD; one before and after ET (A and B), and one RT (C and D), and from one control subject (E). F: Scatter plot of mean number of inflammatory cells in skeletal muscle. Horizontal solid lines indicate mean values. #Difference within group: $P<0.05$.

Figure 4. Representative western blots of (**A**) NOX and GAPDH, (**B**) SOD2 and actin protein content in skeletal muscle from COPD participants (RT or ET) and controls (C). Samples from the same subject were loaded side by side. Baseline (indicated by b) and after (indicated as a) ET and RT. C: Protein expression of NOX and SOD2 in skeletal muscle. Data are in mean (SEM or median (IQR). *Different from control: $P<0.05$, #Difference within group: $P<0.05$.

Table 1. Symptom burden, exercise capacity and body composition.

	Healthy controls	**ET group**		**RT group**	
		Baseline	After training	Baseline	After training
FEV_1, % of predicted	110(3)	55(4)*		57(3)*	
CAT score	4(1)	13(1)*	11(1)*#	13(1)*	10(1)*#
VO_2peak , L/min	2.5(0.2)	1.6(0.1)*	1.7(0.1)*	1.6(0.2)*	1.7±0.2)*
VO_2peak , mL/kg/min	33.2(2.5)	21.3(1.9)*	22.1(1.8)*	21.4(1.9)*	22.7(2.2)*
Maximal workload, W	153(8)	104(8)*	116(10)#	95(11)*	104(11)*#
6MWD, m	673(665-686)	549(20)*	589(14)*#	531(21)*	555(19)*#
FFMI, $kg \cdot m^{-2}$	18.7(0.7)	17.4(0.7)	17.5(0.8)	17.5(0.7)	17.6(0.7)
BMI, $kg \cdot m^{-2}$	25(1)	27(2)	26(1)	27(1)	28(1)

Note: Data in mean (standard error of means) or median (interquartile range). *Different from control: $P<0.05$, #Difference within group: $P<0.05$.
Abbreviations: 6MWD, 6-minute walking distance; BMI, body mass index; CAT, COPD assessment test; FFMI, fat free mass index; FEV_1, forced expiratory volume in 1 second; VO_2, oxygen uptake (1).

Table 2. Leukocyte distribution in COPD patients before and after exercise training.

	Healthy controls	**ET group**		**RT group**	
		Baseline	After training	Baseline	After training
Leukocytes, $x10^9$ cells/l	5.6(0.5)	6.5(6.2-7.8)	7.3(0.5)*	7.9(6.9-9.2)*	8.0(0.4)*
Neutrophils, $x10^9$ cells/l	3.2(0.4)	3.8(3.3-4.1)	4.3(0.4)	4.3(4.1-5.5)*	4.7(0.3)*
Basophils, $x10^9$ cells/l	0.04(0.03-0.05)	0.05(0.01)	0.06(0.01)	0.03(0.02-0.06)	0.05(0.1)
Eosinophils, $x10^9$ cells/l	0.15(0.04)	0.25(0.03)	0.28(0.05)	0.20(0.04)	0.21(0.15-0.33)
Lymphocytes, $x10^9$cells /l	1.9(1.7-1.9)	2.1(0.2)	2.0(0.1)	2.2(0.2)	2.2(0.2)
Monocytes, $x10^9$cells/l	0.6(0.1)	0.7(0.1)	0.7(0.1)	0.7±0.1	0.7(0.1)

Note: Data in mean (standard error of means) or median (interquartile range). *Different from control.