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Resistance exercise training increases skeletal muscle mitochondrial respiration in chronic obstructive pulmonary disease

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Abstract

Background Chronic obstructive pulmonary disease (COPD) is associated with skeletal muscle mitochondrial dysfunction. Resistance exercise training (RT) is a training modality with a relatively small pulmonary demand that has been suggested to increase skeletal muscle oxidative enzyme activity in COPD. Whether a shift into a more oxidative profile following RT also translates into increased mitochondrial respiratory capacity in COPD is yet to be established.

Methods This study investigated the effects of 13 weeks of RT on m. vastus lateralis mitochondrial capacity in 11 persons with moderate COPD [45% females, age: 69 ± 4 years (mean ± SD), predicted forced expiratory volume in 1 s (FEV_1): 56 ± 7%] and 12 healthy controls (75% females, age: 66 ± 5 years, predicted FEV_1: 110 ± 16%). RT was supervised and carried out two times per week. Leg exercises included leg press, knee extension, and knee flexion and were performed unilaterally with one leg conducting high-load training (10 repetitions maximum, 10RM) and the other leg conducting low-load training (30 repetitions maximum, 30RM). One-legged muscle mass, maximal muscle strength, and endurance performance were determined prior to and after the RT period, together with mitochondrial respiratory capacity using high-resolution respirometry and citrate synthase (CS) activity (a marker for mitochondrial volume density). Transcriptome analysis of genes associated with mitochondrial function was performed.

Results Resistance exercise training led to similar improvements in one-legged muscle mass, muscle strength, and endurance performance in COPD and healthy individuals. In COPD, mitochondrial fatty acid oxidation capacity and oxidative phosphorylation increased following RT (+13 ± 22%, $P = 0.033$ and +9 ± 23%, $P = 0.035$, respectively). Marked increases were also seen in COPD for mitochondrial volume density (CS activity, +39 ± 35%, $P = 0.001$), which increased more than mitochondrial respiration, leading to lowered intrinsic mitochondrial function (respiration/CS activity) for complex-1-supported respiration (−12 ± 43%, $P = 0.033$), oxidative phosphorylation (−10 ± 42%, $P = 0.037$), and electron transfer system capacity (−6 ± 52%, $P = 0.027$). No differences were observed between 10RM and 30RM RT, nor were there any adaptations in mitochondrial function following RT in healthy controls. RT led to differential expression of numerous genes related to mitochondrial function in both COPD and healthy controls, with no difference being observed between groups.

Conclusions Thirteen weeks of RT resulted in augmented skeletal muscle mitochondrial respiratory capacity in COPD, accompanied by alterations in the transcriptome and driven by an increase in mitochondrial quantity rather than improved mitochondrial quality.

Keywords Resistance exercise training; Muscle plasticity; Chronic obstructive pulmonary disease; Mitochondrial function

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by persistent airflow limitations that are manifested as dyspnoea and chronic cough.[1] As a consequence, a key pathology of COPD is a reduced aerobic exercise capacity to which in fact also deteriorated skeletal muscle function contributes.[2] Indeed, the reduced whole-body maximal oxygen uptake (VO_{2max}) and the shorter distance covered during a 6 min walking test are partially explained by an attenuated skeletal muscle function.[3]

Specifically, reduced quadriceps muscular strength and endurance, as well as increased fatiguability are frequent in COPD.[4] Furthermore, phenotypic traits commonly observed with COPD include lower thigh muscle cross-sectional area, reduced proportion of m. vastus lateralis (VL) fibre type I, and increased proportion of fibre type IIx.[3,4] Skeletal muscle oxidative capacity is diminished, exemplified by decreased VL oxidative enzyme activity, mitochondrial efficiency, and respiratory capacity, collectively referred to as mitochondrial dysfunction.[5–7] The resulting increased contributions from anaerobic metabolism to muscular ATP synthesis are evident already at low work rates, leading to exacerbated ventilation and aggravating the feeling of breathlessness.[4] This downward disease spiral can be counteracted by exercise training, which accordingly results in a shift towards aerobic metabolism, for example, higher oxidative enzyme activity and mitochondrial function.[8,9] Therefore, exercise training should be an essential part of COPD rehabilitation.[10]

However, due to their pulmonary limitations, individuals with COPD have limited ability to perform whole-body aerobic exercise training at intensities that are required to achieve skeletal muscle adaptations.[11] In accordance with this, more accentuated physiological adaptations were observed when individuals with COPD performed single-limb vs. two-limb cycling training, which arguably is related to the lower systemic physiological demands of one-legged exercise, activating less muscle mass.[12,13] This makes resistance exercise training (RT) a particularly relevant training modality for improving limb muscle function.[14] Indeed, RT allows targeted and maximal exercise of isolated muscle groups without posing large demands on pulmonary ventilation and, as such, is more tolerable for persons with COPD.[15] While RT may not be intuitively associated with improvements in aerobic metabolism, some studies have demonstrated a positive effect of RT on skeletal muscle mitochondrial adaptations in healthy individuals.[16] Moreover, in individuals with COPD, increased citrate synthase (CS) activity and hydroxyacyl coenzyme A dehydrogenase protein levels were reported following 8 weeks of low-load high-repetition RT,[14] suggesting that RT could indeed provide a means of combatting the mitochondrial dysfunction in COPD. Given the time-efficiency and cost-efficiency of RT and the increasing recognition of the impaired mitochondrial phenotype as a contributor to the exercise intolerance in COPD,[17,18] it is worthwhile investigating the efficacy of RT for reversing mitochondrial dysfunction. Therefore, the primary aim of this study was to examine whether RT leads to altered oxidative enzyme activity and expression of mRNA associated with mitochondrial function and whether this translates into improved mitochondrial respiratory capacity.

For healthy adults, high-load low-repetition RT is traditionally recommended for optimal muscle strength and hypertrophy gains, while low-load high-repetition RT is recommended for improving muscular endurance.[19] As mentioned earlier, it has been shown that the latter RT mode improved oxidative enzyme activity in COPD.[14] Whether these adaptations are specific to low-load high-repetition RT or are a generic response to RT remains to be elucidated.

The purpose of this study was to determine the effects of 13 weeks of RT on VL mitochondrial respiratory capacity in persons with COPD and to investigate the potential influence of RT load (low vs. high). Briefly, leg exercises were performed unilaterally, with one leg conducting high-load training and the contralateral leg conducting low-load training. Healthy controls of similar age were included to compare the RT responses between the two populations. We hypothesized that RT would increase mitochondrial respiration in COPD and controls and that the effect would be more pronounced in the low-load training leg.

Materials and methods

The study was approved by the Regional Committees for Medical and Health Research Ethics South-East Norway (reference nr. 2013/1094), pre-registered on clinicaltrials.gov (NCT02598830), and conducted according to the guidelines of the Declaration of Helsinki. The present article reports mitochondrial function that was pre-registered as a secondary outcome of The Granheim COPD double-blind randomized clinical trial (NCT02598830). For a thorough description of the study intervention and the assessment of muscle mass, strength, and endurance performance, as well as the results for the primary objective of the study, the reader is referred to the main article.[20]

Study participants and design

Participants comprised a subset of the individuals enrolled in The Granheim COPD study whose primary objective was to

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investigate the effects of vitamin D_3 supplementation in combination with RT on RT-associated adaptations.[20] Due to the lack of response to vitamin D_3 supplementation in general,[20] and for mitochondrial parameters in particular (Supporting Information, *Figure* S1), the vitamin D_3 and placebo group are presented pooled for the purpose of the herein presented analysis. Due to limited resources, only 23 of the 95 persons enrolled in The Granheim COPD study underwent the mitochondrial assessment battery of the study, selected based on the time of enrolment (the study participants were enrolled at four different time points; the mitochondrial assessment battery was performed with participants enrolled in Week 45, 2016). Of these 23 participants, 11 persons had a clinical diagnosis of stable, moderate COPD [Global Initiative for Obstructive Lung Disease (GOLD) stages II (n = 10) and III (n = 1), predicted forced expiratory volume in 1 s (FEV_1) between 30% and 80%, and FEV_1/forced vital capacity $<$ 70% after reversibility testing[1]] (*Table* 1). Three persons with COPD were current smokers ($<$10 cigarettes·day^{-1}). Twelve healthy non-smoking participants of similar age with normal pulmonary function (predicted FEV_1 $>$ 80%) served as controls. Exclusion criteria were age $<$ 60 years, unstable cardiovascular diseases, physically disabling musculoskeletal diseases, and intake of steroids. All participants completed the study in accordance with the study protocol, except for two persons with COPD; one withdrew due to relocation to another city, and one was excluded from the analyses due to non-adherence to the RT prescription. This participant experienced back pain and could not perform the exercises as prescribed. For a thorough overview of the sample size for each measurement, see *Table* S1. All individuals completed a physical activity log during a regular week prior to the intervention and weekly-spent kilocalories were calculated thereof to assess physical activity levels.[21] All measurements were undertaken prior to and following the RT intervention. Study participants received oral and verbal information about the study and provided informed consent prior to participation.

Resistance exercise training

Participants underwent 13 weeks of RT with two supervised sessions·week^{-1} as detailed in literature.[20] Leg exercises consisted of leg press, knee extension, and knee flexion (Technogym, Italy) and were conducted in three sets. The leg exercises were conducted unilaterally, with one leg exercising with high loads (10 repetitions maximum, 10RM) and the contralateral leg exercising with low loads (30 repetitions maximum, 30RM) to volatile exhaustion. All three sets on one leg were conducted before the other leg was exercised, with all sets being separated by 2 min rest. Loads were increased from session to session, that is, when participants managed to perform more than 12 or 35 repetitions per set for 10RM and 30RM, respectively, and were randomly assigned to each leg. The 10RM and 30RM loads were allocated to the same leg during the entire RT period. Immediately after each training, participants ingested half a protein bar (~15 g protein; Big 100, Proteinfabrikken, Norway).

Leg lean mass and muscle thickness

Leg lean mass was determined using dual-energy X-ray absorptiometry (Lunar Prodigy; GE Healthcare, USA) and was defined as the region distally of the collum femoris. M. rectus femoris and VL thickness were assessed using B-mode ultrasonography (SmartUs EXT-1M; Telemed, Lithuania) with a 39 mm 12 MHz linear array probe as detailed in reference.[20]

One-legged muscle strength and endurance performance, and bicycling aerobic capacity

Maximal muscle strength was determined as one repetition maximum (1RM) in unilateral knee extension (KE) and leg press (Technogym), and KE performance was assessed as the maximal number of repetitions that could be conducted

Table 1 Baseline characteristics

	CONTROLS	COPD	*P*-value
Participants (n, females/males)	12 (9/3)	11 (5/6)	
Age (years)	66 ± 5	69 ± 4	0.104
Body mass (kg)	70 ± 12	71 ± 20	0.740
BMI (kg·m^{-2})	24.5 ± 3.4	24.3 ± 6.1	0.946
FEV_1 (L)	2.78 ± 0.66	1.48 ± 0.32	**<0.001**
Predicted FEV_1 (%)	110 ± 16	56 ± 7	**<0.001**
FVC (L)	3.65 ± 0.75	3.08 ± 0.73	0.079
FEV_1/FVC (%)	76 ± 6	49 ± 7	**<0.001**
VO_{2max} (L·min^{-1})	2.38 ± 0.67	1.54 ± 0.35	**<0.001**
W_{max} (W)	199 ± 46	98 ± 35	**<0.001**
Physical activity level (kcal·week^{-1})	4855 ± 3137	4666 ± 4694	0.687

BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; VO_{2max}, bicycling maximal oxygen uptake; W_{max}, maximal minute power output.
Data are presented as mean ± SD. The p-values in bold are $<$0.05 and significant.

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at a load corresponding to 50% of baseline 1RM. Unilateral maximal isokinetic KE torque was tested with a dynamometer (Humac Norm; CSMi, USA) at three angular speeds (60, 120, and 240°·s^{-1}). A one-legged incremental cycling test to exhaustion (Excalibur Sport; Lode BV, The Netherlands) was performed to assess maximal minute power output (W_{max}) and VO_{2max} (JAEGER Oxycon PRO 280; Carefusion GmbH, Germany) for each leg, while one-legged exercise economy was assessed as O_2 cost of submaximal cycling at a constant load. Two-legged W_{max} and VO_{2max} were determined by an incremental bicycling test.[20]

Skeletal muscle biopsy

VL biopsies were obtained under local anaesthesia (1% lidocaine) from the 30RM leg at baseline and from both legs after RT using the micro-biopsy procedure.[22] Muscle tissue was dissected free of fat and connective tissue and divided into two parts. One part was immediately placed in ice-cold biopsy preservation medium (BIOPS)[23] for *ex vivo* measurements of mitochondrial respiration. The second part was snap-frozen in isopentane and stored at −80°C for later analysis of CS activity and the transcriptome profile.

High-resolution respirometry

The fresh muscle tissue was mechanically dissected and chemically permeabilized as described in literature.[23] One to four milligrams of permeabilized fibres were added to each respirometer chamber (Oxygraph-2k; Oroboros Instruments, Austria) that contained mitochondrial respiration medium 05 plus 20 mM creatine and 280 U·mL^{-1} catalase. Chamber oxygen concentration (nmol·mL^{-1}) and oxygen flux [pmol·(s·mg wet weight)$^{-1}$] were recorded (DatLab; Oroboros, Austria) at 37°C with the titration of various substrates at saturating concentrations (*Table* 2). Respiratory states were normalized to CS activity to assess mitochondrial intrinsic respiratory capacity. Samples were analysed in duplicate in hyper-oxygenated chambers ([O_2] ~ 200–450 nmol·mL^{-1}). Prior to the experiment, respirometers were calibrated for instrumental and chemical background oxygen flux.[23]

Citrate synthase activity

Muscle samples (0.4–5 mg dry weight) were homogenized as detailed elsewhere.[24] Total protein concentrations were determined by BCA assay (Thermo Scientific Pierce, USA). CS activity was assayed in lysates using an assay kit (C3260; Sigma-Aldrich, USA). All activities were normalized to milligrams of protein.

Transcriptome analysis

mRNA transcriptome analysis was performed on a larger number of participants (COPD, *n* = 19; controls, *n* = 34) from The Granheim COPD study,[20] as previously described,[25] comprising all 23 individuals included in the present article. Due to the substantial individual variability of such data, the number of participants was maximized to ensure the quality of data and analyses (i.e. we did not restrict the sample size to the 23 participants from whom we had mitochondrial analyses). Furthermore, as early increases in total RNA seem to be associated with long-term chronic responses to RT,[22] biopsies taken after 3.5 weeks into the RT were also included in these analyses. The MitoCarta v3.0 dataset was used to highlight genes associated with mitochondrial function.[26]

Data analyses and statistics

For a detailed description, see *Data* S1. Prior to analyses, data were evaluated for normality and homogeneity of variance and were log-transformed if required. Baseline differences between COPD and controls were examined using linear regression analysis with sex as a covariate. For one-legged

Table 2 Substrate uncoupler titration protocol

Step	Substrate (concentration)	Inner mitochondrial membrane process
1	Malate (2 mM) and octanoyl carnitine (250 μM)	L_N: leak respiration
2	ADP (5 mM)	P_{FAO}: fatty acid oxidation
3	Pyruvate (5 mM) and glutamate (10 mM)	P_{CI}: complex-1-linked respiration
4	Succinate (10 mM)	P: total oxidative phosphorylation
5	Cytochrome c (10 μM)	Inner mitochondrial membrane integrity
6	FCCP (0.5–1 μM steps)	ETS: electron transfer system

(1) Malate and octanoyl carnitine were titrated into the chambers to induce leak respiration through electron entry in absence of ADP and ATP (L_N), (2) ADP to assess mitochondrial capacity to couple electron transport through electron-transferring flavoprotein to the phosphorylation of ADP to ATP (P_{FAO}), (3) pyruvate and glutamate as substrates of complex I to stimulate complex-I-linked respiration (P_{CI}), (4) succinate to determine total oxidative phosphorylation capacity (P), and (5) cytochrome c to test for the integrity of the mitochondrial membrane. Respiratory data that exhibited >10% increase in oxygen flux following cytochrome c titration were not included in data analysis (9.6% of all measurements). (6) Maximal electron transfer system capacity (ETS) was determined with the addition of the uncoupler carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP). The leak control ratio for fatty acid oxidation (LCR_{FAO}) was computed as L_N/P_{FAO} indicating mitochondrial efficiency to oxidize fat.

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muscle mass, strength, and endurance performance, combined factors were computed from singular outcome measures (see *Table* 3 legend and reference[20]). To address the RT effects on exercise factors and mitochondrial function, linear mixed-effects models were applied. Statistical analysis was performed using the IBM SPSS Version 22 (IBM SPSS, Chicago, USA) and R software (see reference[25] for packages). Figures were made using Prism Software (GraphPad 8, San Diego, USA). Statistical significance was set to a two-tailed *P*-value < 0.05. Data are presented as mean ± SD.

Results

General characteristics

Individuals with COPD showed marked impairments in pulmonary function and displayed lower aerobic exercise capacity compared with the healthy controls prior to the intervention (*Table* 1). There were no differences in body mass, body mass index, or habitual physical activity level between COPD and controls. Four of the individuals with COPD and six of the controls were supplemented with vitamin D_3 during the RT intervention, as well as during the 12 week period leading up to the RT intervention. At the onset of RT, serum 25-hydroxyvitamin D_3 concentrations ($[25(OH)D_3]$) corresponded to 134 ± 18 and 125 ± 14 nmol·L^{-1} in vitamin D_3 supplemented participants (COPD and controls, respectively) and 75 ± 20 and 77 ± 34 nmol·L^{-1} in placebo supplemented participants. The vitamin D_3 supplementation protocol did not affect mitochondrial function compared with placebo, evident as a lack of vitamin D_3 effect of both vitamin D_3 supplementation only and combined vitamin D_3 and RT (*Figure* S1, legend). Adherence to the RT protocol was high and corresponded to a mean of 24.8 completed sessions (min–max: 21–26) in COPD and 25.3 (21–27) in controls.

Muscle mass, strength, and endurance performance

At baseline, one-legged muscle strength and endurance performance were lower in COPD than in controls, while muscle mass tended to be lower (*Table* 3). Briefly, RT led to similar improvements in muscle mass, strength, and endurance performance in COPD and controls, and the RT mode (10RM vs. 30RM) did not affect these improvements. One-legged cycling VO_{2max} tended to be improved in COPD and remained unchanged following RT in controls, while the O_2 cost during steady-state one-legged cycling decreased in both COPD and controls (*Figure* S2). The RT mode did not affect the changes in one-legged VO_{2max} and O_2 cost.

Citrate synthase activity

At baseline, CS activity was 28% lower ($P = 0.005$) in COPD (142.7 ± 36.8 mlU·mg protein^{-1}) than in controls (197.2 ± 40.0 mlU·mg protein^{-1}) (*Figure* 1). In COPD, RT led to increased CS activity by 35–43% ($10RM_{post}$: 185.3 ± 30.0 mlU·mg protein^{-1}, $30RM_{post}$: 197.4 ± 20.6 mlU·mg protein^{-1}, $P = 0.001$), restoring CS activity to healthy levels. Conversely, in controls, RT did not alter CS activity ($10RM_{post}$: 220.8 ± 60.0 mlU·mg protein^{-1}, $30RM_{post}$: 211.4 ± 37.9 mlU·mg protein^{-1}, $P = 0.365$). There was no difference between COPD and controls in the change in CS activity with RT ($P = 0.120$). The RT mode (10RM vs. 30RM) did not modify the effects of RT (or lack thereof) on CS activity in neither COPD nor controls.

Mitochondrial respiratory capacity

In COPD, baseline mass-specific fatty acid oxidation (P_{FAO}), complex-I respiration (P_{CI}), and oxidative phosphorylation (P) were lower (−18%, $P = 0.022$, −20%, $P = 0.020$, and −21%, $P = 0.018$, respectively) and electron transfer system capacity (ETS) tended to be lower (−18%, $P = 0.056$) than in controls, whereas leak respiration (L_N) was similar (−4%, $P = 0.794$) (*Figure* 2, *Table* S2). When respiration was normalized to CS activity (i.e. intrinsic mitochondrial function), the baseline differences between COPD and controls disappeared, except for a tendency towards higher L_N per CS activity (+20%, $P = 0.098$) in COPD. Also, mitochondrial efficiency to oxidize fatty acids (LCR_{FAO}) was similar at baseline ($P = 0.311$) (*Figure* 3, *Table* S2). In COPD, RT led to increased P_{FAO} (+13%, $P = 0.033$) and P (+9%, $P = 0.035$) and tended to lead to increased P_{CI} (+10%, $P = 0.079$) with no differences being evident between RT modes. No alterations were observed for L_N (+7%, $P = 0.340$) and ETS (+11%, $P = 0.115$). Furthermore, in COPD, RT led to reduced mitochondrial respiration/CS activity for P_{CI} (−12%, $P = 0.033$), P (−10%, $P = 0.037$), and ETS (−6%, $P = 0.027$). RT mode tended to impact this reduction, evident as lower intrinsic P (−11%, $P = 0.065$) and ETS (−13%, $P = 0.060$) in the 10RM leg compared with the 30RM leg after RT. In COPD, LCR_{FAO} was not changed. Following RT in controls, L_N, P_{FAO}, P_{CI}, P, and ETS remained unaltered, expressed both as mass-specific respiration and as respiration per CS activity. LCR_{FAO} remained unchanged with RT. There was no interaction between time (pre vs. post RT) and condition (COPD vs. controls) for any of the mitochondrial parameters.

Transcriptome analysis

At baseline, 78 genes associated with mitochondrial function were differentially expressed between COPD and controls

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Table 3 Muscle mass, strength, and endurance performance prior to (PRE) and following (POST) resistance exercise training in CONTROLS and COPD

	PRE				Conditio	POST				CONTROLS Time	COPD Time	Time × Condition
	CONTROLS		COPD		*P*-value	CONTROLS		COPD		*P*-value	*P*-value	*P*-value
	10RM	30RM	10RM	30RM		10RM	30RM	10RM	30RM			
Leg lean mass (kg)	7.63 ± 2.48	7.47 ± 2.41	7.32 ± 2.13	7.28 ± 2.10		7.41 ± 2.10	7.37 ± 2.26	7.34 ± 2.19	7.37 ± 2.24			
M. vastus lateralis thickness (mm)	19.50 ± 2.26	19.85 ± 3.42	18.65 ± 3.02	19.94 ± 4.88		21.07 ± 3.29	21.71 ± 3.02	20.20 ± 2.92	21.94 ± 4.94			
M. rectus femoris thickness (mm)	13.01 ± 2.73	13.13 ± 2.72	10.61 ± 2.36	11.06 ± 3.33		14.20 ± 3.38	14.30 ± 2.91	11.62 ± 1.83	12.89 ± 3.66			
One-legged muscle mass factor (AU)	**0.59 ± 0.14**		**0.54 ± 0.12**		**0.052**	**0.62 ± 0.15**		**0.57 ± 0.12**		**0.010**	**0.001**	**0.404**
1RM KE (kg)	19 ± 7	20 ± 7	15 ± 7	15 ± 7		22 ± 7	22 ± 8	20 ± 8	19 ± 8			
1RM leg press (kg)	111 ± 25	107 ± 27	96 ± 42	97 ± 40		159 ± 56	165 ± 51	146 ± 59	139 ± 62			
Peak torque during KE (Nm)												
60°·s^{-1}	113 ± 30	119 ± 33	101 ± 31	101 ± 41		121 ± 34	126 ± 35	111 ± 42	114 ± 39			
180°·s^{-1}	73 ± 23	69 ± 21	63 ± 20	64 ± 26		78 ± 21	77 ± 19	68 ± 27	74 ± 26			
240°·s^{-1}	62 ± 18	58 ± 18	55 ± 19	59 ± 24		63 ± 18	63 ± 16	59 ± 20	62 ± 23			
One-legged muscle strength factor (AU)	**0.46 ± 0.13**		**0.40 ± 0.14**		**0.001**	**0.52 ± 0.13**		**0.46 ± 0.17**		**<0.001**	**<0.001**	**0.577**
One-legged W_{max} (W)	126 ± 29	127 ± 31	60 ± 20	60 ± 20		131 ± 29	135 ± 30	70 ± 23	70 ± 19			
KE performance (Rep)	16 ± 3	16 ± 3	14 ± 5	15 ± 5		28 ± 7	28 ± 9	15 ± 5	23 ± 7			
One-legged endurance performance factor (AU)	**0.35 ± 0.07**		**0.19 ± 0.05**		**<0.001**	**0.40 ± 0.08**		**0.24 ± 0.06**		**<0.001**	**<0.001**	**0.860**
One-legged VO_{2max} (L·min^{-1})	1.92 ± 0.46	1.95 ± 0.50	1.31 ± 0.29	1.24 ± 0.25	**<0.001**	1.95 ± 0.50	2.01 ± 0.49	1.32 ± 0.27	1.33 ± 0.25	0.233	0.079	0.873
Δ% One-legged O_2 cost						−5 ± 6	−7 ± 6	−13 ± 4	−9 ± 4	**<0.001**	**<0.001**	0.122

1RM, one repetition maximum; 10RM, 10 repetitions maximum; 30RM, 30 repetitions maximum; AU, arbitrary unit; COPD, chronic obstructive pulmonary disease; KE, knee extension; Rep, repetitions; VO_{2max}, maximal oxygen uptake; W_{max}, maximal minute power output.

The *one-legged muscle mass* factor is composed of leg lean mass, m. vastus lateralis, and m. rectus femoris thickness measures. The *one-legged muscle strength* factor includes 1RM KE and leg press and the peak torque for knee extension at 60, 180, and 240°·s^{-1}. The *one-legged endurance performance* factor is composed of values from maximal workload achieved during one-legged cycling and the maximal number of repetitions at 50% of baseline 1RM KE. For each singular outcome measure, each participants' values (pre and post) were normalized to the participant with the highest value recorded during the study, resulting in individual scores ≤ 1. Thereafter, outcome domain factors were calculated as the mean of the normalized values for each variable for each participant and averaged between both legs (10RM and 30RM). One-legged VO_{2max} and O_2 cost were not included in any factors and are displayed individually. Workload to assess O_2 cost was defined as 50% of baseline W_{max} and was therefore different between individuals, but constant for each individual at PRE and POST; CONTROLS: 50 ± 10 W, COPD: 26 ± 2 W. Data are presented as mean ± SD. The *p*-values in bold are <0.05 and significant. Rows in bold represent summary lines.

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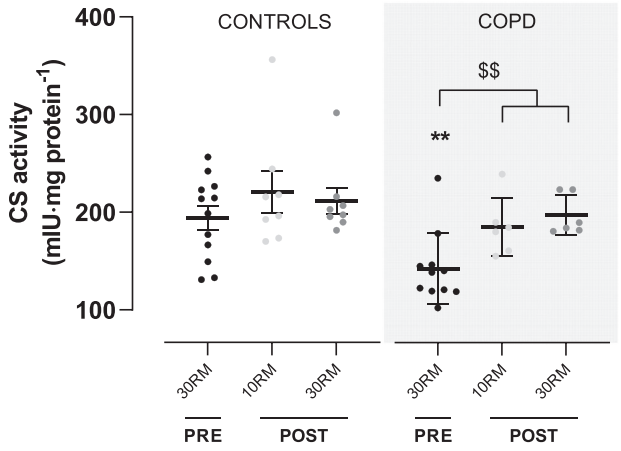
Figure 1 Citrate synthase (CS) activity prior to (PRE) and following 10RM and 30RM (POST) resistance exercise training. Note that skeletal muscle biopsies were obtained from the 30RM leg at PRE and from both legs at POST. Dots illustrate individual values and lines represent mean ± SD. $^{**}P < 0.01$ between CONTROLS and COPD at PRE, $^{\$\$}P < 0.01$ effect of time (PRE vs. 10RM/30RM POST pooled) in COPD. 10RM, 10 repetitions maximum; 30RM, 30 repetitions maximum; COPD, chronic obstructive pulmonary disease.



(*Table* S3); mostly genes related to metabolism.[27] Specifically, COPD showed lower expression of genes related to carbohydrate, fat, and protein metabolism (*Table* 4). Only one mitochondrial gene, *TXNRD2*, was differentially affected by 13 weeks of RT between COPD and controls (*Table* S3), and no MitoPathway categories were differentially changed, indicating similar mRNA responses to RT in COPD and controls. Therefore, COPD and controls were combined in our analyses of the general effects of RT on the gene expression pattern. These analyses revealed that RT led to marked changes in mRNA levels of genes related to mitochondrial function, with 225 (116↑, 109↓) and 228 (117↑, 111↓) genes being differentially expressed after 3.5 and 13 weeks of RT, respectively (*Table* S4). In summary, mostly genes involved in mitochondrial protein translation, substrate metabolism, and electron transfer system were affected by RT. Fifteen of the 34 controls that were included in these analyses and 9 of the 19 individuals with COPD were supplemented with vitamin D_3. Supplementation had no effect on RT-induced alterations in mRNA transcriptome compared with placebo.[20]

Discussion

The main and novel finding of the present study is that m. vastus lateralis mass-specific mitochondrial respiration and oxidative enzyme activity were augmented after 13 weeks of supervised RT in COPD, but not in healthy individuals of similar age. Hence, an exercise training intervention that minimizes the pulmonary effort and taxes the peripheral skeletal muscles maximally successfully normalized the initially deteriorated skeletal muscle mitochondrial phenotype in COPD. These RT-induced improvements in mitochondrial function in COPD were accompanied by the differential expression of numerous genes associated with mitochondrial function. A similar pattern in RT-induced changes in mRNA expression was observed in the healthy controls, although this apparently was not translated into functional mitochondrial adaptations to RT.

Figure 2 Mitochondrial respiratory capacity prior to (PRE) and following 10RM and 30RM (POST) resistance exercise training. Mitochondrial O_2 flux per mg of vastus lateralis muscle tissue with titration of malate and octanoyl carnitine (L_N), ADP (P_{FAO}), glutamate and pyruvate (P_{CI}), succinate (P), and FCCP (ETS) in CONTROLS and COPD patients (shaded). Note that skeletal muscle biopsies were obtained from the 30RM leg at PRE and from both legs at POST. Dots illustrate individual values and lines represent mean ± SD. $^{*}P < 0.05$ between CONTROLS and COPD at PRE 30RM, $^{\$}P < 0.05$ effect of time in COPD. 10RM, 10 repetitions maximum; 30RM, 30 repetitions maximum; COPD, chronic obstructive pulmonary disease.

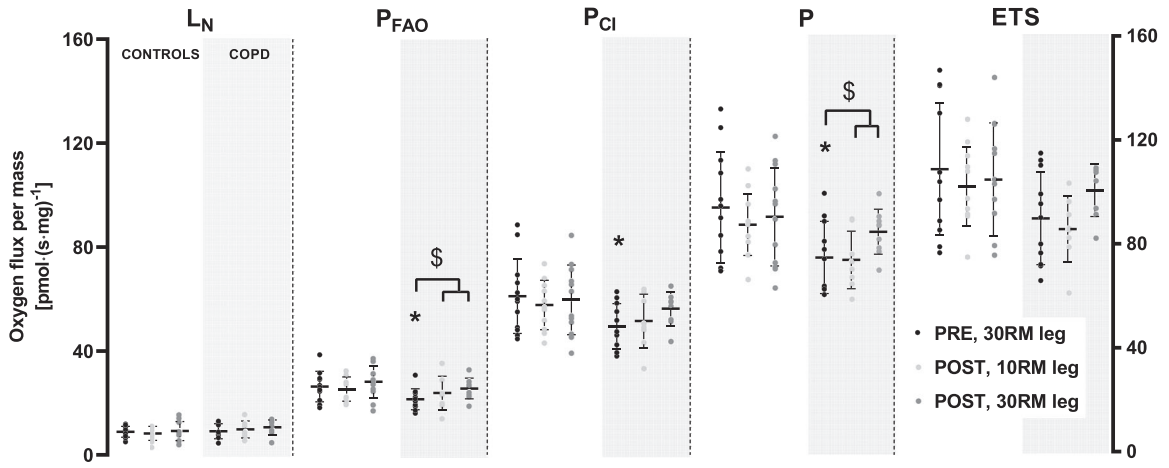


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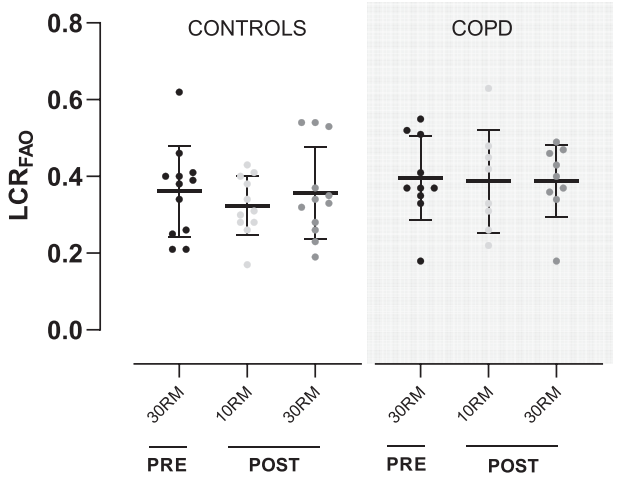
Figure 3 Leak control ratio for fatty acid oxidation (LCR_{FAO}) prior to (PRE) and following 10RM and 30RM (POST) resistance exercise training. Note that skeletal muscle biopsies were obtained from the 30RM leg at PRE and from both legs at POST. Dots illustrate individual values and lines represent mean ± SD. No differences were observed between CONTROLS and COPD, nor was there any effect of time. 10RM, 10 repetitions maximum; 30RM, 30 repetitions maximum; COPD, chronic obstructive pulmonary disease.



In line with previous studies, we found diminished rates of VL mitochondrial respiration in COPD.[5,8] Specifically, P_{FAO}, P_{CI}, P, and ETS were 18–21% lower in COPD than in controls, accompanied by decreased mRNA expression of genes involved in fatty acid oxidation and carbohydrate metabolism. Moreover, CS activity was reduced by 28% in COPD, which is also in accordance with previous studies.[5,6,18,28] CS activity is frequently used as a proxy measure for mitochondrial volume density ($Mito_{VD}$) and is also valid for pre–post comparisons following interventions.[24] When expressed per CS activity, the difference in baseline mitochondrial respiration between COPD and controls disappeared. This confirms that intrinsic mitochondrial function is not compromised by COPD and that the lowered VL respiratory capacity largely results from reduced $Mito_{VD}$, that is, reduced mitochondrial quantity rather than quality.[5,8] In support of an intact mitochondrial quality, the mitochondrial efficiency to oxidize fatty acids was similar in COPD and controls. Intriguingly, previous research has shown that $Mito_{VD}$ is similar between activity level-matched COPD and healthy individuals, suggesting that physical inactivity causes the mitochondrial phenotype in COPD.[11] This has recently been challenged,[29] and indeed, in the present dataset, the lower $Mito_{VD}$ in COPD could not readily be explained by activity levels, as COPD and controls reported similar subjective physical activity levels prior to RT. This rather indicates that the lowered CS activity in COPD was a result of disease-related mechanisms that could involve the long-term exposure of the mitochondria to cellular hypoxia,[30] the augmented skeletal muscle oxidative stress, as well as the increased peripheral inflammatory state.[31]

The main aim of this study was to assess whether a training modality that limits the pulmonary constraints could improve COPD-related deteriorations in mitochondrial profile. Indeed, RT successfully normalized CS activity to healthy levels (controls pre: 194.4 ± 42.3 mlU·mg protein^{-1} vs. COPD post: 191.3 ± 25.3 mlU·mg protein^{-1}; 10RM and 30RM pooled), corresponding to a 39% increase from pre to post RT. This increase is similarly scaled to observations made in healthy, young individuals undergoing endurance exercise training.[24,32] As such, $Mito_{VD}$ shows responsiveness to chronic exercise training stimuli in COPD,[8,14] although this is not a universal observation.[33] The increased $Mito_{VD}$ with RT indicates that there is a potential for reversing the mitochondrial dysfunction of COPD by elevating levels of physical activity, despite the fact that sedentarism does not appear to be a major cause for the mitochondrial phenotype of the disease.[29] Whether this is mediated by an amelioration in the skeletal muscle cellular milieu, for example, reduced inflammation and reactive oxygen species production that could facilitate mitochondrial adaptation, warrants further investigation.

We hypothesized that low-load high-repetition RT could lead to more pronounced mitochondrial adaptations than

Table 4 MitoPathway analysis of genome-wide transcriptome data comparing CONTROLS (n = 34) and COPD (n = 19) at baseline

MitoPathway	Significance category[a]	Rank *P*-value[b]	GSEA *P*-value[c]	NES[d]
Carbohydrate metabolism	Consensus	0.070	0.033	−1.66
Fatty acid oxidation		0.070	0.005	−1.77
Amino acid metabolism	GSEA	0.141	0.087	−1.58
Branched-chain amino acid metabolism		0.229	0.050	−1.64
Metabolism		0.229	0.007	−1.54
Metals and cofactors		0.563	0.087	−1.53
Oxidative phosphorylation		0.960	0.087	−1.50

P-values are adjusted for false discovery rate.
[a]*Consensus* indicates agreement between directional (GSEA) and non-directional (Rank) hypothesis test of over-representation (when *P*-values < 0.1 for both analyses; see *Data* S1 for details).
[b]Rank-based enrichment tests identify MitoPathways that are over-represented among top-ranked genes without a directional hypothesis.
[c]Gene-set enrichment analysis (GSEA) tests for over-representation among top and bottom genes based on $\log_2$ fold-differences × $-\log_{10}$ (*P*-values) when comparing baseline differences between chronic obstructive pulmonary disease (COPD) and CONTROLS.
[d]A negative normalized enrichment score (NES) indicates a MitoPathway with lower expression in COPD compared with CONTROLS.

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high-load low-repetition RT, as it is associated with greater gains in muscular endurance.[19] However, RT mode did not affect changes in CS activity, neither in COPD nor in controls. For COPD, a potential explanation may be that any contractile activity provokes large perturbations in skeletal muscle homeostasis that may lead to a generic initial training response, as is typically seen in untrained individuals.[34] For controls, the explanation is likely more complex, as they did not exhibit RT-induced response in CS activity to either of the training modes. However, the control individuals started the intervention with higher baseline CS activity, and as such, the training protocol may have led to less pronounced metabolic perturbations, and thus less downstream adaptations.

Our study demonstrates that 13 weeks of RT results in improved mitochondrial respiratory capacity in individuals with COPD. In accordance with our hypothesis, we found increased mass-specific P_{FAO} and P following RT in COPD, as well as a tendency towards increased P_{CI}. The observed 9–13% improvement in mitochondrial respiration was, however, lower than the ~25% increase commonly observed after endurance exercise training in healthy, young individuals,[32] and the 40% increment in P_{CI} previously observed after endurance-like high-intensity KE training in COPD.[8] It could thus be argued that the aerobic stimulus is more accentuated by endurance-like KE training compared with RT. However, we did not observe differences in RT-induced mitochondrial adaptations between 10RM and 30RM training, with the latter approximating endurance-like exercise training. Furthermore, there was no effect of RT on CS activity and mitochondrial respiration in the healthy controls, despite the substantial mitochondrial reprogramming, as implied by changes in the mRNA transcriptome. Hence, although there are indications on the mRNA level that RT may potentially elicit mitochondrial adaptations, this did not translate into improved mitochondrial respiration in the controls. From these results, it can be derived that the RT stimulus was too low to induce mitochondrial protein translation in the controls with a healthy mitochondrial profile. An increase in RT frequency may potentially evoke alterations in the mitochondrial phenotype, as observed in healthy, young individuals.[35] A common view is that RT-induced muscular hypertrophy is more pronounced than the mitochondrial biogenesis with RT, which may thus 'dilute' the mitochondrial adaptations.[16] In line with this, the observed increase in VL thickness in COPD and controls in the present study corresponded to 10% and 9%, respectively, arguably masking an even greater increase in total VL mitochondrial capacity. Importantly, the augmented mitochondrial respiratory capacity in COPD was accompanied by functional improvements induced by RT, for example, enhanced one-legged muscle endurance performance and reduced submaximal O_2 cost. Whereas these improvements were also present in the controls, the unaltered mitochondrial respiration suggests other mechanisms underlying the enhanced muscle endurance performance in the healthy individuals.

Lastly, when expressed per CS activity, P_{CI}, P, and ETS were reduced after RT in COPD, indicating lowered intrinsic mitochondrial function. This is not a unique phenomenon and lowered mitochondrial quality has previously been shown after 2–6 weeks of exercise training in healthy individuals.[24,36] Altogether, the present findings suggest that in COPD, $Mito_{VD}$ is a key determinant of the increased mass-specific respiratory capacity observed after exercise training, with the increase in CS activity being more pronounced than the increase in mitochondrial respiratory capacity.

Methodological considerations

First, as we did not include a non-exercising COPD control group, disease progression over the course of the RT intervention may have affected our measure of RT-associated changes in oxidative capacity. Of note, this scenario seems unlikely, as pulmonary function and scores of the COPD assessment test were preserved from before to after the intervention.[27] Second, the comparison of RT responses between COPD and control individuals was likely limited by the relatively small sample size. It thus remains possible that individuals with COPD are more amenable to RT-associated alterations in oxidative enzyme activity and mitochondrial respiration than healthy individuals. This perspective warrants further investigation. We presently limit our main conclusion to the finding that mitochondrial respiratory capacity is increased with RT in COPD and unaltered in healthy controls. Third, the comparison of the efficacy of the two RT modes may have been affected by the lack of biopsy sampling from the 10RM leg prior to RT; that is, RT-associated changes in the 10RM leg were inferred using baselines values from the 30RM leg. Together with the relatively small sample size, this may have reduced the statistical power to identify differential responses to the two RT modes. Importantly, however, it should be emphasized that 10RM and 30RM training was compared using a within-subject protocol, with the two training modes being allocated to the two legs in a randomized manner, ensuring equal distribution of the dominant leg between the RT modes. Moreover, the within-subject comparison of 10RM vs. 30RM arguably reduced variance caused by factors such as genetics, diet, sleep, and habitual levels of physical activity. Fourth, it could be speculated that RT-associated adaptations in one leg may have been influenced by training of the other leg. While the presence of this so-called cross-education effect remains controversial, it is likely restricted to neuromuscular adaptations.[37,38] For muscle biological variables such as mRNA abundance and oxidative enzyme activity, the cross-education effect seems unlikely.[37] Of note, to minimize the likelihood of any such cross-education effect, also for muscular performance tests, several measures were implemented in the current study, including thorough familiarization to strength tests.[20] Fifth,

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some of the included participants were supplemented with vitamin D_3 prior to and during the intervention, while others received placebo. In the vitamin D_3 supplementation group, this resulted in elevated [25(OH)D_3], the storage form of vitamin D_3, prior to the RT intervention. The active form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$], has been shown to increase mitochondrial oxygen consumption in human skeletal muscle cells *in vitro*.[39] In the present dataset (The Granheim COPD study), [1α,25$(OH)_2D_3$] remained similar between the supplementation and the placebo group over the course of the RT intervention.[20] In line with these findings, the present dataset did not indicate effects of vitamin D_3 supplementation on RT-induced changes in mitochondrial respiration. Lastly, it is an ongoing debate whether the altered mitochondrial phenotype seen in COPD is caused by disuse or disease-related characteristics.[5,11,29] Whereas our data on habitual physical activity levels measured at baseline point towards the latter, it should be emphasized that these data were retrieved using a questionnaire and that a more robust method, such as live-tracking of activity levels, should be applied to confirm our findings.

Conclusions

Resistance exercise training is a potent intervention to restore mitochondrial function in COPD. We present novel evidence that RT leads to improvements in mitochondrial respiratory capacity, seemingly determined by increased CS activity rather than augmented quality of the mitochondria. Further research should focus on whether these adaptations in mitochondrial phenotype are related to RT-induced amelioration of COPD-related pathophysiology. Our observations provide further evidence that RT is a well-tolerated, time-efficient, and efficacious exercise training mode that induces beneficial alterations in VL oxidative capacity in COPD and is hence a promising therapeutic tool.

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Conflict of interest

All authors declare that they have no conflict of interest.

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Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Effect of vitamin D_3 supplementation (D+) and placebo (D-) on resistance exercise training (RT)-induced changes in mitochondrial function.
Figure S2. VO_2 of steady-state submaximal one-legged cycling prior to (PRE) and following 10 RM and 30RM (POST) resistance exercise training (RT).
Table S1. Sample size of each assessment.
Table S2. Mitochondrial respiratory capacity prior to (PRE) and following (10RM/30RM) resistance exercise training.
Table S3. Baseline and resistance exercise training-associated differences in gene expression between COPD (*n* = 19) and CONTROLS (*n* = 34).
Table S4. Resistance exercise training-associated changes in gene expression averaged over study groups (COPD, *n* = 19 and CONTROLS, *n* = 34).

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