Independent effects of endurance training and weight loss on peak fat oxidation in moderately overweight men: a randomized controlled trial

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1Department of Biomedical Sciences, University of Copenhagen, Denmark; and 2Center for Healthy Aging, University of Copenhagen, Denmark

Submitted 7 August 2014; accepted in final form 20 January 2015

Nordby P, Rosenkilde M, Ploug T, Westh K, Feigh M, Nielsen NB, Helge JW, Stallknecht B. Independent effects of endurance training and weight loss on peak fat oxidation in moderately overweight men: a randomized controlled trial. J Appl Physiol 118: 803–810, 2015. First published January 22, 2015; doi:10.1152/japplphysiol.00715.2014.—Endurance training increases peak fat oxidation (PFO) during exercise, but whether this is independent of changes in body weight is not known. The aim of the present study was to investigate the effects of endurance training with or without weight loss or a diet-induced weight loss on PFO and on key skeletal muscle mitochondrial proteins involved in fat oxidation. Sixty moderately overweight, sedentary but otherwise healthy men were randomized to 12 wk of training (T), diet (D), training and increased caloric intake (T-iD), or continuous sedentary control (C). Isoenergetic deficits corresponding to 600 kcal/day were comprised of endurance exercise for T and caloric restriction for D. T-iD completed similar training but was not in 600 kcal deficit because of dietary replacement. PFO and the exercise intensity at which this occurred (FatMax) were measured by a submaximal exercise test and calculated by polynomial regression. As intended by study design, a similar weight loss was observed in T (−5.9 ± 0.7 kg) and D (−5.2 ± 0.8 kg), whereas T-iD (−1.0 ± 0.5 kg) and C (0.1 ± 0.6 kg) remained weight stable. PFO increased to a similar extent with 42% in T (0.16 g/min; 95% confidence intervals (CI): 0.02; 0.30, P = 0.02) and 41% in T-iD (0.16 g/min; 95% CI: 0.01; 0.30, P = 0.04) compared with C, but did not increase in D (P = 0.96). In addition, the analysis of covariance showed that changes in both PFO (0.10 g/min; 95% CI: 0.03; 0.17, P = 0.03) and FatMax (6.3% VO2max; 95% CI: 1.4; 11.3, P < 0.01) were independently explained by endurance training. In conclusion, endurance training per se increases PFO in moderately overweight men.

fat metabolism; skeletal muscle; exercise; diet

Obesity and type 2 diabetes are predisposed by physical inactivity, and impairments in fat oxidation during sedentary living might be a causal link in the development of these morbid conditions (19, 40). Almost 75 years ago it was reported that habitual endurance training improves fat oxidation (9), and since then numerous studies have shown that fat oxidation during exercise is increased after endurance training (5, 12, 17, 23). However, under experimental maintenance of energy balance (i.e., weight stability) the effects of endurance training are somewhat controversial as submaximal fat oxidation at the same absolute exercise intensity improves (39), whereas 24-h fat oxidation has been reported not to increase after endurance training (24). Therefore, some of the improvements that occur in fat oxidation with endurance training could be affected by weight loss and/or changes in body composition.

During exercise substrate metabolism primarily depends on exercise intensity, such that absolute rates of fat oxidation first increase and then decline with increasing intensity, whereas rates of carbohydrate oxidation progressively increases with exercise intensity (4, 8, 29). This is known as the “crossover concept” of exercise substrate metabolism (8). The dynamics of fat oxidation with increasing exercise intensity can be depicted as a bell-shaped relationship (13), and based on this relationship Jeukendrup and colleagues (1) have proposed two measures of whole body fat oxidation capacity: peak fat oxidation (PFO) and the intensity at which this occurs (FatMax), which are calculated from incremental submaximal exercise workloads based on measurements of respiratory gaseous exchange and indirect calorimetry. In cross-sectional studies we have previously shown that PFO is higher in well-trained male subjects compared with less-trained counterparts (27) and that a high whole body fat oxidation capacity during exercise may convey protection toward an unfavorable cardiometabolic phenotype in overweight sedentary men (31). More recently we found that PFO increased 21 ± 4% with 12 wk of moderate dose endurance exercise (~30 min/day) and 28 ± 4% with a high dose of exercise (~60 min/day) in moderately overweight men (32) in spite of similar reductions in body fat between the two doses of exercise (30). Also, it has been found that PFO increases with dietary-induced weight loss in overweight subjects (36). However, the independent effects of endurance training with or without simultaneous weight loss or an isoenenergetic diet-induced weight loss on PFO have not been elucidated. Skeletal muscle is the primary site for fat oxidation during exercise (20), and both regular endurance training (35) and chronic restrictions in caloric intake (10) affect muscle substrate metabolism and a number of mitochondrial proteins involved in these processes. Thus we hypothesize that changes in a number of skeletal muscle mitochondrial proteins are associated with changes in PFO. Therefore, the aim of the present study was to investigate the effect of endurance training with or without weight loss or weight loss per se on PFO as well as on key skeletal muscle mitochondrial proteins involved in fat oxidation.

METHODS

Study design. Male participants were eligible for participation based on a body mass index (BMI) of 25–30 kg/m2, body fat percentage >25%, peak oxygen uptake (VO2peak) <45 ml·min−1·kg−1, but otherwise healthy (fasting blood glucose <6.1 mmol/l and blood pressure <140/90 mmHg) and no first degree relatives with Type 2 diabetes. The primary findings and the overall

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design of this study have been presented and described in detail elsewhere (26), but here we present the findings in relation to PFO during exercise. The study was registered at (www.clinicaltrials.gov; identifier: NCT01090869), approved by the ethics committee of the Capital Region of Denmark (H-KF-2006-6443), and adhered to the Helsinki Declaration.

Participants were screened before inclusion for the above mentioned criteria including a familiarization $V_\text{O}_{2\text{peak}}$ test and measures of body composition. Eligible participants then took part in three separate test days. On the first test day after an overnight fast (≥12 h), body composition was measured with dual-energy $X$-ray absorptiometry (DPX-IQ $X$-ray bone densitometer 4.7e, Lunar, Madison, WI) and a cycle ergometer test, to measure PFO, and FatMax was performed (27). On the second test day participants performed a $V_\text{O}_{2\text{peak}}$ test. Both exercise tests were performed on an electronically braked bicycle (Ergoline 800, Ergometrics, Bitz, Germany), and respiratory gaseous exchange was measured with an indirect calorimetric system (Oxycon Pro, Jaeger, Würzburg, Germany). Finally, on the third test day, pulmonary gaseous exchange was measured with an indirect calorimetric system every 5–10 min (ABL 625, Radiometer, Copenhagen, Denmark) to determine $V_\text{O}_{2\text{peak}}$ and PFO, respectively, and throughout both tests pulmonary gaseous exchange was measured.

The submaximal exercise test used to determine PFO has been described previously in lean trained (27) and untrained subjects (31); briefly, an 8-min warm-up period to ensure steady state is followed by 3-min increments of 30 W until RER exceeds 1.0 for a full 3-min workload. In the present study the workload was 60 W during the warm-up period and was followed by 30-W increments. Periperal insulin sensitivity was measured with a hyperinsulinemic isoglycemic clamp administering exogenous insulin as a primed-continuous infusion at a rate of 40 mU·min$^{-1}$·m$^{-2}$ simultaneously with a variable 20% (wt/vol) glucose infusion adjusted according to plasma glucose measurements every 5–10 min (ABL 625, Radiometer, Copenhagen, Denmark) to maintain the individual fasting plasma glucose level as described and presented in more detail elsewhere (26).

**Skeletal muscle tissue analysis.** Biopsies were obtained from muscle vastus lateralis by the Bergström technique (6) applying suction, frozen in liquid nitrogen, and stored at −80°C. Preparation of the muscle homogenates has been described previously (26), and protein concentrations were determined by the bicinchoninic acid method (Pierce, Rockford, IL). Ten to fifteen micrograms of muscle protein diluted in Laemmli buffer was separated by SDS-PAGE on 12.5 or 4–15% Criterion gels (Biorad, Herlev, Denmark) and electrophoretically transferred to polyvinylidene difluoride membranes in a tank buffer system (48 mM Tris, 39 mM glycine, and 20% methanol). Membranes were blocked in 5% BSA plus 2.5% skimmed milk powder in TS buffer [10 mM Tris (pH 7.4), 150 mM NaCl] and incubated for 90 and 60 min with primary and HRP-labeled secondary antibodies (DAKO, Glostrup, Denmark), respectively, diluted in blocking solution. Antigen-antibody complexes were visualized by enhanced chemiluminescence (ECL, GE Healthcare, Hillerød, Denmark) and a digital camera system (LAS-3000, Luminescent Image Analyser, FujiFilm, Japan), and quantitated by the analysis software “Multi Gauge” (ver. 3.0, FujiFilm). Signal strength was expressed in relative units as percent of an “internal standard” loaded in quadruplicate on each gel. As internal standard we used aliquots from a pool of muscle homogenates generated from a different group of subjects than those included in the present study. Six different primary antibodies were used: monoclonal mouse antibodies against subunits of mitochondrial Oxphos complex II (MS203 from MitoSciences, Eugene, Oregon), complex III (MS304), complex IV (MS405) and complex V (MS507), β-hydroxacyl-CoA dehydrogenase (HADH) (Abnova, Taipei City, Taiwan), and citrate synthase (CS) (Sigma-Aldrich, Brøndby, Denmark).

**Calculations and statistical analysis.** Fat and carbohydrate oxidation during the different exercise intensities were calculated with standard stoichiometric equations for respiratory gaseous exchange (11), disregarding protein oxidation during this short bout of exercise (28). Substrate oxidation was calculated during the last 90 s of each incremental step in the graded exercise test, and standard polynomial curve fitting was obtained for each individual to assess PFO and the exercise intensity at which it occurred, termed FatMax (1). This study is an efficacy study, and the outcomes were assessed using per protocol analysis. Because of a RER >1.0 already after the first workload in pretesting of four subjects (T-ID: 1; D: 1; C: 2), PFO could not be determined, and all data from these subjects have subsequently been excluded from the data analysis as the primary data are not available.

Descriptive pre- and postintervention data are described as means ± SE. The differences in postintervention values between the randomization groups are presented as adjusted least squares means with two-sided 95% CI. Within group changes were evaluated by a paired t-test, and to evaluate main effects of the intervention between-group differences were assessed by ANCOVA with postintervention values as the dependent variable and baseline values and group assignment as
covariates. All pairwise comparisons were adjusted by the Tukey procedure. In addition, the overall effects of endurance training (T and T-iD) or weight loss (D and T) and the interaction between endurance training and weight loss were evaluated by ANCOVA with postintervention data as the dependent variable, training and weight loss as qualitative covariates, and baseline data as quantitative covariates. This is also presented by least squares means with 95% CI, and this analysis was performed to dissociate outcomes resulting from endurance training and weight loss. Predictors of changes in PFO were determined by simple bivariate correlations (Pearson) for both the training (T and T-iD) and weight loss groups (T and D). A level of $P \leq 0.05$ was considered significant.

RESULTS

The subjects and the response to the prescribed interventions have been presented in more detail elsewhere (26). Briefly, registrations of dietary intake showed dietary compliance to the intervention (Table 1). In D, energy intake was lower compared with C ($-640$ kcal; $-1,150; -136, P < 0.01$) and T ($-590$ kcal; $-1,080; -100, P = 0.01$). In T-iD, energy intake was higher compared with C ($790$ kcal; $270; 1,310, P < 0.001$) and T ($850$ kcal; $320; 1,380, P < 0.01$). In accordance with the intentional study design, energy intake remained constant within T and C ($P > 0.12$) and energy intake did not differ between these two groups ($P = 0.99$). The decrease in EI in D was achieved by decreasing dietary fat intake (vs. C: $-5$ E%; $-10; -1, P = 0.03$), to some extent alcohol consumption ($P = 0.06$ vs. C), and increasing the relative contribution of dietary carbohydrate (vs. C: $7$ E%; $-1,13; P = 0.02$) whereas intake of dietary protein was unchanged (P ($0.65$ vs. C) (Table 1). In T-iD, the increased dietary intake was obtained by increasing mainly dietary carbohydrate (vs. C $7$ E%; $-1,12; P = 0.01$), most likely at the expense of dietary fat (within group difference: $-3$ E%; $-6; -0.5, P = 0.03$) (Table 1). In addition, excellent compliance with the training interventions was observed; T: $97 \pm 2\%$ (exercise energy expenditure: $576 \pm 11$ kcal/day); and T-iD: $95 \pm 2\%$ (563 $\pm 15$ kcal/day). Average exercise intensity did not differ between T ($72 \pm 2\%$Vo2peak) and T-iD ($67 \pm 2\%$Vo2peak) ($P = 0.14$).

Although the full sample of subjects was not included in the present paper, the findings regarding body composition and Vo2peak (Table 2) were similar to those previously published (26). Body weight and body fat was reduced in T and D compared with C ($P < 0.001$). Also, body fat was reduced within T-iD ($-1.9 \pm 0.3$ kg, $P < 0.001$), but not compared with C ($P = 0.12$). FFM increased $2.0$ kg ($0.7; 3.2, P < 0.001$) in T compared with C, and tended to do so in T-iD (vs. C: $1.2$ kg; $-0.1; 2.5, P = 0.08$). Within D, FFM was reduced from pre- to postintervention ($-1.0 \pm 0.3$ kg, $P < 0.01$), but this was not significant compared with C ($P = 0.29$). In quantitative terms Vo2peak improved $15\%$ independent of weight loss in the two training groups ($4.6$ ml O2·kg$^{-1}$·min$^{-1}$; $1.2; 8.0, P < 0.01$) and there was no effect of weight loss on Vo2peak ($P = 0.26$).

Fat oxidation capacity. The capacity to oxidize lipids during submaximal exercise improved with endurance training, as changes in PFO was observed with endurance training independent of weight loss ($0.10$ g/min; $0.03; 0.17, P = 0.03$) as based on the ANCOVA with training and weight loss as qualitative covariates. Furthermore, compared with C, PFO increased with $42\%$ in T ($0.16$ g/min; $0.02; 0.30, P = 0.02$) and $41\%$ in T-iD ($0.16$ g/min; $0.01; 0.30, P = 0.04$). This difference was also apparent in T compared with D ($0.14$ g/min; $0.01; 0.28, P = 0.03$), but the increase in PFO in T-iD only tended to be different from D ($0.14$ g/min; $-0.005; 0.28, P = 0.06$) (Table 2 and Fig. 1). Adjusted for changes in FFM, PFO also increased within T ($2.2 \pm 0.5$ mg/kg FFM/min; $P < 0.01$) and T-iD ($3.1 \pm 0.6$ mg/kg FFM/min, $P < 0.001$), but in the groupwise comparison this was only statistically significant in T vs. C ($2.2$ mg/kg FFM/min; $0.2; 4.2, P = 0.03$). Moreover, endurance training (with or without weight loss) was independently associated with changes in PFO corrected for changes in FFM ($P < 0.01$), whereas this was not the case for weight loss (with or without endurance training) ($P = 0.24$).

The increased capacity for lipid oxidation during submaximal exercise was also apparent in other ways: the number of submaximal workloads completed with a RER $\leq 1.0$ increased in T and T-iD (T vs. C: $2.7; 0.2: 5.2, P = 0.03$; T-iD vs C: $3.2; 0.6: 5.9, P = 0.01$) (Fig. 1). Additionally, FatMax increased $10\%$ within T ($P < 0.01$) and $11\%$ within T-iD ($P < 0.001$), and these increases could also be independently explained by endurance training ($P = 0.01$) but not by weight loss ($P = 0.43$) (Table 2 and Fig. 1). At the same submaximal workloads pre- and postintervention carbohydrate oxidation decreased within T at $90$ W ($-0.34$ g/min; $-0.59; -0.09, P = 0.01$), $120$ W ($-0.65$ g/min; $-0.99; -0.30, P < 0.01$), and $150$ W ($-0.97$ g/min; $-1.43; -0.51, P < 0.01$) and within T-iD at $60$ W ($-0.23; -0.41 P = 0.02$), $90$ W ($-0.51 g/min; -0.79; -0.23, P < 0.01$) and $120$ W ($-0.76 g/min; -1.00; -0.53, P < 0.001$) (Fig. 1). At $60$ W, carbohydrate oxidation did not differ between pre- and postintervention within T ($P = 0.21$). At all submaximal workloads carbohydrate oxidation did not differ from D and C in both T ($P > 0.26$ for all comparisons) and T-iD ($P > 0.40$ for all comparisons).

### Table 1. Dietary intake

<table>
<thead>
<tr>
<th></th>
<th>T (n = 12)</th>
<th>D (n = 11)</th>
<th>T-iD (n = 11)</th>
<th>C (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake, kcal/day</td>
<td>2420 ± 130</td>
<td>2690 ± 190</td>
<td>3220 ± 305</td>
<td>2770 ± 160</td>
</tr>
<tr>
<td>Dietary macronutrients</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Δ carbohydrate intake, % total EI</td>
<td>0.6 ± 2.2</td>
<td>6.8 ± 2.9*++</td>
<td>2.6 ± 2.1†</td>
<td>-5.8 ± 1.7</td>
</tr>
<tr>
<td>Δ fat intake, % total EI</td>
<td>-2.7 ± 1.6</td>
<td>-5.8 ± 2.0*++</td>
<td>-3.2 ± 1.7*</td>
<td>2.3 ± 1.7</td>
</tr>
<tr>
<td>Δ protein intake, % total EI</td>
<td>0.8 ± 1.1</td>
<td>3.2 ± 1.3*</td>
<td>0.4 ± 1.1</td>
<td>0.9 ± 1.3</td>
</tr>
<tr>
<td>Δ alcohol intake, % total EI</td>
<td>0.1 ± 1.1</td>
<td>-4.2 ± 1.8++</td>
<td>0.1 ± 1.1</td>
<td>2.7 ± 1.3</td>
</tr>
</tbody>
</table>

Data are means ± SE, and the level of significance is $P < 0.05$. *Significant change within group. †Significantly different from C. §Significantly different from D. ‡Significantly different from T-iD. C, control group; D, diet group; EI, energy intake; T, training group; T-iD, training increased diet group.
Skeletal muscle mitochondrial proteins. The changes in skeletal muscle mitochondrial enzymes are presented in Fig. 2. Endurance training independently increased mitochondrial respiratory chain complex II ($P < 0.001$) and IV ($P = 0.02$), but not complex III ($P = 0.16$) and V ($P = 0.17$). Mitochondrial complex II also increased in T and T-iD compared with D ($P < 0.001$) and C ($P < 0.01$). Skeletal muscle protein content of CS increased within both T-iD (100 ± 90%, $P = 0.04$) and in T (82 ± 28%, $P = 0.03$), but despite these large numerical increases this was not significant compared with D ($P > 0.6$ for both) or C ($P > 0.3$ for both), nor was the change independently increased by endurance training ($P = 0.09$). Likewise, there was no intergroup differences for HADH, although changes in the amount of this protein tended to increase with training ($P = 0.06$).

Associations with training-induced changes in PFO. For $n = 44$, intervention-induced changes in VO$_{2peak}$ were associated with changes in PFO ($R^2$: 0.42, $P < 0.01$), but this was neither replicated in the training groups alone (T and T-iD) nor for T and T-iD combined ($P = 0.43$). Likewise, changes in FFM were associated with changes in PFO ($R^2$: 0.43, $P < 0.01$) but not separately in T and T-iD ($P = 0.58$). Changes in dietary fat intake did not correlate with changes in PFO ($P = 0.87$). Additionally, changes in HADH ($R^2$: 0.49, $P < 0.01$) and mitochondrial protein complex II ($R^2$: 0.43, $P < 0.01$) and IV ($R^2$: 0.31, $P < 0.05$) were positively associated with changes in PFO. Furthermore, changes in whole body peripheral insulin sensitivity as measured with the hyperinsulemic isoglycemic clamp technique correlated positively with changes in peak fat oxidation in the full sample ($R^2$: 0.11, $P = 0.03$, $n = 44$), although this association was only borderline statistically significant when PFO was adjusted for FFM (PFO/FFM; $R^2$: 0.09, $P = 0.054$, $n = 44$). When PFO was adjusted for changes in VO$_{2max}$ changes in whole body peripheral insulin sensitivity were not associated with changes in PFO ($P = 0.23$).

DISCUSSION

The main finding of the present paper is a clear effect (~40%) of endurance training both with and without weight loss on PFO, but no effect of diet-induced weight loss, in moderately overweight men. Hence, we found that whole body fat oxidation capacity during exercise is increased by endurance training per se. Moreover, PFO occurred at higher exercise intensity (FatMax) after training, as has also been demonstrated in previous exercise trials (25, 34). The separate and combined effects of endurance training and diet-induced weight loss on fat oxidation during submaximal steady-state exercise have previously been investigated in a 16-wk intervention study of older obese subjects, and it was shown that fat oxidation during 1 h of moderate-intensity exercise increased after endurance training alone (without weight loss) and in conjunction with diet-induced weight loss, but not as a result of diet-induced weight loss per se (2). The training-induced changes in PFO in the present study (~0.16 g/min) are comparable to those obtained in a recent study from our research group in a similar, albeit different, group of moderately overweight men. Daily endurance training of a moderate (300 kcal) or high dose (600 kcal) were prescribed for 12 wk. PFO increased 0.09 g/min with moderate- and 0.14 g/min with high-dose training (32). In that study, subjects’ body weight on

Table 2. Body composition and training variables

<table>
<thead>
<tr>
<th></th>
<th>T (n = 12)</th>
<th>D (n = 11)</th>
<th>T-iD (n = 11)</th>
<th>C (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>28.4 ± 1.4</td>
<td>32.6 ± 1.8</td>
<td>32.2 ± 2.2</td>
<td>32.4 ± 1.9</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>94.5 ± 2.3</td>
<td>90.9 ± 1.9</td>
<td>96.4 ± 2.6</td>
<td>90.6 ± 2.9</td>
</tr>
<tr>
<td>Post</td>
<td>88.6 ± 2.2††</td>
<td>85.7 ± 2.4††</td>
<td>95.4 ± 2.6*</td>
<td>90.8 ± 2.7</td>
</tr>
<tr>
<td>Body fat, kg</td>
<td>28.5 ± 1.4</td>
<td>29.6 ± 1.5</td>
<td>27.5 ± 1.5</td>
<td>26.1 ± 1.4</td>
</tr>
<tr>
<td>Post</td>
<td>20.8 ± 1.7††‡</td>
<td>25.1 ± 1.9*†</td>
<td>25.6 ± 1.5*</td>
<td>26.4 ± 1.0</td>
</tr>
<tr>
<td>Fat free mass, kg</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pre</td>
<td>66.0 ± 2.0</td>
<td>61.7 ± 1.4</td>
<td>68.9 ± 1.7</td>
<td>64.6 ± 2.1</td>
</tr>
<tr>
<td>Post</td>
<td>67.8 ± 2.1††‡</td>
<td>60.6 ± 1.3‡</td>
<td>69.8 ± 1.6*§</td>
<td>64.4 ± 2.0</td>
</tr>
<tr>
<td>VO$_{2peak}$, ml O$_2$/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>3650 ± 140</td>
<td>3240 ± 100</td>
<td>3590 ± 180</td>
<td>3380 ± 180</td>
</tr>
<tr>
<td>Post</td>
<td>4170 ± 140†‡‡</td>
<td>3250 ± 120</td>
<td>4060 ± 150*§</td>
<td>3540 ± 160</td>
</tr>
<tr>
<td>VO$_{2peak}$, ml O$_2$/kg/min</td>
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<tr>
<td>Pre</td>
<td>38.5 ± 1.1</td>
<td>35.9 ± 1.5</td>
<td>37.5 ± 2.2</td>
<td>37.5 ± 2.2</td>
</tr>
<tr>
<td>Post</td>
<td>47.1 ± 1.3*†‡</td>
<td>38.1 ± 1.6*</td>
<td>42.8 ± 1.9*†</td>
<td>39.3 ± 2.1</td>
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<tr>
<td>Peak fat oxidation, g/min</td>
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</tr>
<tr>
<td>Pre</td>
<td>0.30 ± 0.03</td>
<td>0.28 ± 0.04</td>
<td>0.19 ± 0.02</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>Post</td>
<td>0.46 ± 0.03*†‡</td>
<td>0.31 ± 0.04</td>
<td>0.41 ± 0.04*§</td>
<td>0.29 ± 0.04</td>
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<td>Peak fat oxidation, mg fat/kg FFM/min</td>
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<tr>
<td>Pre</td>
<td>4.5 ± 0.4</td>
<td>4.5 ± 0.6</td>
<td>2.7 ± 0.3</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>Post</td>
<td>6.7 ± 0.4†‡</td>
<td>5.0 ± 0.6</td>
<td>5.8 ± 0.5*</td>
<td>4.5 ± 0.6</td>
</tr>
<tr>
<td>FatMax, %VO$_{2max}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>36 ± 3</td>
<td>44 ± 3</td>
<td>34 ± 3</td>
<td>39 ± 3</td>
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<tr>
<td>Post</td>
<td>45 ± 2*</td>
<td>39 ± 3</td>
<td>47 ± 2*</td>
<td>40 ± 2</td>
</tr>
</tbody>
</table>

Data are means ± SE, and the level of significance is $P < 0.05$. *Significant change within group. †Significantly different from T-iD. §Significantly different from C. ‡Significantly different from D.
average was approximately reduced by 3 kg independent of training dose (30), and an additive effect of weight loss on PFO could not be excluded. Our two studies differ methodologically because in the previous study PFO was determined 24 h after the last exercise bout, whereas in the present study PFO was measured 3 to 4 days after the intervention had ended. During exercise, skeletal muscle is the major site for fat oxidation (21), and a positive association exists between FFM and PFO (32, ...
37), wherefore a part of the training-induced increase in PFO could have been due to increases in FFM. However, when corrected for changes in FFM the endurance training effect on PFO remained.

Other recent studies have not provided a clear picture of the independent effects of exercise training and changes in body weight on PFO. Twelve weeks of either high (80 –90% of maximal watt output) or moderate-intensity interval training (60 – 80%) enhanced PFO in sedentary women (BMI: 17–33 kg/m²) (3). However, in obese men PFO did not increase after 4 wk of high-intensity interval training but increased after continuous moderate-intensity training (38). Scharhag-Rosenberger et al. (34) observed an increase in PFO after 12 but not 6 mo of endurance training (3 days/wk) in sedentary men and women. Notably, in the mentioned studies there was no weight loss as a consequence of training. Tsujimoto et al. (36) found increases in PFO with caloric restriction only when adjusted for FFM (i.e., PFO/FFM). The study did not include a control group, and the effects observed could thus be due to customization to the testing procedures. Also, numerical changes in

Fig. 2. Skeletal muscle protein content of citrate synthase (A), β-hydroxyacyl-CoA dehydrogenase (HADH) (B), and mitochondrial enzyme complexes II–V (C–F) at baseline (PRE) and at the end of a 12-wk intervention (POST) with training-induced weight loss (T, n = 11), diet-induced weight loss (D, n = 11), training without weight loss (T-iD, n = 11), and sedentary controls (C, n = 10). Representative blots of specific proteins are shown in each panel for one subject in T, D, T-iD, and C groups. *Significant change within group. †Significantly different from change in C after the 12-wk intervention. §§Significantly different from change in D after the 12-wk intervention. $Significant effect of endurance training per se. Data are means ± SE, and the level of significance is P < 0.05.
PFO in the diet group of the present and the Tsujimoto studies were comparable (0.03 and 0.04 g/min, respectively). In the present study we are able to dissect the individual influence of either training or weight loss on PFO, and we demonstrate that PFO is increased with endurance exercise independent of weight loss. The clinical importance of PFO has not yet been determined. We have previously shown in a cross-sectional study that a high PFO is associated with a metabolically healthy phenotype (31). In the present study a positive, albeit weak, association was observed between changes in whole body peripheral insulin sensitivity and changes in PFO. Clearly, more studies are needed to determine the physiological relevance of PFO across different populations and after lifestyle modifications, as presented in the present study.

Studies of a cross-sectional nature as well as the above mentioned training intervention studies have shown large intersubject variability in fat oxidation capacity (14, 37). The underlying mechanisms might include molecular pathways implicated in fatty acid metabolism in skeletal muscle during exercise. Whole body fat oxidation is positively associated with in vitro mitochondrial fat oxidation (33). In the present study we measured protein levels of several skeletal muscle mitochondrial enzymes. Mitochondrial respiratory chain enzyme complex II and IV were increased by endurance exercise independently of weight loss, and there was a trend for an increase also in HADH. We have previously shown that mitochondrial enzyme expression (CS and complex II–V) was increased and positively associated with changes in PFO after endurance training in a similar population of overweight subjects (32). These findings were only partly reproduced in the present study, probably because of a lack of statistical power. Nevertheless, the positive association between changes in PFO and VO\textsubscript{2peak}, albeit more unspecific, underpins the observations between metabolic adaptations induced by endurance training and improvements in PFO (32).

The present study benefits from a randomized design including relevant control groups and a high training compliance ensuring robust conclusions about PFO, but there are some limitations. Underreporting of energy and dietary fat intake is common in overweight subjects (7, 22), but the inclusion of a control group and groupwise comparisons hereof limits the influence of this well-known bias. Fat oxidation during exercise can be influenced by experimental manipulation of dietary fat intake (16), but the reported fat intake was not associated with changes in PFO in the present study. Furthermore, substrate oxidation was measured under fasted conditions, and it is not clear whether the exercise training–induced improvements in PFO would persist in the postabsorptive state (12). Moreover, we did not provide standardized pretest diets, wherefore substrate oxidation is affected by the habitual diets of the subjects; nevertheless, a single day protocol as employed in the present study ensures less bias in day-to-day variation of food intake and physical activity. During the submaximal exercise test, measurements of blood lactate would have been preferable to evaluate metabolic steady state. The degree of negative energy balance between groups could be different because of a quantitative larger loss of body fat in T than D (30). Despite weight stability, T-ID was also most likely in negative energy balance because of loss of more energy-dense fat tissue compared with observed gain in FFM (15). From a theoretical viewpoint, the different levels of negative energy balance between T, D, and T-ID could have implications for whole body fat oxidation, but in the present study PFO increased to the same extent in T and T-ID, with no increase in D. Also, the different interventions were highly controlled, and subjects were daily to adhere to exercise and diet prescriptions, making the weight loss and exercise conditions comparable.

In the present study we employed a robust study design, and subjects were carefully controlled to dissociate effects of endurance training with or without weight loss or weight loss effects per se. We conclude that 3 mo of daily endurance training, independently of weight loss but not diet-induced weight loss, increases PFO in moderately overweight men.

ACKNOWLEDGMENTS
All participants in the trials are acknowledged, and Gerda Hau, Jeppe Bach, Regitze Kraunøe, and Thomas Beck contributed with expert technical assistance.

GRANTS
Financial support for this study was obtained from the Danish National Research Council; The Ministry of Culture, Committee on Sports Research; the Academy of Muscle Biology, Exercise and Health Research (AMBEHR); the Novo Nordisk Foundation; the Danish Diabetes Association; Aase og Ejnar Danielsens Fond; Oda og Hans Svenningsens Fond; Fonden til Legevidenskabens Fremme; Else og Mogens Wedell-Wedellsborgs Fond, Fondet af 17.12.1981; Beckett-Fonden and Direktør J. Madsen og hustru O. Madsens Fond and Fitness.dk. Salary of Mads Rosenkilde was funded by the University of Copenhagen Excellence Programme for Interdisciplinary Research (www.go.ku.dk). The financial sponsors had no role in designing or conducting the study, in the collection, analysis, or interpretation of data, or in the preparation of the manuscript.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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