

## Similar qualitative and quantitative changes of mitochondrial respiration following strength and endurance training in normoxia and hypoxia in sedentary humans

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<sup>2</sup>D. Swarovski Research Laboratory, Department of Visceral, Transplant and Thoracic Surgery, Innsbruck Medical University, Innsbruck, Austria; <sup>3</sup>Department of Sport Science, Medical Section, University of Innsbruck, Innsbruck, Austria;

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**Pesta D, Hoppel F, Macek C, Messner H, Faulhaber M, Kobel C, Parson W, Burtscher M, Schocke M, Gnaiger E.** Similar qualitative and quantitative changes of mitochondrial respiration following strength and endurance training in normoxia and hypoxia in sedentary humans. *Am J Physiol Regul Integr Comp Physiol* 301: R1078–R1087, 2011. First published July 20, 2011; doi:10.1152/ajpregu.00285.2011.—Endurance and strength training are established as distinct exercise modalities, increasing either mitochondrial density or myofibrillar units. Recent research, however, suggests that mitochondrial biogenesis is stimulated by both training modalities. To test the training “specificity” hypothesis, mitochondrial respiration was studied in permeabilized muscle fibers from 25 sedentary adults after endurance (ET) or strength training (ST) in normoxia or hypoxia [fraction of inspired oxygen ( $F_{iO_2}$ ) = 21% or 13.5%]. Biopsies were taken from the musculus vastus lateralis, and cycle-ergometric incremental maximum oxygen uptake ( $\dot{V}O_{2max}$ ) exercise tests were performed under normoxia, before and after the 10-wk training program. The main finding was a significant increase ( $P < 0.05$ ) of fatty acid oxidation capacity per muscle mass, after endurance and strength training under normoxia [2.6- and 2.4-fold for endurance training normoxia group (ET<sub>N</sub>) and strength training normoxia group (ST<sub>N</sub>);  $n = 8$  and 3] and hypoxia [2.0-fold for the endurance training hypoxia group (ET<sub>H</sub>) and strength training hypoxia group (ST<sub>H</sub>);  $n = 7$  and 7], and higher coupling control of oxidative phosphorylation. The enhanced lipid oxidative phosphorylation (OXPHOS) capacity was mainly (87%) due to qualitative mitochondrial changes increasing the relative capacity for fatty acid oxidation ( $P < 0.01$ ). Mitochondrial tissue-density contributed to a smaller extent (13%), reflected by the gain in muscle mass-specific respiratory capacity with a physiological substrate cocktail (glutamate, malate, succinate, and octanoylcarnitine). No significant increase was observed in mitochondrial DNA (mtDNA) content. Physiological OXPHOS capacity increased significantly in ET<sub>N</sub> ( $P < 0.01$ ), with the same trend in ET<sub>H</sub> and ST<sub>H</sub> ( $P < 0.1$ ). The limitation of flux by the phosphorylation system was diminished after training. Importantly, key mitochondrial adaptations were similar after endurance and strength training, regardless of normoxic or hypoxic exercise. The transition from a sedentary to an active lifestyle induced muscular changes of mitochondrial quality representative of mitochondrial health.

strength training; human skeletal muscle; permeabilized fibers; OXPHOS capacity; coupling control; fatty acid oxidation; high-resolution respirometry

DIMINISHED MITOCHONDRIAL COMPETENCE is a result of the sedentary lifestyle characteristic of modern and rapidly aging societies, with far-reaching consequences on health care, quality of life, and life expectancy (40). Mitochondria account for aerobic ATP production by oxidative phosphorylation. Consequently, mitochondrial performance is crucial for organ function, whereas mitochondrial dysfunction plays a major role in the development of degenerative diseases, including type 2 diabetes, dyslipidemia, and sarcopenia (20, 46, 47, 63). Aerobic exercise is known as an effective preventive measure to counteract these maladies (26, 36, 49). The predominant view on the effect of aerobic training relates to the increase of mitochondrial density. It is well established that these beneficial adaptations are achieved by endurance training (ET) (13). In contrast, it is widely held that strength training (ST) increases anaerobic power and muscle mass without specifically inducing mitochondrial biogenesis (58). ST improves endurance performance by increased leg strength (42, 58). The beneficial effect of ST, however, on aerobic performance remains controversial (7, 8, 28, 29). In our study, we show a qualitative shift toward intramuscular lipid oxidation by both ET and ST programs attended by healthy sedentary subjects.

Well-trained athletes can maximize their aerobic capacity only with an emphasis on high-volume training (38). A potentially beneficial effect of ST on mitochondrial biogenesis is unexpected on the basis of the “AMPK-Akt switch” hypothesis (1), suggesting mutually exclusive mechanisms mediating adaptations specific to ET vs. ST. Therefore, most investigations on exercise-induced stimulation of mitochondrial biogenesis focus on ET, whereas the effect of ST on stimulation of mitochondrial biogenesis has been little addressed in studies of sedentary subjects. A much higher compliance to training programs in patients with sedentary lifestyles and progressive obesity, however, might be achieved with time-efficient ST compared with conventional high-volume ET.

Recent molecular evidence indicates that the pathways activated in response to the distinct contractile stimuli of strength vs. endurance exercise show significant overlap (16, 44), in contrast to the “AMPK-Akt switch” hypothesis (14). Short-duration, high-intensity exercise training (11, 22) yields pronounced and beneficial mitochondrial adaptations in terms of an improved oxidative phenotype. Similar effects are reported with ST in nonathletic men and women, resulting in increased ergometric capacity [60+ years of age (3, 61)], and increased

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mtDNA copy number (chronic kidney patients; Ref. 3). To address the question of whether different training modalities elicit divergent or comparable adaptations of mitochondrial function in apparently healthy sedentary subjects, we measured mitochondrial respiratory control and capacity in muscle biopsies before and after 10 wk of ST or ET.

Hypoxia is used during exercise or at rest ("live high-train low") to induce changes in gene expression in skeletal muscle (62), or elicit specific hypoxic responses such as an increase in erythropoietin and red cell mass (41). Results on the role of hypoxia on mitochondrial function in response to ET are inconsistent and a matter of debate (2, 21, 27, 51, 57). Few studies address the impact on mitochondrial respiratory capacity of ST during hypoxia. Long-term exposure to hypoxia is undoubtedly detrimental to mitochondrial function (35). Whether or not short-term hypoxia or ischemia attenuates the effect of ET or ST remains elusive, strongly dependent on the tissue studied, the experimental design, and time of exposure.

We compared functional mitochondrial adaptations to endurance or strength training performed under normoxia or hypoxia. Mitochondrial performance was analyzed in permeabilized skeletal muscle fibers using high-resolution respirometry (for review, see Ref. 24). A substrate-uncoupler-inhibitor titration protocol was applied to assess qualitative and quantitative mitochondrial changes in response to these training protocols. Tissue mass-specific mitochondrial respiratory capacity (per muscle mass) increased when a physiological substrate-cocktail was applied, but fatty acid oxidation increased overproportionally in all groups. These results suggest that ST provides a relevant alternative to ET in sedentary subjects and patients in which compliance to or options for high-volume training are limited. After attending different training programs, the uniform sedentary group was transformed into a moderately active group representative of a diversity of exercise preferences in an active lifestyle. In clinical diagnosis, mitochondrial disease should primarily be evaluated with reference to an active control group representative of mitochondrial health (47).

## METHODS

**Subjects.** Twenty-five young sedentary volunteers participated in the present study (Table 1). After being informed about potential risks of the study, all subjects gave written informed consent. The study was approved by the ethics committee of the Medical University of Innsbruck (AN3433 271/4.12). The subjects were divided into four groups performing 1) endurance training under normoxia at a fraction of inspired oxygen ( $F_{I_{O_2}}$ ) of 21% (ET<sub>N</sub>), 2) ET under hypoxia at  $F_{I_{O_2}} = 13.5\%$  (ET<sub>H</sub>), corresponding to an altitude of ~4,000 m, 3) strength training under normoxia (ST<sub>N</sub>), and 4) ST under hypoxia (ST<sub>H</sub>; Table 2).

All groups performed three fully supervised exercise training sessions per week for 10 wk. To be included in the final analysis,

subjects had to complete 30 sessions within 10 wk. The subjects were assigned to one of the four groups stratified to account for similar baseline  $\dot{V}O_{2max}$  in the ET<sub>N</sub> and ET<sub>H</sub> groups, and similar isometric leg strength values estimated from an isometric leg test in the ST<sub>N</sub> and ST<sub>H</sub> groups. The high dropout rate in the ST<sub>N</sub> group of four (not due to study-related reasons) and consequently the small number of subjects ( $n = 3$ ) lead to a considerable statistical type II error in this group. Therefore, ST<sub>N</sub> was omitted from statistical testing, but the results are shown to indicate tendencies.

**Training.** ET and ST programs were designed following Chilibeck et al. (12, 13), who demonstrated significant training effects by using these programs. ET consisted of two weekly continuous sessions at 75% maximum heart rate ( $HR_{max}$ ) and one session of three sets of interval training at 95% of  $HR_{max}$  on a cycle ergometer (Ergobike Medical 8, Daum Electronics, Fuerth, Germany). The duration of the continuous sessions was increased from 35 to 55 min by 5-min steps after every second week. ET intervals consisted of 3 min of training and 2 min of recovery. The number of the 3-min intervals was increased from two during weeks 1–3, to three during weeks 4–7, to four intervals during weeks 8–10. Training in normoxia and hypoxia was matched by heart rate, reflecting the same relative intensity.

ST was performed three times a week at the same days as the ET. ST consisted of three different exercises for the legs, calf raise, leg curl, and leg extension. At the beginning of the training, weight was adjusted for the subjects to perform two sets with 12 repetitions at about 80% of their one repetition maximum (1-RM). Every 2 wk, the weight was adjusted to match the progress of the subject. The number of sets was increased incrementally from one to four. Before every training session, subjects performed a 10-min warm-up on an ergometer at 80–100 W. Also, one warm-up set was performed at every machine at 50% of the 1-RM.

Hypoxic training was performed in normobaric hypoxia (Hypoxic room systems; Hypoxico, Köln, Germany). The ambient conditions of the hypoxia room were monitored and controlled continuously to maintain  $F_{I_{O_2}}$  (13.5%) and a low concentration of  $CO_2$  (<0.1%) during the entire training session.

To account for the Hawthorne effect (subjects might modify aspects of their lifestyle in response to the fact that they are being part of a scientific study), subjects were instructed to continue their normal dietary and physical activity habits throughout the study. This was evaluated during the study with a detailed training diary and in a retrospective survey upon cessation of the study. According to this inquiry, subjects complied well with the specified aims.

**Aerobic capacity and isometric strength.**  $\dot{V}O_{2max}$  was assessed using a standardized incremental cycle ergometer (Ergoselect, Ergoline, Bitz, Germany) test to exhaustion. A warm-up of 4 min at 50 W was followed by steps of 25 W every minute until exhaustion. Isometric leg strength was assessed using a custom-made Isometric Leg Tester.

**Muscle biopsy sampling.** Muscle biopsy sampling from the musculus vastus lateralis was carried out using of a 5-mm needle (Dixons Surgical Instruments, Essex, UK) for percutaneous biopsy sampling (6) of muscle tissue optimized with a suction-enhancement technique. Samples were taken before and after the 10-wk training program. The muscle tissue was immediately transferred into ice-cold relaxing medium (BIOPS) containing 10 mmol/l  $Ca^{2+}$ /EGTA buffer, 20

Table 1. Subject characteristics at baseline

	Endurance training normoxia (ET <sub>N</sub> )	Endurance training hypoxia (ET <sub>H</sub> )	Strength training normoxia (ST <sub>N</sub> )	Strength training hypoxia (ST <sub>H</sub> )
Age, yr	27.8 ± 7.4	28.8 ± 7.0	24.2 ± 4.9	24.6 ± 4.0
$\dot{V}O_{2max}$ , ml·kg <sup>-1</sup> ·min <sup>-1</sup>	38.0 ± 7.7	41.5 ± 8.6	44.4 ± 7.0	41.9 ± 6.5
Height, cm	183.1 ± 7.2	181.8 ± 5.9	180.0 ± 5.9	181.3 ± 7.3
Weight, kg	91.10 ± 14.6	89.6 ± 21.4	75.7 ± 10.4	79.9 ± 13.0

All values are expressed as means ± SD.

Table 2. Respiratory flux, flux control ratios, FCR, coupling control ratios, mtDNA content in vastus lateralis,  $\dot{V}O_{2max}$ , and body mass index before and after endurance or strength training

	Pre/post	ET <sub>N</sub> pre/post	ET <sub>H</sub> pre/post	ST <sub>N</sub> pre/post	ST <sub>H</sub> pre/post
<i>n</i>	25	8	7	3	7
<i>J<sub>s</sub></i> : ETF <sub>L</sub> (OctM)	6.0 ± 2.0	7.3 ± 1.7	7.1 ± 1.8	8.0 ± 0.9	6.4 ± 2.0
	10.7 ± 2.9**	11.9 ± 2.7**	9.9 ± 1.9*	9.1 ± 0.8	10.9 ± 4.0*
<i>J<sub>s</sub></i> : ETF <sub>P</sub> (OctM)	13.0 ± 5.3	14.9 ± 5.9	12.4 ± 2.9	15.9 ± 11.3	10.1 ± 1.9
	28.6 ± 7.4**	33.0 ± 6.4**	25.7 ± 5.5**	30.8 ± 8.9	25.6 ± 8.0**
<i>J<sub>s</sub></i> : CI+II <sub>P</sub> (GMSOct)	76.8 ± 15.6	79.9 ± 15.8	75.0 ± 16.6	80.5 ± 3.6	73.6 ± 19.2
	86.3 ± 17.9**	107.1 ± 16.6*	94.3 ± 19.2#	101.1 ± 25.1	94.9 ± 26.8#
<i>J<sub>r</sub></i> : CI+II <sub>E</sub> (GMSOct)	89.9 ± 21.1	97.5 ± 20.5	84.7 ± 19.8	87.8 ± 5.8	87.3 ± 27.7
	103.5 ± 19.5**	112.9 ± 10.1*	99.8 ± 18.6	103.3 ± 19.7	96.7 ± 26.7
<i>FCR</i> : ETF <sub>L</sub> /CI+II <sub>E</sub>	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.09 ± 0.01	0.07 ± 0.01
	0.10 ± 0.02**	0.11 ± 0.03*	0.10 ± 0.02	0.09 ± 0.01	0.11 ± 0.02**
<i>FCR</i> : ETF <sub>P</sub> /CI+II <sub>E</sub>	0.15 ± 0.06	0.15 ± 0.05	0.15 ± 0.03	0.18 ± 0.13	0.12 ± 0.03
	0.28 ± 0.04**	0.29 ± 0.04**	0.26 ± 0.04**	0.30 ± 0.07	0.27 ± 0.04**
<i>FCR</i> : CI <sub>P</sub> /CI+II <sub>E</sub>	0.52 ± 0.07	0.50 ± 0.07	0.54 ± 0.06	0.53 ± 0.11	0.53 ± 0.07
	0.48 ± 0.05*	0.47 ± 0.05	0.49 ± 0.05	0.51 ± 0.01	0.48 ± 0.07
<i>FCR</i> : CII <sub>E</sub> /CI+II <sub>E</sub>	0.53 ± 0.07	0.51 ± 0.06	0.54 ± 0.05	0.55 ± 0.10	0.51 ± 0.10
	0.58 ± 0.05**	0.57 ± 0.06	0.56 ± 0.04	0.60 ± 0.08	0.59 ± 0.03
CI <sub>P</sub> /CII <sub>E</sub>	1.00 ± 0.12	0.98 ± 0.15	1.00 ± 0.14	0.96 ± 0.09	1.03 ± 0.09
	0.84 ± 0.11**	0.83 ± 0.13	0.87 ± 0.12	0.86 ± 0.11	0.81 ± 0.10**
<i>L/P</i> (ETF)	0.58 ± 0.16	0.52 ± 0.15	0.58 ± 0.13	0.64 ± 0.32	0.63 ± 0.13
	0.39 ± 0.10**	0.37 ± 0.09**	0.40 ± 0.09*	0.32 ± 0.11	0.42 ± 0.10**
<i>P/E</i> (CI+II)	0.85 ± 0.09	0.83 ± 0.11	0.89 ± 0.06	0.92 ± 0.09	0.86 ± 0.09
	0.96 ± 0.08**	0.95 ± 0.11*	0.94 ± 0.07	0.97 ± 0.10	0.98 ± 0.08#
mtDNA	34.8 ± 8.0	33.2 ± 10.4	34.6 ± 8.5	40.2 ± 5.2	34.4 ± 5.6
	36.0 ± 13.8	32.2 ± 10.7	31.4 ± 13.2	44.5 ± 9.3	41.3 ± 17.8
$\dot{V}O_{2max}$	40.8 ± 7.5	38.0 ± 7.7	41.5 ± 8.6	44.4 ± 8.0	41.9 ± 6.9
	44.0 ± 7.8**	41.7 ± 9.3*	45.0 ± 7.1	47.7 ± 9.7	44.0 ± 6.7*
BMI	25.6 ± 5.3	27.0 ± 4.3	26.9 ± 7.6	21.4 ± 3.9	23.9 ± 3.6
	24.6 ± 4.3	26.4 ± 4.0	25.2 ± 5.7	22.1 ± 4.1	22.7 ± 2.9

All values are expressed as means ± SD. Respiratory flux (*J*) is expressed in pmol O<sub>2</sub>·s<sup>-1</sup>·mg<sup>-1</sup> wet weight. Flux control ratios (*FCR*) are *J<sub>s</sub>* normalized to *J<sub>r</sub>*. LEAK/oxidative phosphorylation (OXPHOS) (*L/P*) and OXPHOS/electron transfer system (OXPHOS/ETS) (*P/E*) are coupling control ratios. Mitochondrial DNA (mtDNA) content is expressed in mitochondrial genome equivalents per milligram.  $\dot{V}O_{2max}$  is given in ml·min<sup>-1</sup>·kg<sup>-1</sup>. Body mass index (BMI) is given in kg/m<sup>2</sup>. Substrate states are ETF (octanoylcarnitine+malate, OctM), CI (glutamate+malate), CI+II (glutamate, malate, succinate, and octanoylcarnitine, GMSOct), and CII (succinate+rotenone). Coupling states of LEAK respiration, OXPHOS and ETS capacity are indicated by subscripts *L*, *P*, *E*. Significant differences pre vs. post: \*\**P* < 0.01; \**P* < 0.05; #*P* < 0.1.

mmol/l imidazole, 50 mmol/l K<sup>+</sup>-4-morpholinoethanesulfonic acid (Mes), 0.5 mmol/l dithiothreitol, 6.56 mmol/l MgCl<sub>2</sub>, 5.77 mmol/l ATP; 15 mmol/l phosphocreatine at pH 7.1 (60), or transferred to liquid nitrogen for mtDNA analysis.

**Muscle fiber preparation.** Subsamples of 5 mg wet weight (*W<sub>w</sub>*) were transferred into BIOPS onto a small petri dish on an ice-cold metal plate and separated using forceps with sharp tips. To ensure complete permeabilization the fibers were incubated by gentle agitation at 4°C in BIOPS solution containing 50 µg/ml saponin for 30 min. Fibers were washed for 10 min at 4°C in ice-cold mitochondrial respiration medium [MiR06; 0.5 mM EGTA, 3 mM MgCl<sub>2</sub>, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH<sub>2</sub>PO<sub>4</sub>, 20 mM HEPES, 110 mM sucrose and 1 g/l BSA essentially fatty acid free, adjusted to pH 7.1 (25), 2,800 units/mg solid catalase lypophilized powder], and wet weight of the fibers was measured on a microbalance (Mettler Toledo, Greifensee, Switzerland). With this method, the cholesterol-rich plasma membrane is selectively permeabilized, leaving intracellular membrane structures, such as mitochondria intact, and the entire mitochondrial population can be studied in the muscle sample (4).

**High-resolution respirometry.** Two to three milligrams *W<sub>w</sub>* were used per respirometer chamber (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria) containing 2 ml MiR06 at 37°C. Oxygen concentration (µM = nmol/ml) and oxygen flux (pmol·s<sup>-1</sup>·mg<sup>-1</sup>; negative time derivative of oxygen concentration, divided by muscle mass per volume) were recorded using DatLab software (Oroboros Instruments). The Oxygraph-2k is a two-chamber titration-injection respirometer with a limit of detection of volume-specific oxygen flux of 1 pmol·s<sup>-1</sup>·ml<sup>-1</sup> (25). Four instruments were operated in parallel. Instrumental background oxygen flux was corrected online, account-

ing for sensor oxygen consumption and oxygen diffusion between the medium and the chamber boundaries. The oxygen concentration in the chamber was maintained between 250 and 400 µM to avoid oxygen limitation of fiber respiration. Intermittent reoxygenations were achieved by injecting 200 mM hydrogen peroxide solution into the medium containing high catalase activity (48).

In the substrate-uncoupler-inhibitor titration protocol (Fig. 1), the following substrates were added (final concentrations): Malate (2 mM) and octanoylcarnitine (0.2 mM) to support electron entry from fatty acid β-oxidation through electron-transferring flavoprotein (ETF) and Complex I (CI) to coenzyme Q (2). Higher octanoylcarnitine concentrations were without effect on respiration (9, 48, 52). In the absence of ADP and ATP, resting respiration mainly compensates for the proton leak (LEAK respiration, *L*). Active respiration was stimulated by ADP (2.5 mM; lipid oxidative phosphorylation (OXPHOS) capacity or state 3). Further addition of CI substrate glutamate (10 mM) yields reduced nicotinamide adenine dinucleotide (NADH), which feeds electrons into CI (NADH-ubiquinone oxidoreductase). Succinate (10 mM) was added to stimulate CI+II-linked respiration (where CI+II is convergent electron input through CI and CII simultaneously in state 3 at high but not saturating ADP concentration), providing convergent electron input into the Q-junction simultaneously through CI (NADH) and CII (succinate). This combination of substrates is required for reconstitution of tricarboxylic acid (TCA) cycle function and represents a substrate cocktail required to achieve physiological respiratory capacity (24). Adding another 2.5 mM ADP after glutamate or succinate resulted in ADP-saturated respiration (5 mM) to obtain CI or CI+II-linked OXPHOS capacity [CI<sub>P</sub> or CI+II<sub>P</sub>; higher concentrations of ADP did not

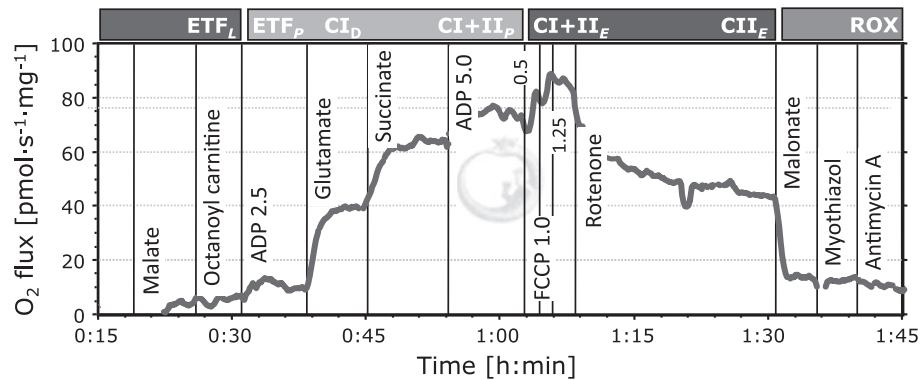


Fig. 1. High-resolution respirometry with permeabilized fibers from a biopsy of human vastus lateralis (2.8 mg wet weight; 37°C). Oxygen flux (pmol O<sub>2</sub>·s<sup>-1</sup>·mg<sup>-1</sup> wet weight) in the substrate-uncoupler-inhibitor titration protocol inducing coupling and substrate control states sequentially. ETF<sub>L</sub>, octanoylcarnitine and malate with convergent electron supply through electron transferring flavoprotein (ETF) and Complex I (CI); LEAK respiration, L, in the absence of ADP and ATP; ETF<sub>P</sub>, +2.5 mM ADP; fatty acid oxidative phosphorylation (OXPHOS) capacity at saturating [ADP], limitation of flux by electron input through ETF. CI<sub>D</sub>, +Glutamate, stimulating CI-linked flux such that ETF is not limiting; D: state 3 at high [ADP], which is not saturating at high flux. CI+II<sub>P</sub>, +Succinate, yielding a physiological CI+II substrate cocktail (P: 5 mM ADP supports CI+II OXPHOS capacity). CI+II<sub>E</sub>, FCCP titrations, ETS capacity at 1.25 μM. CII<sub>E</sub>, +Rotenone, after inhibition of CI, ETS capacity is supported by succinate (CII). ROX, residual oxygen consumption after addition ETS inhibitors (malonate for CII; myothiazol and antimycin A for CIII).

stimulate respiration further (48)]. Cytochrome *c* (10 μM) was added to test the integrity of the outer mitochondrial membrane (39). The increase of flux with cytochrome *c* was on average  $3.3 \pm 0.4\%$ . Electron transfer system capacity (CI+II<sub>E</sub>) was reached by stepwise addition of the uncoupler carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) at 0.025-μM steps. Optimum FCCP concentrations for maximum flux were 1.25 to 2 μM. The addition of rotenone (0.5 μM; inhibitor of CI) induced a slow decline of respiration to succinate-supported ETS capacity [CII<sub>E</sub>, where *E* denotes electron transfer system capacity (ETS)]. Sequential addition of malonic acid and antimycin A (inhibitors of CII and CIII; 5 mM and 2.5 μM, respectively) inhibited flux, but full inhibition to the level of residual oxygen consumption (ROX) was not reached in the present time-limited experimental regime (Fig. 1), hence correction of fluxes for ROX was not justified.

**Quantitative mtDNA analysis.** Approximately 7 mg of biopsy material was used for mtDNA quantification. After 3 h of proteinase K treatment, DNA from the muscle tissue was extracted in 50 μl Tris-EDTA buffer using a commercially available kit (Qiagen EZ1 DNA investigator kit). The sample was diluted 1:100, and 143 bp mtDNA was quantified, as reported previously (45).

**Statistical analysis.** Statistics were performed using PASW Statistics 18, Release Version 18.0.0 (SPSS, 2009, Chicago, IL). Between-group effects (strength-endurance and normoxia-hypoxia) were evaluated by a repeated-measures ANOVA; pre- and postconditions were analyzed using a paired-samples *t*-test and a nonparametric test (Wilcoxon rank test) if data were not normally distributed. Averages were obtained from the two respirometric replicates for each biopsy. Results for each training group are shown as mean ± SD. The significance level was set at  $P < 0.01$ ,  $P < 0.05$ , and values of  $P < 0.1$  were considered as trends. Because this is an explorative study, no correction for multiple testing was applied.

## RESULTS

### Fatty Acid Oxidation Capacity

The main finding of the present study was an increase in fatty acid OXPHOS capacity in muscle as a result of both endurance or strength training (Fig. 2A). Muscle mass-specific flux with octanoylcarnitine and malate (ETF<sub>P</sub>) was  $13.0 \pm 5.3$  pmol·s<sup>-1</sup>·mg<sup>-1</sup> in the sedentary controls ( $n = 25$ ), increasing 2.0- to 2.6-fold ( $P < 0.01$ ) after training (Table 2). The

increase of lipid OXPHOS capacity in the hypoxic groups was less pronounced than for normoxic training ( $P < 0.05$ ).

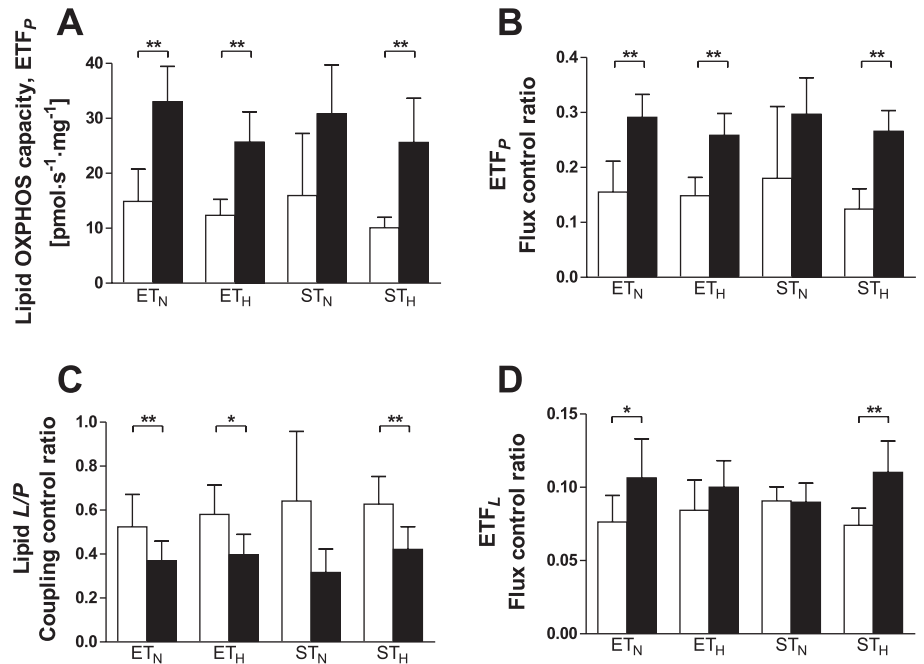
When normalizing lipid oxidation capacity for the internal reference state of physiological ETS capacity (CI+II<sub>E</sub>), the resulting flux control ratios, *FCR*, reveal important changes in mitochondrial quality (Fig. 2B). The normalized lipid oxidation capacity was significantly increased from  $0.15 \pm 0.06$  in the untrained state to  $0.29 \pm 0.04$  (ET<sub>N</sub>),  $0.26 \pm 0.04$  (ET<sub>H</sub>), and  $0.27 \pm 0.04$  (ST<sub>H</sub>; Table 2).

LEAK respiration as a fraction of OXPHOS capacity declined after training (*L/P*; Fig. 2C). The respiratory acceptor control ratio ( $RCR = P/L$ ) is an index of coupling control at a specific substrate supply. Before training, the RCR was  $1.9 \pm 0.6$  for lipid oxidation (Fig. 2C). This value is low due to the low OXPHOS capacity supported by fatty acid oxidation. After training, the RCR increased to  $2.8 \pm 0.7$  (ET<sub>N</sub>,  $P < 0.01$ ),  $2.7 \pm 0.8$  (ET<sub>H</sub>,  $P < 0.05$ ),  $3.4 \pm 1.0$  (ST<sub>N</sub>,  $P < 0.05$ ), and  $2.5 \pm 0.7$  (ST<sub>H</sub>,  $P < 0.01$ ). The dominant effect of lipid oxidation capacity on the RCR is shown in Fig. 2D. Despite the increased RCR, muscle mass-specific LEAK respiration, ETF<sub>L</sub>, increased after training to a larger extent than physiological ETS capacity (CI+II), resulting in an enhanced ETF<sub>L</sub>/CI+II<sub>E</sub> flux control ratio (significant for ET<sub>N</sub> and ST<sub>H</sub>; Fig. 2D).

**Ergometric performance, OXPHOS capacity, and mitochondrial density.**  $\dot{V}O_{2max}$  increased overall from  $40.8 \pm 7.5$  in untrained subjects to  $44.0 \pm 7.8$  ml·min<sup>-1</sup>·kg<sup>-1</sup> after training ( $P < 0.01$ ). Subgroup analyses showed a significant increase for ET<sub>N</sub> ( $P < 0.05$ ) and ST<sub>H</sub> ( $P < 0.01$ ; Table 2).  $\dot{V}O_{2max}$  correlated with skeletal muscle OXPHOS capacity (CI+II<sub>P</sub>) for the merged endurance groups ( $r = 0.49$ ,  $P < 0.01$ ; Fig. 3A) and strength groups ( $r = 0.49$ ,  $P < 0.05$ ; Fig. 3B). To investigate a possible effect of variation in BMI (higher scope of weight loss at higher BMI), we reanalyzed the data after excluding the five subjects with the highest BMI, resulting in matched body mass of subjects at baseline (compare Table 2). This was without effect on the results.

The physiological OXPHOS capacity (CI+II substrate cocktail; Fig. 1) was consistently increased by training by a factor of 1.3 from  $76.8 \pm 15.6$  to  $86.3 \pm 17.9$  pmol·s<sup>-1</sup>·mg<sup>-1</sup>,

Fig. 2. Enhancement of fatty acid oxidative capacity and mitochondrial coupling control after endurance or strength training under normoxia or hypoxia. **A:** increased muscle mass-specific lipid OXPHOS capacity,  $ETF_P$  ( $\text{pmol O}_2 \cdot \text{s}^{-1} \cdot \text{mg}^{-1}$ ), with octanoylcarnitine and malate. **B:**  $ETF_P$  flux control ratio, normalized for physiological ETS capacity ( $ETF_P/CI+II_E$ ) shows the significance of qualitative change for improved lipid OXPHOS capacity. **C:** decreased LEAK control ratio,  $L/P$  (inverse RCR) with octanoylcarnitine and malate indicates a tighter coupling control between oxidation and phosphorylation in response to exercise training. **D:**  $ETF_L$  flux control ratio, normalized for physiological ETS capacity ( $ETF_L/CI+II_E$ ), integrates the effects of the dominant substrate control (B) and coupling control (C). Open bars denote pre training, while solid bars denote posttraining.  $ET_N$ , endurance training normoxia;  $ET_H$ , endurance training hypoxia;  $ST_N$ , strength training normoxia;  $ST_H$ , strength training hypoxia.  $**P < 0.01$ ;  $*P < 0.05$ . Statistical tests were omitted for  $ST_N$  ( $n = 3$ ).



reaching significance in the  $ET_N$  group (Fig. 4A). Analysis of the  $P/E$  coupling control ratio ( $CI+II$ ) revealed another aspect of qualitative changes of mitochondria in response to training, increasing from  $0.85 \pm 0.09$  to  $0.96 \pm 0.08$  (Table 2; Fig. 4B).

Physiological ETS capacity increased after training 1.15-fold, whereas mtDNA content was increased only 1.03-fold, without reaching significance (Table 2). The correspondingly narrow range of mitochondrial content, therefore, renders detection of a significant correlation between ETS capacity and mtDNA difficult (Fig. 4, C and D).

**Diagnosis of qualitative and quantitative changes of mitochondrial function.** Training resulted in quantitative and qualitative alterations of mitochondrial respiratory function in skeletal muscle (Figs. 2 to 4). We addressed the question, therefore, to which extent the increase in tissue mass-specific lipid OXPHOS capacity (oxygen flux per unit mus-

cle mass, Fig. 2A) was elicited by a qualitative shift of respiratory control vs. a general increase of mitochondrial density (quantity of mitochondria; mitochondrial biogenesis, degradation, or dilution). Flux control ratios,  $FCR$ , remain invariant and independent of mt-density if training yields merely more of the same mitochondria.  $FCR$  are independent of measurements of tissue mass, protein content, or other reference values, if derived from a substrate-uncoupler-inhibitor titration with sequential activation of different metabolic states within an experimental run (Fig. 1). Thus, we take advantage of the statistical power provided by normalization of respiration for an “internal” functional marker. The  $FCR$  has an upper and lower limit of 0.0 and 1.0 if the respiratory reference state (reference flux,  $J_r$ ) is chosen to represent maximum activity (24) (physiological ETS capacity,  $CI+II_E$ ; Fig. 2B).

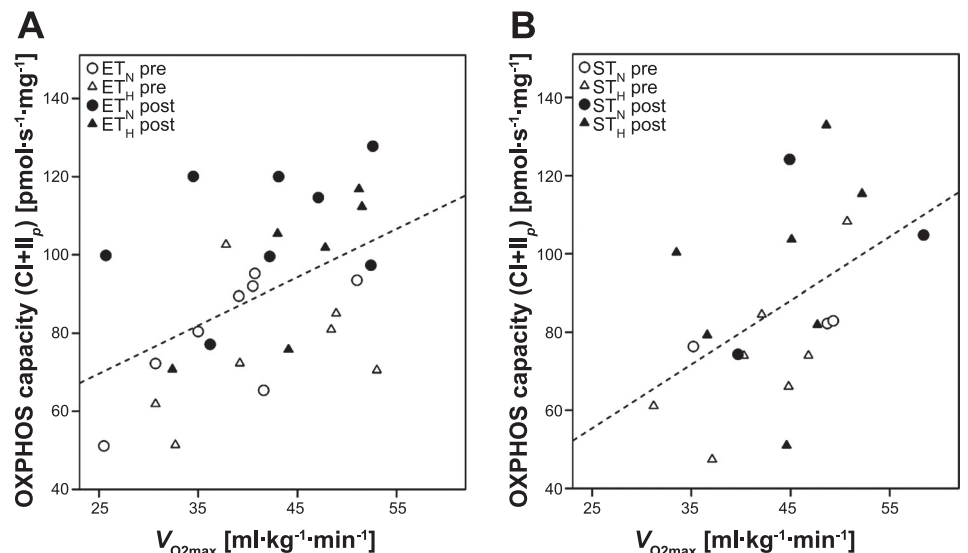


Fig. 3. Relation between skeletal muscle OXPHOS capacity,  $CI+II_p$  ( $\text{pmol O}_2 \cdot \text{s}^{-1} \cdot \text{mg}^{-1}$ ), and whole body oxygen uptake,  $\dot{V}O_{2\max}$  ( $\text{ml O}_2 \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ). Mitochondrial OXPHOS capacity was significantly correlated with  $\dot{V}O_{2\max}$  both in the combined endurance training groups (A:  $r = 0.49$ ,  $P < 0.01$ ) and strength training groups (B:  $r = 0.49$ ,  $P < 0.05$ ).

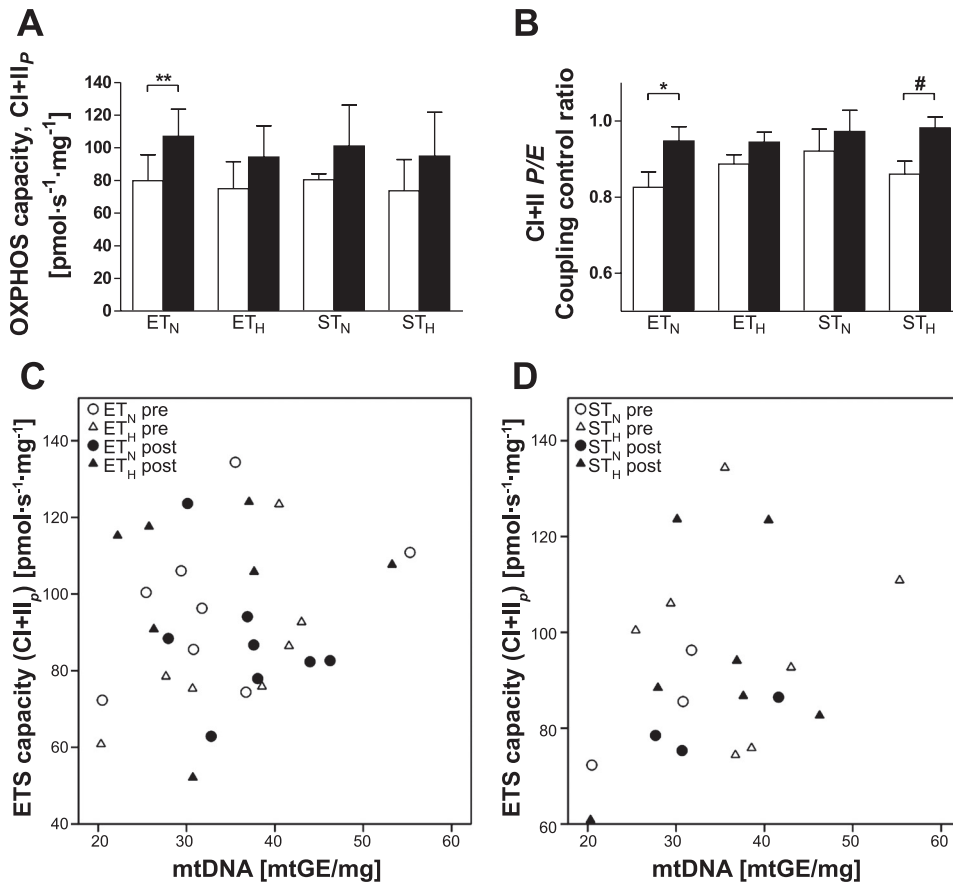


Fig. 4. OXPHOS capacity, ETS capacity, and mtDNA content as a function of training. *A*: muscle mass-specific OXPHOS capacity,  $CI+II_p$  ( $\text{pmol O}_2 \cdot \text{s}^{-1} \cdot \text{mg}^{-1}$ ), with a physiological substrate cocktail at saturating ADP concentration increased consistently in all groups ( $P < 0.01$  in  $ET_N$ ). *B*: phosphorylation system control ratio,  $P/E$ , increased in all groups ( $P < 0.05$  in  $ET_N$ ), contributing to the increase of OXPHOS capacity due to a slightly higher effect on OXPHOS compared with ETS capacity, indicating a decreased limitation by the phosphorylation system after training. *C* and *D*: muscle mass-specific ETS capacity,  $CI+II_E$  ( $\text{pmol O}_2 \cdot \text{s}^{-1} \cdot \text{mg}^{-1}$ ) plotted as a function of mtDNA content of the tissue both in the combined endurance training groups ( $C$ :  $r = 0.31$ ,  $P = 0.09$ ) and strength training groups ( $D$ :  $r = 0.39$ ,  $P = 0.09$ ). See Fig. 2 for abbreviations. \* $P < 0.05$ , \*\* $P < 0.01$ ; # $P < 0.01$  for values that were considered trends.

$J_r$  integrates system-relevant and proportional mitochondrial elements as far as possible to provide a functional marker of mitochondrial quantity. The CI-linked OXPHOS capacity was an invariant proportion of  $J_r$  ( $CI+II_E$ ; Fig. 5A). Similarly, the  $CII_E$  flux control ratio showed no significant changes irrespective of training modality (Fig. 5B). Consistent with collectively higher  $CII_E$  flux control ratios after training (Table 2), there was a decline of the  $CI_p/CII_E$  flux ratio (reaching significance in the  $ST_H$  group, Fig. 5C). Considering the shift in the  $P/E$  ratio (Fig. 4B) and the minor variations of CI- and CII-flux control ratios, the tissue mass-specific physiological ETS capacity ( $CI+II_E$ ) qualifies as a reference flux.  $J_r$  is, therefore, a functional marker of mitochondrial density, with largely conserved functional units for CI-, CII-, and CI+II-related electron input as part of the electron transfer system.

$J_s$  is the tissue mass-specific flux in a specified respiratory state,  $s$  (e.g., fatty acid OXPHOS capacity with octanoylcarnitine + malate,  $ETF_p$ ). The corresponding  $FCR$  is  $j_s = J_s/J_r$  (Fig. 2B). If pre- and posttraining conditions (labeled 1 and 2) differ exclusively in mitochondrial content, then  $J_{s1}/J_{s2} = J_{r1}/J_{r2}$ , reflecting the mt-density ratio before and after training, and  $j_{s1} = j_{s2}$ , or  $j_{s1}/j_{s2} = 1$ . Then, the qualitative effect,  $y_{\text{qual}}$ , is zero, as expressed by

$$y_{\text{qual}} = \frac{1 - j_{s1}/j_{s2}}{1 - J_{s1}/J_{s2}} \quad (1)$$

The difference between  $j_{s2}$  and  $j_{s1}$  provides a sufficient proof for a qualitative modification of mitochondria in the tissue ( $y_{\text{qual}} > 0$ ). Invariant  $J_s$  is excluded from the analysis ( $y_{\text{qual}}$

would be undefined, Eq. 1). But preserved  $J_r$  defines the opposite extreme when mitochondrial density remains constant, the ratios  $j_{s1}/j_{s2}$  and  $J_{s1}/J_{s2}$  are equal, hence  $y_{\text{qual}} = 1$  (Eq. 1) and  $y_{\text{dens}} = 0$  (Eq. 2),

$$y_{\text{dens}} = \frac{j_{s1}/j_{s2} - J_{s1}/J_{s2}}{1 - J_{s1}/J_{s2}} \quad (2)$$

The rationale (Eqs. 1 and 2;  $y_{\text{dens}} + y_{\text{qual}} = 1$ ) is simply explained by deriving another form of Eq. 2. From Table 2,  $J_{s1}$  and  $J_{s2}$  ( $ETF_p$ ) are 13.0 and 28.6  $\text{pmol} \cdot \text{s}^{-1} \cdot \text{mg}^{-1}$ , indicating a 2.2-fold increase of fatty-acid specific flux. In contrast,  $CI+II_E$  increased from  $J_{r1}$  of 89.9 to  $J_{r2}$  of 103.5  $\text{pmol} \cdot \text{s}^{-1} \cdot \text{mg}^{-1}$ , by a small but significant factor ( $J_{r2}/J_{r1}$ ) of only 1.15.  $J_{s2,\text{dens}}$  is the partial density-dependent magnitude of  $J_{s2}$ , and equals  $J_{s1} \cdot J_{r2}/J_{r1}$ . The density-dependent effect,  $y_{\text{dens}}$ , is then obtained by dividing the partial density-dependent change,  $J_{s2,\text{dens}} - J_{s1}$ , by the total change, which yields Eq. 2 in merely a different form

$$y_{\text{dens}} = \frac{J_{s1} \cdot (J_{r2}/J_{r1} - 1)}{J_{s2} - J_{s1}} \quad (3)$$

This analogous form of Eq. 2 can also be used for the quality-dependent effect:

$$y_{\text{qual}} = \frac{J_{s2} - J_{s1} \cdot (J_{r2}/J_{r1})}{J_{s2} - J_{s1}} \quad (4)$$

Inserting the numerical results for fatty acid OXPHOS capacity (Table 2),  $y_{\text{qual}}$  and  $y_{\text{dens}}$  were 0.87 and 0.13, respectively, revealing the predominant importance of qualitative mitochon-

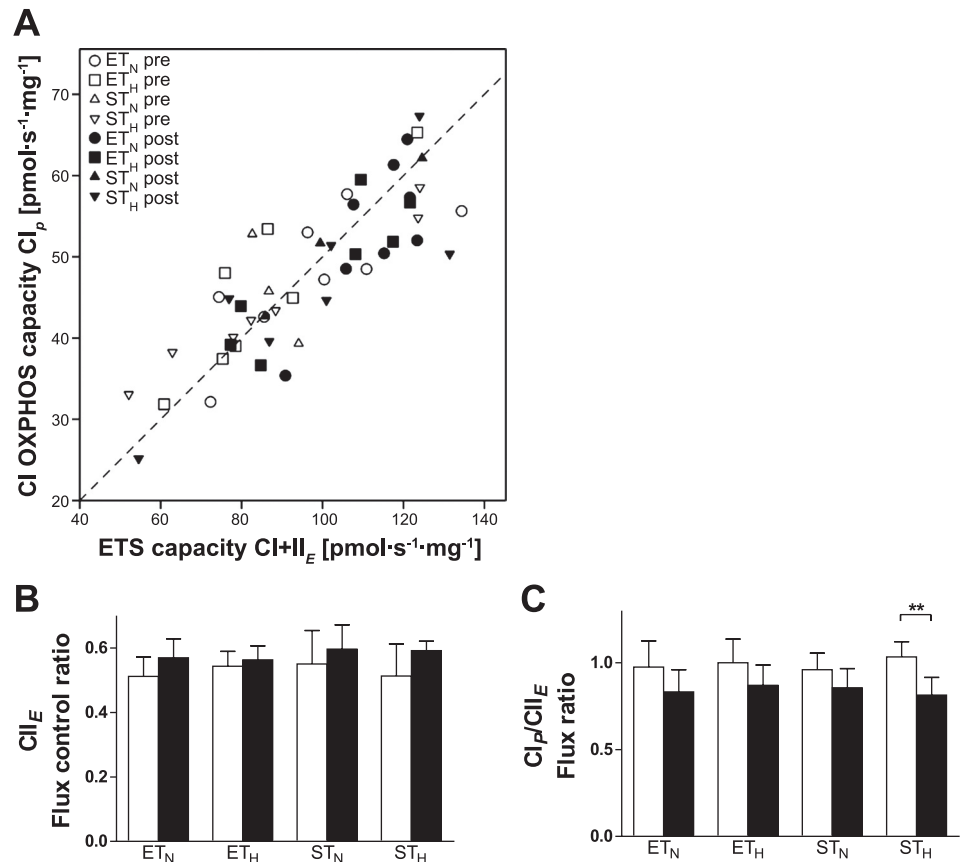


Fig. 5. Constant proportions of CI- and CII-linked respiratory capacities. **A:** OXPHOS capacity,  $CI_P$  ( $\text{pmol O}_2 \cdot \text{s}^{-1} \cdot \text{mg}^{-1}$ ) (glutamate, malate, and octanoylcarnitine, saturating [ADP]), varied in direct proportion to  $CI+II_E$  ( $\text{pmol O}_2 \cdot \text{s}^{-1} \cdot \text{mg}^{-1}$ ), resulting in a preserved  $CI_P$  flux control ratio in all groups. Dashed line represents total fit,  $r = 0.83$ . **B:** preserved  $CI_{II_E}$  flux control ratio (succinate+rotenone; normalized for  $CI+II_E$ ) in all groups. **C:** significant difference in the  $CI_P/CI_{II_E}$  flux ratio was only found in the  $ST_H$  group ( $P < 0.01$ ).  $***P < 0.01$ . See Fig. 2 for abbreviations.

drial change for enhanced fatty acid oxidation capacity after training.

## DISCUSSION

An increase of mitochondrial density in mammalian skeletal muscle is considered to be the only evolutionary and acclimatory response to an active lifestyle and training (64). Apart from changes in mitochondrial content, however, the plasticity of mitochondrial functional properties is well documented by enzymatic and respirometric analyses of the effects of endurance training (19, 32, 67). Recently developed protocols for the analysis of mitochondrial respiration (48) reveal the importance of shifts in substrate and coupling control of oxidative phosphorylation. In the present study, an increase of mitochondrial density after 10-wk mild exercise training in sedentary subjects was indicated by increased muscle mass-specific ETS capacity (1.15-fold) measured with a cocktail of physiological substrates for carbohydrate and lipid metabolism. However, the lipid oxidation capacity was elevated by 2.2-fold, i.e., to a significantly higher extent than the physiological respiratory capacity. Taken together, these results demonstrate that the increase of lipid oxidation capacity was predominantly due to qualitative changes of the mitochondria as an adaptive response to exercise.

The fate of oxidation of medium-chain fatty acids (MCFA) in response to exercise is controversial. While Sidossis et al. (56) reported similar MCFA oxidation in endurance-trained and sedentary subjects when infusing labeled substrates, Jong-Yeon et al. (37) found enhanced MCFA oxidation in skeletal

muscle homogenates from endurance-trained vs. sedentary subjects. In our study, octanoylcarnitine was used as a substrate for fatty acid oxidation. In contrast to octanoate, octanoylcarnitine is transported across the mitochondrial membrane by carnitine palmytoyltransferase (CPT) but then enters the common beta-oxidation pathway (15, 17). Our results on increased MCFA oxidation may be due to enhanced transport into the mitochondria or increased beta-oxidation capacity. In any case, increased lipid oxidation capacity is a favorable condition and related to health benefits (10, 50).

The increase of muscle mass-specific lipid oxidation capacity was less pronounced after hypoxic compared with normoxic exercise but was similar after ET and ST. These two distinct training modalities resulted in similar mitochondrial adaptations. Substantial overlap in signaling pathways has raised doubts about the general applicability of the AMPK-Akt hypothesis (1). Burgomaster et al. (11) report similar increases in muscle oxidative capacity induced by 6 wk of low-volume "sprint" interval training and traditional high-volume ET. Comparable to ST, this high-intensity training comprises repeated brief sessions of intermittent exercise performed at a high workload. The AMPK-PGC-1 $\alpha$  pathway is activated after four 30 s all-out exercise bouts (22). Similar remodeling occurs after low-intensity endurance exercise training (37a). Furthermore, mtDNA copy number increases after a 12-wk classic strength training program targeted at 80% of the 1-RM in patients with chronic kidney disease (3). mtDNA copy number is a well-established marker for mitochondrial biogenesis, but it does not provide information on mitochondrial function.

mtDNA correlates with functional mitochondrial markers in patients with type 2 diabetes and controls, with muscle mass-specific mtDNA content, citrate synthase activity, and CI+II ETS capacity being higher by a factor of 1.25, 1.24, and 1.27, respectively, in healthy controls with a BMI of 28 compared with diabetic patients with a BMI of 32 (9). In our exercise study, training did not result in identical increases of mtDNA and CI+II ETS capacity, but our data do not exclude such a relationship due to the small increase of mitochondrial content. The present study extends the important findings of Balakrishnan et al. (3) by revealing an increased lipid oxidation capacity after conventional ST. Whereas improvement of the oxidative phenotype by ET has long been established, the present study clarifies that oxidative enhancement results from ST and high-intensity, low-volume interval training (22). These findings can be summarized by a generalized concept applying to sedentary subjects, that metabolic challenges independent of training modality trigger an effective stimulus for enhancing skeletal muscle oxidative capacity.

Tissue mass-specific OXPHOS capacity with octanoylcarnitine increased by 91% with training, whereas the corresponding LEAK respiration was elevated by only 44%. Therefore, coupling control of mitochondrial respiration increased in response to training, leading to an amplified dynamic range of mitochondrial lipid oxidation. This is expressed by an increased respiratory acceptor control ratio (RCR), which is the ratio between OXPHOS capacity ( $P$ ; state 3) and LEAK respiration ( $L$ ). Two mechanisms can explain this increase of RCR ( $P/L$ ) for fatty acid oxidation, a higher degree of coupling (decrease of  $L$ ) and higher OXPHOS capacity (increase of  $P$ ). Our results indicate a predominant effect of lipid oxidation capacity on coupling control. A training-induced increase of the RCR with CI-linked substrates has been interpreted in terms of higher coupling (59, 67). In contrast, mitochondrial uncoupling is reported in endurance trained vs. sedentary subjects, based on constant ATP synthesis but a 50% increased TCA cycle activity in muscle in vivo (5). Under these conditions, muscle mitochondrial density is expected to double in line with peak oxygen consumption. Assuming that most mitochondria under resting conditions function close to state 4 ( $L$ ), the LEAK respiration, and hence TCA cycle activity per unit of tissue mass, would nearly double as a result of increased mitochondrial density. Despite the increase of total (tissue mass-specific) proton leak, LEAK respiration normalized for mitochondrial content would remain constant (constant leakiness of the mitochondrial membrane) or even decline at improved mitochondrial coupling after training. The apparently controversial results of the effect of ET on coupling, therefore, can be explained by a simple concept taking into account a higher muscle mass-specific LEAK respiration at higher mitochondrial density.

The RCR (or  $L/P$  ratio) is a valid index of coupling control when OXPHOS capacity ( $P$ ) and ETS capacity ( $E$ ) are identical. This is the case for lipid oxidation, when the phosphorylation system is not limiting. OXPHOS capacity with octanoylcarnitine was only 12% to 30% of ETS capacity after the addition of CI+II substrates ( $FCR$   $ETF_P$ ; Table 2). At maximum physiological flux, the phosphorylation system exerts control over oxidative phosphorylation in human muscle tissue, as expressed by  $P/E$  ratios less than 1.0 (24). The physiological  $P/E$  ratio increased significantly in the  $ET_N$  group.

This indicates a higher increase in the capacity of the oxidative phosphorylation system than in the capacity for electron transfer. A possible mechanism is an increase in adenine nucleotide translocase (ANT), ATP synthase and inorganic phosphate transporter. ANT is increased after ET, however, the increase of ANT protein is not significantly higher than that of citrate synthase activity (19). Whether or not the 10-wk training effects described here are representative of a new steady state in an active lifestyle or represent a new acclimatization in the transition to a new steady state requires further investigation.

Because of a high apparent  $K_m$  for ADP (up to 0.5 mM) in permeabilized muscle fibers (55), high ADP concentrations of 5 mM are required to overcome diffusion limitations and limitations by tubulin-regulated conductance of the outer mitochondrial membrane (53) and to reach >90% kinetic saturation of flux. The increased maximal capacity for oxidative phosphorylation due to increased mitochondrial density in trained muscle results in a decreased rate of substrate flux per individual mitochondrion at any given tissue mass-specific rate of ATP hydrolysis. The adjustment of the rate of ATP synthesis to an increased demand enables the muscle to maintain an adequate ATP/ADP ratio in relation to increased energy demand. This leads to an increased muscle mass-specific mitochondrial ATP production rate at a given ADP concentration (23).

In general, hypoxia diminishes ATP production, in part, by lowering the activity of the electron transfer system by activating HIF-1. Because of a decreased electron transport, overproduction of ROS during hypoxia can be avoided (65). Bakkman et al. (2) found increased OXPHOS capacity in the normoxic group but no increase in the hypoxic group after 4 wk of training in a low-pressure chamber. In our study, we found a significant increase in ETS capacity only in the  $ET_N$  group. OXPHOS capacity was increased significantly in the  $ET_N$  group ( $P < 0.01$ ) with a trend in the  $ET_H$  and  $ST_H$  groups ( $P < 0.1$ ). Tendencies were similar in all groups, regardless of normoxia and hypoxia, except for the lipid oxidation capacity. This might be related to the shift of the well-established substrate preference, improving the yield of ATP per mole of oxygen consumed by using glucose in preference to fatty acids during hypoxic exposure (30, 31). The small effect of hypoxia might be due to the short time of hypoxic exposure (maximum of 3 h/wk) or due to the more potent stimulus resulting from exercise training in the sedentary subjects. When using the intermittent hypoxic approach (training in hypoxia), Vogt et al. (62) found similar systemic adaptations between the normoxic and hypoxic groups but changes at the molecular level in the muscle, such as HIF or myoglobin mRNA, occurred only in the hypoxic groups. No performance increase was observed in well-trained athletes when using 7 sessions of intermittent hypoxic exposure (18). When applying the supposedly more effective: "live high-train low" model (hypoxia during rest but training at or near sea level), performance increases by only ~0.5%, even in highly trained individuals (33, 34). Moreover, hypoxic training does not appear to be more advantageous for increasing muscle oxidative potential than equivalent normoxic training in sedentary human subjects.



### Perspectives and Significance

In conclusion, this investigation shows that 10 wk of exercise training can provoke a significant shift in skeletal muscle substrate utilization toward fatty acid oxidation. Unexpectedly, key mitochondrial adaptations were similar in the ET and ST group. This is of great interest for several reasons: especially in the elderly, quality of life can be decreased by the inability to perform many activities of daily living due to sarcopenia and muscle weakness (54). ST increases muscular strength and size and can be used successfully to significantly improve quality of life by improvement of glucose metabolism and muscle strength and functional mobility in elderly. ST could also be seen as a favorable training modality for our time-poor society. In contrast to traditional high-volume ET, ST classified as high-intensity/low-volume training seems to be a “time-efficient” strategy to improve skeletal muscle oxidative capacity. The World Health Organization’s recommendations on physical activity for health suggest a minimum of 150 min ET or at least 75 min of vigorous-intensity training per week (66). The diagnostic perspective gained from analysis of mitochondrial competence after exercise training challenges the definition of the control group (43): Are sedentary subjects healthy?

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