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The potential for mitochondrial fat oxidation in human skeletal muscle influences whole body fat oxidation during low-intensity exercise

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Sahlin K, Mogensen M, Bagger M, Fernström M, Pedersen PK. The potential for mitochondrial fat oxidation in human skeletal muscle influences whole body fat oxidation during low-intensity exercise. *Am J Physiol Endocrinol Metab* 292: E223–E230, 2007. First published August 22, 2006; doi:10.1152/ajpendo.00266.2006.—The purpose of this study was to investigate fatty acid (FA) oxidation in isolated mitochondrial vesicles (mit) and its relation to training status, fiber type composition, and whole body FA oxidation. Trained ($\dot{V}O_{2\,peak}$ 60.7 ± 1.6, n = 8) and untrained subjects (39.5 ± 2.0 ml·min^{-1}·kg^{-1}, n = 5) cycled at 40, 80, and 120 W, and whole body relative FA oxidation was assessed from respiratory exchange ratio (RER). Mit were isolated from muscle biopsies, and maximal ADP stimulated respiration was measured with carbohydrate-derived substrate [pyruvate + malate (Pyr)] and FA-derived substrate [palmitoyl-L-carnitine + malate (PC)]. Fiber type composition was determined from analysis of myosin heavy-chain (MHC) composition. The rate of mit oxidation was lower with PC than with Pyr, and the ratio between PC and Pyr oxidation (MFO) varied greatly between subjects (49–93%). MFO was significantly correlated to muscle fiber type distribution, i.e., %MHC I (r = 0.62, P = 0.03), but was not different between trained (62 ± 5%) and untrained subjects (72 ± 2%). MFO was correlated to RER during submaximal exercise at 80 (r = −0.62, P = 0.02) and 120 W (r = −0.71, P = 0.007) and interpolated 35% $\dot{V}O_{2\,peak}$ (r = −0.74, P = 0.004). ADP sensitivity of mit respiration was significantly higher with PC than with Pyr. It is concluded that MFO is influenced by fiber type composition but not by training status. The inverse correlation between RER and MFO implies that intrinsic mit characteristics are of importance for whole body FA oxidation during low-intensity exercise. The higher ADP sensitivity with PC than that with Pyr may influence fuel utilization at low rate of respiration.

oxidative phosphorylation; training

FATTY ACID (FA) OXIDATION contributes significantly to whole body energy turnover during low- to moderate-intensity exercise, and a high rate of FA oxidation is of importance for performance during prolonged exercise. Furthermore, both fuel utilization and fuel storage are abnormal in patients with type 2 diabetes (21) and obesity (23). Despite intensive research, the mechanisms that regulate the relative contribution of fat and carbohydrate (CHO) in substrate oxidation remain unclear.

FA oxidation is reduced, both in absolute and relative terms, at high exercise intensities, but there is no clear effect of exercise intensity on relative FA oxidation at low intensities [<50% of peak oxygen consumption ($\dot{V}O_{2\,peak}$)] (9, 50). Previous studies have shown that there are large interindividual variations in FA utilization both at rest and during exercise (14, 16), and there is evidence that FA oxidation is influenced by factors such as preceding diet, muscle glycogen content, exercise intensity, training status, training volume, and muscle fiber composition (14, 51). A recent cross-sectional study of 300 subjects showed that a mere 12% of the variation in FA oxidation after 4-h fast could be explained by differences in training status, physical activity level, and sex, and it was concluded that differences in FA utilization between subjects remain largely unexplained (51).

Control of FA oxidation may be exerted at each of a number of steps in the chain between delivery and metabolism to acetyl-CoA (27, 43). It has been known for many years that plasma levels of FA will influence FA oxidation (9). However, there is convincing evidence that part of the control resides beyond the delivery of FA, i.e., at the mitochondrial level. First, analysis of muscle biopsies taken after exercise demonstrate that the concentration of FA was higher at high than at moderate exercise intensities despite reduced rate of FA oxidation (22). Second, studies with labeled medium- (MCFA) and long-chain FA (LCFA) demonstrate that oxidation of LCFA is reduced at higher intensities, whereas that of MCFA increases (41). The inward transport of LCFA, but not of MCFA, is dependent upon the carnitine carrier system, and the results therefore suggest that mitochondrial transport is a limiting factor of LCFA oxidation. Recent findings indicate that LCFA translocase (FAT/CD36) is present in mitochondria in both rat (8) and human skeletal muscle (6, 19) and that the protein is important for FA oxidation (8, 19). Whole body FA oxidation correlated to mitochondrial FA oxidation, and it was suggested that increased mitochondrial FAT/CD36 protein is important for increasing whole body FA oxidation (19). In summation, several independent findings suggest that FA oxidation is controlled at the mitochondrial level. The recent finding that intrinsic mitochondrial characteristics may influence whole body FA oxidation is intriguing, but further studies are required to confirm this idea.

There is a clear fiber type difference in FA metabolism. Slow-twitch fibers (vs. fast-twitch fibers) have higher activities of enzymes involved in FA oxidation (5, 31) and higher content of stored triglycerides (12). Studies in rat muscle (24) demonstrate that FA oxidation is higher in homogenates of oxidative muscles than glycolytic muscles. Recently, we (26) demonstrated that, in mitochondria isolated from rat soleus (predominantly composed of slow-twitch fibers), the rate of FA

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oxidation was high and similar to that of CHO-derived substrates. However, in mitochondria from fast-twitch fibers, FA oxidation rate was only 58% of that with CHO-derived substrates (26). The results demonstrate that the higher rate of FA oxidation in slow-twitch muscles is not only due to a higher mitochondrial volume density but that inherent mitochondrial characteristics contribute. It is not known whether fiber type composition also influences mitochondrial FA oxidation rate in human skeletal muscle.

It is well known that FA oxidation during exercise is augmented by training (17, 18). This is normally explained by an increased mitochondrial volume and a concomitant increased activity of oxidative enzymes (18). Sidossis et al. (42) have shown that trained individuals (vs. untrained subjects) have an increased in vivo oxidation of LCFA but a similar oxidation rate of MCFA. Measurements in muscle homogenates demonstrated that trained subjects have a higher oxidation rate of both LCFA and MCFA (20). Training is also known to cause qualitative changes in mitochondrial respiration, such as decreased ADP sensitivity (47) and increased stimulatory effect of creatine (49, 55). Both of these changes may influence fuel utilization and thus FA oxidation in vivo. Only a few studies have investigated the effect of training on FA oxidation in mitochondrial vesicles (24, 27, 52). In rodents it has been shown that training increases FA oxidation rate when expressed both per weight of muscle (27) and per mitochondrial protein (24), demonstrating that both the quantity and the quality of mitochondria are of importance. However, the rate of FA oxidation in isolated mitochondria from human skeletal muscle appears not to be affected by endurance training when expressed per mitochondrial protein (52). Further studies are required to elucidate the effect of training on FA metabolism at the mitochondrial level.

The purpose of this study, therefore, was to investigate in humans *1*) whether the relative rate of mitochondrial FA oxidation (MFO) in vitro is related to training status and fiber type composition and *2*) whether the rate of whole body FA oxidation is influenced by MFO.

METHODS

Subjects. Eight trained and five untrained healthy male subjects participated in the study. Subjects agreed to participate in the experiment after having been informed of the purpose and potential risks involved. The project was approved by the local ethics committee at the Odense University Hospital (VF20040035) and conforms to the Declaration of Helsinki II. Inclusion criteria for the trained group were a $\dot{V}O_{2\,peak}$ above 55 ml $O_2 \cdot min^{-1} \cdot kg^{-1}$ and a high level of habitual physical activity. Inclusion criteria for the untrained group were a $\dot{V}O_{2\,peak}$ <45 ml $O_2 \cdot min^{-1} \cdot kg^{-1}$ and a low level of habitual physical activity. Subjects were instructed to refrain from exhaustive exercise, use of alcohol (24 h prior to tests), and use of coffee, tea, and tobacco (3 h prior to tests) and had a light meal containing cereal or bread products 2–5 h prior to the test. The experiment was designed primarily to investigate work efficiency and its relation to mitochondrial efficiency (25), and the main group consisted of 18 subjects (9 trained and 9 untrained). Subjects in the main group that were in a postprandial state (had been eating a meal <2 h before the test, $n = 3$) or were overnight fasted ($n = 2$) were excluded. The rationale for this is that nutritional state is known to influence fuel utilization during exercise, and it is important that involved subjects have similar pretest status.

Experimental protocol. $\dot{V}O_{2\,peak}$ was determined with a standardized graded cycle exercise test. During the test, oxygen uptake was measured in 15-s intervals with a mixing chamber online system (Oxycon Pro; Jaeger, Hoechberg, Germany). $\dot{V}O_{2\,peak}$ was defined as the highest value attained.

Subjects performed a submaximal exercise test (≥48 h after the $\dot{V}O_{2\,peak}$ test) at three different absolute work rates. After adjusting the ergometer, the subjects rested on the ergometer (3 min), performed loadless pedaling (5 min), and cycled at 40, 80, and 120 W (5 min at each work rate). Respiratory data ($\dot{V}O_2$ and $\dot{V}CO_2$ measured with Oxycon Pro) as well as heart rate (Polar Electro, Kempele, Finland) were measured at rest (while sitting on the bicycle) and continuously (every 15 s) during submaximal exercise. Steady state was reached within 3 min of exercise, and the average of values obtained between 3 and 4.5 min of exercise was used to calculate respiratory exchange ratio (RER) and FA oxidation (13). RER at 35% $\dot{V}O_{2\,peak}$ was obtained by interpolation. Coefficient of variation for RER, calculated from two different tests in the same subject, was 4.5, 3.8, and 3.8% at 40, 80, and 120 W, respectively.

Muscle biopsies and preparation of mitochondria. On a separate day, muscle biopsies were taken from m. vastus lateralis with a Bergström needle modified for suction. After local anesthesia (2–3 ml, 20 mg/ml; Carbocain, AstraZeneca, Sweden), insertions were made at one-third of the distance between patella and anterior superior iliac spine. One biopsy was taken from each leg, and a small part of each biopsy was used for determination of fiber type distribution. The remaining part of the muscle was pooled and divided into two parts. One part (10–15 mg) was rapidly frozen in liquid nitrogen and stored at −80°C for later determination of enzyme activities and uncoupling protein-3 (UCP3) protein content. The remaining part (100–250 mg) was weighed and used for mitochondrial isolation as described previously (48). Briefly, the muscle sample was finely cut with scissors, rinsed thoroughly with isolation medium (100 mM sucrose, 100 mM KCl, 50 mM Tris·HCl, 1 mM KH_2PO_4, 0.1 mM EGTA, and 0.2% BSA, pH 7.40), incubated for 2 min in 0.2 mg/ml bacterial protease (Subtilisin Carlsberg, EC 232-752-2, type VIII; Sigma Chemical), and homogenized for 2 min in a water-cooled glass homogenizer with a motor-driven (180 rpm) Teflon pestle (radial clearance 0.15 mm). After dilution with three volumes of protease-free isolation medium, the homogenate was centrifuged at 750 *g* (10 min) and the preserved supernatant centrifuged at 10,000 *g* (10 min). The pellet was washed and resuspended in a suspension medium (~0.5 μl/mg initial muscle) containing (in mmol/l) 225 mannitol, 75 sucrose, 10 Tris, 0.1 EDTA, and 0.2% BSA, pH 7.40. All of the above procedures were carried out at 0–4°C. Part of the mitochondrial suspension was used for assessment of respiratory function, and the remaining part was frozen in liquid nitrogen and stored at −80°C for later assay of citrate synthase (CS) activity.

Mitochondrial respiratory activity. Mitochondrial oxygen consumption was measured polarographically using a Clark-type electrode in a temperature-controlled (25°C) chamber (DW1 oxygraph; Hansatech Instruments, Norfolk, UK) equipped with magnetic stirring. The oxygraph medium contained (in mmol/l) 225 mannitol, 75 sucrose, 10 Tris, 10 KCl, 10 K_2HPO_4, 0.1 EDTA, and 0.8 $MgCl_2$, pH 7.0. Respiration was measured with a CHO-derived substrate containing pyruvate (5 mmol/l) and L-malate (2 mmol/l) (Pyr) or alternatively with a FA-derived substrate containing palmitoyl-L-carnitine (10 μmol/l) and L-malate (2 mmol/l) (PC). Preliminary tests showed that these concentrations were sufficient to ensure maximal rate of respiration. Both palmitoyl-L-carnitine and pyruvate were dissolved in 0.5% BSA. State 3 respiration was initiated by adding K-ADP dissolved in oxygraph medium (final concentration 0.3 mmol/l). Mitochondrial FA oxidation, measured as maximal ADP-stimulated respiration with PC, was expressed in relation to that with Pyr and termed MFO.

Mitochondrial ADP sensitivity. ADP sensitivity was determined as previously described (26). Briefly, after determination of the maximal

respiration (state 3) and respiration without ADP (state 4), the oxygen tension was regained and submaximal respiration initiated by low-rate ADP infusion. This was accomplished by the use of a microdialysis pump (CMA/Microdialysis CMA/102; CMA/Microdialysis, Solna, Sweden) with a pumping rate ranging from 0.1 to 20 μl/min. Three submaximal respiratory measurements (low, medium, and high rates) were performed. A sample (50–80 μl) was rapidly withdrawn from the chamber during ADP infusion, eliciting a respiration of ~50% of state 3. The sample was immediately mixed with 20 μl perchloric acid (2.5 M) and the exact sample volume determined from the increase in weight. Samples were centrifuged (16,100 *g*, 5 min), and the supernatant was neutralized with $KHCO_3$ and centrifuged again. The concentration of ADP was measured with HPLC assay and the apparent K_m for ADP calculated by assuming Michaelis-Menten kinetics.

Hydroxyacyl-CoA dehydrogenase and CS activity. The activities of hydroxyacyl-CoA dehydrogenase (HAD) and CS were determined in freeze-dried muscles samples. CS activity was also measured in the mitochondrial suspension buffer and used as a reference base for mitochondrial respiration.

Freeze-dried samples were trimmed free of blood and connective tissue, powdered, and homogenized in a Triton solution (containing in mmol/l: 50 Na_2HPO_4, 1 EDTA-Na_2, 0.05% Triton X-100, pH 7.0) using a glass-glass homogenizer. Homogenized muscles or dissolved mitochondria were assayed for CS activity spectrophotometrically at 25°C (3). Homogenized muscles were also assayed for HAD activity spectrophotometrically at 25°C (32).

Fiber type distribution. Myosin heavy-chain (MHC) composition was analyzed as previously described (11) and modified for humans (4). Briefly, muscle homogenate (80 μl) was mixed with 200 μl of sample buffer (10% glycerol, 5% 2-mercaptoethanol, 2.3% SDS, 62.5 mmol/l Tris, and 0.2% bromophenol blue at pH 6.8), boiled in water bath for 3 min, and loaded (10–40 μl) on an SDS-PAGE gel [8% polyacrylamide (100:1 acrylamid:bisacrylamid), 30% glycerol, 67.5 mmol/l Tris base, 0.4% SDS, and 0.1 mol/l glycine]. Gels were run at 80 V for ≥42 h at 4°C and MHC bands made visible by staining with Coomassie. The gels were scanned (Linoscan 1400 scanner; Heidelberg Instruments, Heidelberg, Germany) and MHC bands quantified densitometrically (Phoretix 1D, nonlinear; Phoretix, Newcastle, UK). MHC II was identified with Western blot using monoclonal antibody (Sigma M 4276) with the protocol Xcell IITM (Invitrogen, Carlsbad, CA). Due to technical reasons, MHC determination was not successful in one untrained subject.

UCP3 protein content. Determination of UCP3 protein content has been described in detail previously (46). Briefly, portions of freeze-dried muscle were homogenized in ice-cold lysis buffer containing (in mmol/l) 2 HEPES, 1 EDTA, 5 EGTA, 10 $MgCl_2$, 50 β-glycerophosphate, 1 Na_3VO_4, 2 DTT, 1% Triton X-100, 20 μg/ml leuptin, 50 μg/ml aprotinin, and 40 μg/ml PMSF, pH 7.40. The protein concentration was determined (BCA protein assay 23223 Pierc Cat. 1610737; Bio-Rad Laboratories, Hercules, CA). Homogenates were solubilized in Laemmli sample buffer (62.5 mmol/l Tris·HCl, 2% SDS, 25% glycerol, 0.01% bromphenol blue, and 5% β-mercaptoethanol) and denaturized by boiling. A constant amount of protein was added per lane (100 μg) on 12% polyacrylamide gels and separated by SDS-PAGE for 60 min at 135 V. The separated polypeptides were transferred to a PVDF membrane at 10 V for 60 min, and blocked in Tris-buffered saline (TBS) with 5% nonfat milk. Membranes were incubated overnight with polyclonal antibody against UCP3 (Chemicon AB3046), diluted 1:1,000, washed, and incubated with secondary antibody goat antirabbit (IgG-HRP, no. sc-2030; Santa Cruz Biotechnology). The membrane was again washed and incubated with the chemiluminescence detection reagent ECL, No. RPN 2106 (Amersham). Finally, an X-ray film was exposed to the membrane for 50 min. The optical density of the bands was quantified by using Molecular Analyst 1.5 (Bio-Rad). UCP3 protein content was made in doublets for every individual.

Statistics. Data given in the text and tables are presented as means ± SE. Differences between trained and untrained subjects were tested for statistical significance with Student's unpaired *t*-test or paired *t*-test when comparisons were made within the same subject. Correlation between two variables was tested with Pearson Correlation analysis. The difference in metabolic parameters between groups (training status) and conditions (40, 80, and 120 W) were tested for statistical significance with a two-way ANOVA with repeated measures. If a main difference was detected the location of significance was determined with post hoc test. Statistical significance was accepted at $P < 0.05$.

RESULTS

Subject characteristics and exercise parameters. Trained subjects had a significantly higher aerobic power both at the whole body level (54% higher $\dot{V}O_{2\,peak}$, $P < 0.001$; Table 1) and at the muscle level (30% higher CS activity, $P = 0.01$; and 41% higher HAD activity, $P = 0.04$). CS activity was significantly related to the proportion of type I fibers (%MHC I) in the trained group ($r = 0.84$, $P < 0.01$) but not in the untrained group or in the group as a whole (combined trained and untrained subjects). UCP3 protein (expressed per CS activity) was more than twofold higher in untrained subjects, whereas fiber type distribution, measured as %MHC I, was not significantly different between trained (range 22–57%) and untrained subjects (range 46–60%).

$\dot{V}O_2$ increased during exercise but was not different between the trained and the untrained group at any of the three submaximal work rates (Table 2). Exercise at the highest work rate (120 W) corresponded to relative intensities of 39 and 55% of $\dot{V}O_{2\,peak}$ for the trained and the untrained group, respectively. Ventilation increased during exercise, but the ventilatory

Table 1. *Subject characteristics*

	T ($n = 8$)	UT ($n = 5$)	Statistics (T vs. UT)
$\dot{V}O_{2\,peak}$, ml O_2·min^{-1}·kg^{-1}	60.7±1.6	39.5±2.5	$P < 0.0001$
Age, yr	25.4±0.9	23.2±0.9	NS
Height, cm	174±1	179±3	NS
Weight, kg	72.6±2.3	88.8±13.2	NS
%MHC I	44.9 (22–60.4)	49.3 (40–53.6) n=4	NS
CS, units·g dry wt^{-1}·min^{-1}	121±3	93±10	$P = 0.01$
HAD, units·g dry wt^{-1}·min^{-1}	116±7	82±16	$P = 0.04$
UCP3, arbitrary units/mU CS	1.8±0.4	4.1±0.9	$P = 0.02$

Values are means ± SE. $\dot{V}O_{2\,peak}$, peak oxygen consumption; T, trained; UT, untrained; MHC I, %myosin heavy chain isozyme I; CS, citrate synthase; HAD, hydroxyacyl-CoA dehydrogenase; UCP3, uncoupling protein-3; NS, not significant. UCP3 was expressed per mU CS, using CS activity as a marker of mitochondrial volume.

Table 2. *Cardiorespiratory and metabolic parameters during exercise*

	40 W	80 W	120 W
$\dot{V}O_2$, ml/min			
Trained	974±51	1,328±59†	1,698±49†‡
Untrained	1,062±78	1,397±68†	1,832±67†‡
$\dot{V}O_2$, %$\dot{V}O_{2\ peak}$			
Trained	22±0.6	30±0.7	39±1.1
Untrained	32±1.3*	42±3.1*	55±3.6*
$\dot{V}E$, l/min			
Trained	25.1±1.3	32.2±1.6†	40.3±1.6†‡
Untrained	26.3±1.4	34.2±1.3†	44.5±1.1†‡
$\dot{V}E/\dot{V}O_2$, l/l O_2			
Trained	25.8±0.6	24.2±0.7†	23.7±0.6†
Untrained	25.1±1.2	24.7±1.3	24.5±1.2
HR, bpm			
Trained	102±4	111±4	126±5
Untrained	114±5*	130±7*	147±6*
RER			
Trained	0.88±0.01	0.88±0.01	0.90±0.01‡
Untrained	0.83±0.02	0.85±0.02	0.90±0.02‡
Whole body FA oxidation, g/min			
Trained	0.19±0.02	0.27±0.04	0.28±0.04
Untrained	0.30±0.04	0.35±0.05	0.32±0.06

Values are means ± SE from 8 trained and 5 untrained subjects. HR, heart rate; RER, respiratory exchange ratio; FA, fatty acid. *Significantly different from trained subjects; †significantly different from that at 40 W; ‡significantly different from that at 80 W.

Table 3. *Mitochondrial respiratory parameters*

	T (n = 8)	UT (n = 5)	Statistics (T vs. UT)
Palmitoyl-L-carnitine			
State 3, mmol O_2·min^{-1}·kg m.$^{-1}$	1.5±0.1*	1.1±0.1*	P = 0.03
State 3, mmol O_2·min^{-1}·U CS^{-1}	52.6±2.4*	54.3±3.1*	NS
RCI	8.4±0.5*	8.5±0.2*	NS
P/O ratio	2.37±0.03*	2.30±0.08*	NS
Apparent K_m, μM ADP	15.5±3.4*	14.2±3.1*	NS
Pyruvate			
State 3, mmol O_2·min^{-1}·kg m.$^{-1}$	2.4±0.1	1.6±0.1	P < 0.001
State 3, nmol O_2·min^{-1}·U CS^{-1}	87.1±4.3	75.6±4.8	NS
RCI	13.4±1.0	12.1±1.3	NS
P/O ratio	2.47±0.03	2.49±0.09	NS
Apparent K_m, μM ADP	28.2±4.2	21.1±3.5	NS

Values are means ± SE. State 3 is the maximal ADP-stimulated respiration per kg muscle mass (m.) or per mitochondrial volume (U/CS). Respiratory control index (RCI) = state 3/state 4. P/O ratio is the amount of ADP phosphorylated to ATP per used oxygen atoms. Apparent K_m (μM ADP) is the amount of ADP required to increase respiration to 50% of state 3. *Significantly lower than with pyruvate.

equivalent ($\dot{V}E/\dot{V}O_2$) for oxygen remained constant at the three work rates. RER was not significantly different during exercise at 40 and 80 W but increased significantly at 120 W. Whole body fat oxidation (kJ/min) was not influenced by training status at the low work rates investigated in this study.

Mitochondrial characteristics. Mitochondrial yield, calculated as the fraction of muscle CS activity recovered in isolated mitochondria, averaged 15 and 16% for the trained and untrained subjects, respectively. Mitochondrial state 3 respiration was, when expressed per kilogram muscle mass, significantly higher in trained vs. untrained subjects with both PC (36% higher) and Pyr (50% higher) (Table 3). However, there was no difference between trained and untrained subjects when respiration was expressed per CS activity (i.e., marker of mitochondrial volume). Mitochondrial respiration was lower with PC than with Pyr, but there was no significant difference in MFO (relative FA oxidation) between trained (62 ± 5%) and untrained subjects (72 ± 2%). Both MFO and respiration expressed per CS activity are measures of mitochondrial quality, and the results from this study demonstrate that mitochondrial quality is not different between trained and untrained subjects. MFO showed a large intersubject variation (range 49–93%) and correlated significantly to fiber type composition, measured as %MHC 1 (r = 0.62, P = 0.03; Fig. 1), but not to HAD/CS activity (r = −0.33, P > 0.05) or to UCP3 protein [neither when expressed as arbitrary units per muscle protein (r = 0.30) or per CS activity (r = 0.28)].

Respiration with Pyr elicited, when compared with PC, higher values of P/O ratio (+6%), respiratory control index (+50%) and apparent K_m for ADP (+66%), but there was no effect of training status on these parameters (Table 3).

Whole body substrate utilization and mitochondrial quality. There was a significant correlation between relative whole body substrate oxidation (expressed as RER) and relative FA oxidation in isolated mitochondria (expressed as MFO). This was evident when RER was measured during exercise at 80 (r = −0.62, P = 0.024; Fig. 2*A*) and 120 W (r = −0.71, P = 0.006; Fig. 2*B*) and interpolated to 35% $\dot{V}O_{2\ peak}$ (r = −0.74, P = 0.004; Fig. 3). RER was not correlated to MFO at rest (r = −0.26, P = 0.43), during exercise at 40 W (r = −0.47, P = 0.1), or to muscle fiber type composition (%MHC I). The absolute rate of whole body fat oxidation (kJ/min) was not correlated with mitochondrial quality measured as MFO or PC oxidation.

DISCUSSION

The major finding in the present study was that whole body relative FA oxidation during low-intensity exercise was correlated to relative mitochondrial FA oxidation (MFO) determined in vitro. MFO was correlated to type I fiber composition

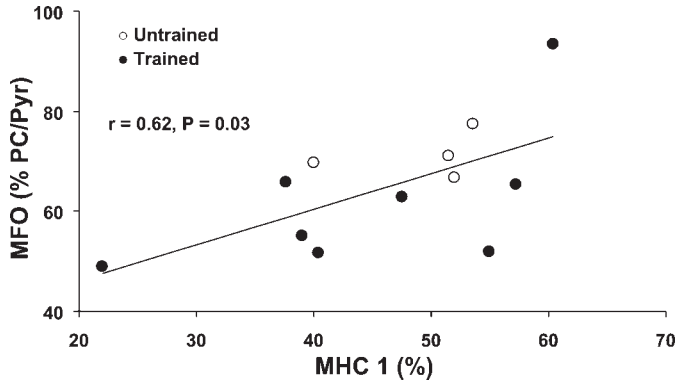

Fig. 1. Correlation between relative mitochondrial fat oxidation (MFO) and muscle fiber type composition [%myosin heavy chain isozyme I (MHC I)]. MFO was expressed as the ratio between state 3 respiration with palmitoyl-L-carnitine + malate (PC) and pyruvate + malate (Pyr; percentage of that with Pyr). Values are from 8 trained and 4 untrained subjects (r = 0.62, P = 0.03). Determination of MHC composition was not successful in 1 untrained subject due to technical reasons.

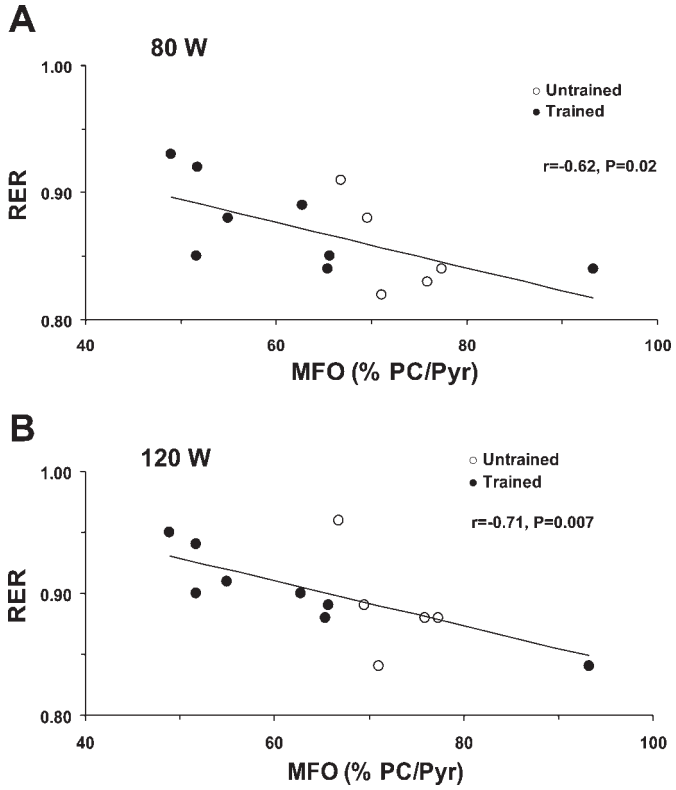

Fig. 2. Correlation between relative fat oxidation in vivo [respiratory exchange ratio (RER)] and that in mitochondria (MFO). MFO was expressed as the ratio between state 3 respiration with PC and Pyr (percentage of that with Pyr).

in skeletal muscle but was not different between trained and untrained subjects. Furthermore, the sensitivity of mitochondrial respiration to ADP was significantly higher for PC than for Pyr.

Whole body FA oxidation in relation to mitochondrial FA oxidation. A novel finding in the present study was that whole body relative FA oxidation was correlated with that in isolated mitochondria. The correlation was observed during exercise at 80 and 120 W and was even stronger when interpolated to the same relative intensity (i.e., 35% $\dot{V}O_{2\,max}$). The absence of a correlation at 40 W and at rest may be explained by the relatively low contribution of muscle respiration to whole body respiration at these low rates of energy expenditures.

The rationale for comparing RER with MFO is that RER is a measure of the relative rates of substrate utilization at the whole body level, and MFO is a measure of the relative substrate utilization at the mitochondrial level. MFO is related to mitochondrial quality and expresses the maximal mitochondrial respiratory rate with PC in relation to that with Pyr. MFO is measured in vitro during standardized conditions, whereas RER reflects substrate utilization in vivo during submaximal conditions and is thus influenced by the prevailing metabolic and physiological conditions. The absolute rate of whole body fat oxidation was not related to mitochondrial quality (MFO or PC oxidation) in this study. This may be due to the fact that absolute fat oxidation is strongly influenced by the metabolic rate, which varies between subjects due to differences in body weight and work efficiency. Preceding diet is known to influence fuel utilization during exercise (9, 28). In the present study, dietary conditions on the experimental days were similar between subjects, and subjects were instructed not to perform exhaustive exercise 24 h prior to the tests. However, previous studies have shown that differences in preexercise muscle glycogen markedly affect FA oxidation during exercise (36). The diet and training was not controlled beyond the day preceding the experiments, and the possibility that differences in muscle glycogen may have influenced RER cannot be excluded. The effect of diet and/or muscle glycogen stores on MFO is not known and may be a subject for future studies.

The present results suggest that mitochondrial quality may affect fuel utilization in vivo during low-intensity exercise. Our results are consistent with that of a recent study by Holloway et al. (19), where mitochondrial FA oxidation correlated with whole body fat oxidation (kJ/min) during exercise at 60% $\dot{V}O_{2\,max}$. There are important differences in experimental design between studies. In the study by Holloway et al., rate of mitochondrial FA oxidation was measured as oxidation of labeled palmitate in non-ADP stimulated mitochondria. In the present study, palmitoyl-L-carnitine oxidation was measured during maximal ADP stimulation, which is similar to the conditions prevailing during exercise. Despite the differences in experimental design, both studies suggest that mitochondrial characteristics of FA oxidation influence whole body FA oxidation during submaximal exercise.

Control of mitochondria FA oxidation. The rate of FA oxidation increases during exercise, in parallel with the increase in energy demand. FA oxidation reaches a peak at an intensity of 44–65% $\dot{V}O_{2\,max}$ (1, 2, 29) and decreases when the intensity is increased further. The reduction in FA oxidation at high exercise intensities can only in part be prevented by experimental elevation of plasma FA (37) and demonstrates that FA oxidation in addition to FA delivery is limited by intramuscular factors. Potential intramuscular sites of limitation include FA transport over sarcolemma (7), rate of intramuscular lipolysis (35), and inward transport and metabolism of FA within mitochondria (34, 43, 44). The accumulation of LCFA in the cytosol during intensive exercise despite reduced rates of FA oxidation (22) provides strong evidence that the mitochondrion is a major site of limitation.

As pointed out at the beginning of this article, several lines of evidence suggest that mitochondrial inward transport of LCFA is a limiting step in FA oxidation. Carnitine palmitoyl-

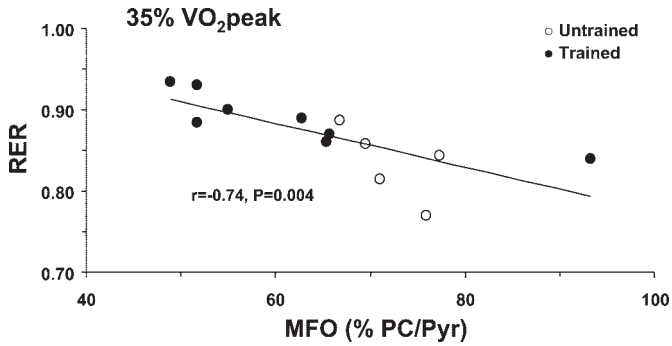

Fig. 3. Correlation between relative fat oxidation in vivo (RER) and that in mitochondria (MFO). MFO was expressed as the ratio between state 3 respiration with PC and Pyr (percentage of that with Pyr). RER at 35% of peak oxygen consumption ($\dot{V}O_{2\,peak}$) was obtained by interpolating RER measured at 40, 80, and 120 W to the work rate corresponding to 35% $\dot{V}O_{2\,peak}$.

transferase I (CPT I) is a key enzyme in carnitine-mediated transport of LCFA and is allosterically inhibited by malonyl-CoA (M-CoA). M-CoA remains unchanged in human skeletal muscle during exercise (30), but a decreased sensitivity of CPT I to M-CoA (19) may contribute to increased FA oxidation during exercise. However, FA oxidation was, in the present study, investigated with palmitoyl-L-carnitine, which bypasses CPT I. Our results suggest that at least part of the control of mitochondrial FA oxidation is located downstream of CPT I.

FAT/CD36 is a transmembrane protein with a suggested role in the cellular uptake of LCFA (54). Studies using immunofluorescence microscopy in human skeletal muscle demonstrate high FAT/CD36 expression in endothelial cells, weaker expression in sarcolemma, and no apparent staining in the interior of the muscle cells (54). In contrast, recent studies have presented evidence of abundant expression of FAT/CD36 protein in mitochondria isolated from both rat (8) and human skeletal muscle (6). The protein immunoprecipitated with CPT I and the mitochondrial fraction was shown not to be contaminated with membranes from sarcolemma or sarcoplasmic reticulum (8). When FAT/CD36 was inhibited there was an almost complete arrest of LCFA oxidation (6, 8), but not of MCFA oxidation, verifying a functional role of the protein in FA metabolism. Furthermore, studies in mitochondria isolated from human skeletal muscle showed that oxidation of PC was inhibited (6) when FAT/CD36 was blocked, which suggests that the protein is located downstream of CPT I. Although an attractive candidate for control of FA oxidation at the mitochondrial level, further studies are required to confirm the presence and function of FAT/CD36 in mitochondria.

It is well established that whole body fat oxidation has a lower maximal rate than that of carbohydrate. The reason(s) for the lower energetic power with fat is not clear but may include pathway interactions at the mitochondrial level, including amplification of the M-CoA inhibition of CPT I by acidosis (44) and limiting levels of carnitine (10, 38). The present study demonstrates that the maximal rate of FA oxidation is also lower than that of pyruvate at the mitochondrial level (on average 66%), which is consistent with previous studies (33, 52, 53). The lower rate of FA oxidation (vs. pyruvate oxidation) observed at the mitochondrial level cannot be explained by control of CPT I.

The intersubject variation in MFO was large (49–93%) and was correlated to the distribution of type I fibers. This is consistent with findings in rat skeletal muscle, where MFO averaged 95% in soleus (predominantly type I fibers) but only 58% in extensor digitorum longus (EDL) muscle (predominantly type II fibers) (26). The activity of β-oxidation (i.e., HAD activity) was much lower in EDL than in soleus, and it was suggested that the low rate of PC oxidation in EDL was related to limitation of β-oxidation (26). However, the present results demonstrate that MFO is not correlated with HAD activity or with HAD/CS ratio in human skeletal muscle, and the hypothesis that the capacity of β-oxidation limits mitochondrial PC oxidation thus appears unlikely.

Relative FA oxidation is high at low rates of energy flux and decreases at high exercise intensities. This has generally been explained by an inhibition of FA oxidation during high-intensity exercise (see *Control of mitochondria FA oxidation*). An additional explanation, which receives some support from this study, is that feedback activation of oxidative phosphorylation (OxPhos) by increases in ADP might be stronger during FA than during pyruvate oxidation. Increase in ADP is a major trigger of OxPhos and provides a direct link between energy demand and energy supply. The present study demonstrates that the sensitivity of OxPhos for ADP was higher (lower apparent K_m) for PC than for Pyr. Similar results have been obtained in mitochondria isolated from rat skeletal muscle (15, 26, 53). There is evidence that isolated mitochondria have much higher ADP sensitivity than those in permeabilized muscle fibers (39), and it has been argued that mitochondrial functional properties are changed during the isolation process (39). However, experiments in rat cardiac muscle (45) show that apparent K_m for ADP is also much higher for pyruvate (236 ± 24 μM) than for PC (36 ± 8 μM) in permeabilized fibers, thus supporting the present results in isolated mitochondria. The mechanism for the substrate difference in ADP sensitivity of OxPhos is not clear, but the physiological implication is that small increases in ADP (low energy demand) would preferentially stimulate FA oxidation.

Effect of training. It is well known that FA oxidation during exercise at moderate to high intensities is augmented by training (17, 18). This is normally explained by an increased mitochondrial volume and a concomitant increased activity of oxidative enzymes (18). In the present study we have investigated whether mitochondria from endurance-trained subjects have a higher potential for FA oxidation, when mitochondrial volume is controlled for. We could not find any differences in MFO between trained and untrained subjects, which is consistent with previous studies in human muscles. In a longitudinal training study, mitochondrial ATP production during FA oxidation increased after training when related to muscle weight but remained unchanged when related to mitochondrial protein (52). Similar findings were reported in a cross-sectional study, where palmitate oxidation per CS activity was similar in muscle homogenates from trained and untrained subjects (20). These studies in humans are in contrast to studies in rodents showing an increased mitochondrial ability to oxidize FA after endurance training (24, 27).

A suggested function of UCP3 protein is to facilitate outward transport of FA and thus protect mitochondria from lipotoxicity (40). In agreement with this hypothesis, Schrauwen et al. (40) have, in a series of studies, shown that UCP3 is increased during conditions of increased FA availability and/or during conditions of decreased mitochondrial FA oxidation. The present data (Table 1) show that UCP3 protein was more than twofold higher in mitochondria from untrained subjects. However, there was no correlation between UCP3 protein and MFO, indicating that the expression of UCP3 protein is not a simple reflection of low mitochondrial FA oxidation potential.

Physiological perspectives. Neither RER, measured during low-intensity exercise, nor MFO was different between trained and untrained subjects. This might appear contradictory to present ideas, since endurance training is known to augment FA oxidation during exercise when comparisons are made at the same absolute work rate. However, FA oxidation is controlled by different factors in the different domains of exercise intensity. During exercise at moderate to high intensity, whole body FA oxidation is influenced by aerobic training status, whereas during exercise at low intensity, as investigated in the present study, intrinsic mitochondrial characteristics (i.e., MFO) appear to be more important for whole body FA oxida-

tion than training status. An important new finding in the present study was that FA oxidation potential at the mitochondrial level (MFO) influences whole body FA oxidation during low-intensity exercise. MFO varied greatly between subjects and was correlated to the abundance of type I fibers but not to training status or to UCP3 protein content.

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