Endurance Training and Obesity: Effect on Substrate Metabolism and Insulin Sensitivity

MICHELLE C. VENABLES and ASKER E. JEUKENDRUP

Human Performance Laboratory, School of Sport and Exercise Sciences, The University of Birmingham, Edgbaston, UNITED KINGDOM

ABSTRACT

VENABLES, M. C. and A. E. JEUKENDRUP. Endurance Training and Obesity: Effect on Substrate Metabolism and Insulin Sensitivity. *Med. Sci. Sports Exerc.*, Vol. 40, No. 3, pp. 495–502, 2008. **Purpose:** Obesity and type 2 diabetes mellitus are disease states associated with hallmark features such as insulin resistance and an impaired ability to oxidize lipids. It has recently been reported that an optimal exercise intensity for fat oxidation (FATmax) exists; we hypothesize that continuous exercise training at this specific intensity can lead to greater improvements in fat oxidation and insulin sensitivity than a eucaloric interval training program. **Methods:** In a counterbalanced, crossover design, eight sedentary, obese, but otherwise healthy male participants performed two 4-wk blocks of endurance training, either at a predetermined intensity eliciting maximal fat oxidation (TP_{CON}) or at 5-min intervals of ± 20% FATmax (TP_{INT}). During the week preceding the exercise training and 48 h after the final exercise bout, an OGTT, $\dot{V}O_{2max}$ test, steady-state exercise, and measurements of body composition were undertaken. Diet was controlled the day before all trials (50% carbohydrate, 35% fat, and 15% protein; ~2900 kcal·d^{-1}). Variables were compared using two-way repeated-measures analyses of variance. **Results:** It was shown that fat oxidation rates were increased by 44% after TP_{CON} (0.24 ± 0.01 vs 0.35 ± 0.03 g·min^{-1}, $P < 0.05$) but not after TP_{INT}, and the whole-body insulin sensitivity index was increased by 27% after TP_{CON} ($P < 0.05$). These changes occurred despite no change in body weight, body mass index (BMI), waist to hip ratio (WHR), percent body fat (%BF), or $\dot{V}O_{2max}$. **Conclusions:** A continuous exercise training protocol that can elicit high rates of fat oxidation increases the contribution of fat to substrate oxidation during exercise and can significantly increase insulin sensitivity compared with a eucaloric interval protocol. **Key Words:** EXERCISE INTENSITY, FAT OXIDATION, INSULIN SIGNALING, GLUCOSE TOLERANCE

BASIC SCIENCES

Obesity is a condition commonly associated with elevated plasma fatty acids (FA), as well as insulin resistance and hyperinsulinemia, two important risk factors in the development of type 2 diabetes mellitus and cardiovascular disease. There is a great deal of evidence to show that intramuscular triglyceride (IMTG) concentrations are elevated in obesity and type 2 diabetes mellitus (15,16, 33,42), possibly because of an inability to oxidize lipids within the skeletal muscle (11,19,23,25,34); therefore, the accumulation of IMTG may be the link between obesity and insulin resistance (15,23,30,32). However, there is emerging evidence to suggest that the presence of elevated levels of plasma FA, diacylglycerols (DAG), and fatty acyl–CoA within skeletal muscle are the direct link to insulin resistance. Studies in which plasma FA concentrations have been acutely raised have led to insulin resistance in human skeletal muscle within 6 h (3,6), whereas reductions in plasma FA concentrations in individuals with chronically elevated levels have been shown to improve insulin resistance overnight (36). Furthermore, inactivation of fatty acid transport protein 1 (FATBP1), which is important in FA uptake and subsequent conversion to fatty acyl–CoA within the skeletal muscle, prevents the insulin resistance observed with high-fat feeding and lipid infusions in mice (24).

Physical activity is a simple, effective means by which insulin sensitivity can be improved in lean, obese, and diabetic groups. Not only does acute exercise improve insulin sensitivity for up to 48 h (31), regular exercise training can induce long-term changes within the skeletal muscle. However, there is no consensus regarding the type, intensity, and duration of exercise required to improve insulin sensitivity. Some studies suggest that only high-intensity exercise can improve insulin sensitivity (8,22,40), whereas others have shown that mild- to moderate-intensity exercise can be just as effective (22,29). More recently, Houmard et al. (18) have shown that it is the duration of exercise that is important in determining improvements in insulin sensitivity, regardless of intensity.

Address for correspondence: Asker E. Jeukendrup, Ph.D., School of Sport and Exercise Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT; E-mail: A.E.Jeukendrup@bham.ac.uk.
Submitted for publication May 2007.
Accepted for publication August 2007.

0195-9131/08/4003-0495/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0b013e31815f256f

TABLE 1. Participant characteristics before and after training.

	TP_{CON}		TP_{INT}	
	Pre	Post	Pre	Post
Age (yr)	39 ± 7	39 ± 7	40 ± 7	40 ± 7
Weight (kg)	99.0 ± 9.1	98.8 ± 8.7	100.1 ± 9.2	99.5 ± 8.9
BMI ($kg \cdot m^{-2}$)	32.5 ± 2.6	32.4 ± 2.5	32.9 ± 2.8	32.6 ± 2.7
WHR	0.93 ± 0.05	0.93 ± 0.04	0.93 ± 0.04	0.93 ± 0.04
BF (%)	28.7 ± 3.8	28.6 ± 3.3	29.9 ± 3.7	29.5 ± 3.4
$\dot{V}O_{2max}$ ($mL \cdot min^{-1}$)	3264 ± 427	3273 ± 414	2955 ± 513	2967 ± 593
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	33.4 ± 4.7	34.6 ± 4.2	31.1 ± 5.2	31.1 ± 6.1

Values are expressed as means ± SD (N = 8). BMI, body mass index; WHR, waist to hip ratio; BF, body fat; FM, fat mass; FFM, fat-free mass; $\dot{V}O_{2max}$, maximal oxygen uptake.

We recently developed and validated a protocol to determine the exercise intensity at which fat oxidation is maximal (1,47). Here, we have hypothesized that continuous exercise training at this specific intensity can lead to greater improvements in fat oxidation and insulin sensitivity than a eucaloric interval training program.

METHODS

Participants

Eight obese male participants (see Table 1 for characteristics) completed the study. All participants were classed as sedentary, which, for the purposes of the study, was defined as participating in no more than 2 h of nonvigorous exercise per week. All participants were deemed healthy, as assessed by the school's general health questionnaire, and a physical exam was conducted by a qualified physician. Participants were excluded from the study if they were taking any medication that could alter fat or carbohydrate metabolism, if they were diabetic, or if they were hypertensive (diastolic BP > 90 mm Hg). All were informed of the purpose and nature of the study and the potential risks involved, after which their written informed consent was given. The protocol was approved by the ethics committee of the School of Sport and Exercise Sciences, The University of Birmingham, United Kingdom.

Experimental Design

In a counterbalanced, crossover design, all participants undertook two eucaloric 4-wk blocks of exercise, separated by 6 wk of nonexercise. At least 1 wk before the start of the endurance training periods, and 48 h after completion of the final exercise bout, all participants underwent an oral glucose tolerance test (OGTT), measurements of body composition, maximal oxygen uptake ($\dot{V}O_{2max}$), and substrate oxidation (Fig. 1).

Diet and Activity

On the days prior to testing, participants were fed a diet totaling approximately 2900 $kcal \cdot d^{-1}$, the macronutrient composition of the diet being 50% carbohydrate, 35% fat, and 15% protein. Throughout the course of the study, the participants were asked to maintain their normal dietary habits, with slight adjustments if needed to remain weight-stable. For 3 d of each week, participants were asked to record both dietary intake and physical activity patterns.

Measurements

OGTT. The participants arrived at the laboratory in the morning (between 7:30 and 9:00 a.m.) after an overnight fast (10 h). On arrival, standard measures of height and weight were taken, after which subjects were placed in a reclined position. A flexible 20-gauge Teflon catheter (Quickcath, Becton Dickinson, Plymouth, UK) was inserted into an antecubital vein of the arm. A three-way stopcock (PVB Medizintechnik, Kirchseean, Germany) was attached to this to allow for repeated blood sampling during the 2-h glucose tolerance test. A resting blood sample (5 mL; t = 0) was taken, immediately after which participants ingested a 25% glucose beverage (75 g of glucose (Meritose-200; Amylum UK Ltd, London, UK) made up with water to a volume of 300 mL). Further blood samples (5 mL) were collected with subjects seated at 15, 30, 45, 60, 90, and 120 min. The catheter was kept patent by flushing with 2–3 mL of isotonic saline (0.9%, Baxter, Norfolk, UK) after each blood sample collection and at 75 and 105 min.

Body composition. Body composition was determined using the four-site skinfold method of Durnin and Womersley (13), with the addition of waist and hip girth measurements. Waist and hip measurements were taken using a flexible, nonelastic tape, with the subject in a relaxed standing position with the arms folded across the thorax. Waist girth was taken at the level of the narrowest point between the umbilicus and

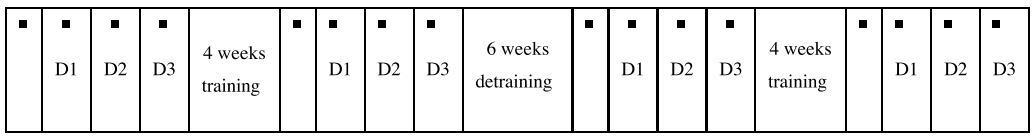

FIGURE 1—Schematic of study protocol. ■ Days on which diet was controlled; D1 = OGTT and body composition, D2 = maximal oxygen uptake test, and D3 = 30-min steady-state exercise at 50% $\dot{V}O_{2max}$.

TABLE 2. Oxygen uptake and maximal fat oxidation rates during the graded exercise to exhaustion protocol.

	TP_{CON}		TP_{INT}	
	Pre	Post	Pre	Post
MFO ($g \cdot min^{-1}$)	0.39 ± 0.03	0.40 ± 0.03	0.38 ± 0.03	0.40 ± 0.03
$\dot{V}O_2$ at MFO ($mL \cdot min^{-1}$)	1423 ± 61	1459 ± 65	1400 ± 90	1355 ± 109
$\%\dot{V}O_{2max}$ at MFO (%)	44 ± 2	43 ± 2	45 ± 2	46 ± 4

Values are means ± SE (N = 8). MFO, maximal fat oxidation rate; $\dot{V}O_2$, oxygen uptake.

xiphoid process, and hip girth was taken at the level of the greatest circumference of the buttocks region, which usually corresponds anteriorly to the level of the symphysis pubis (2).

Maximal oxygen uptake and substrate oxidation. Maximal oxygen uptake ($\dot{V}O_{2max}$), maximal fat oxidation rate (MFO), and the intensity at which MFO occurred (FATmax) were determined using a graded exercise test to exhaustion on a treadmill (Woodway® PPS 70sport-I, Weil am Rhein, Germany). The protocol described has been used previously to determine substrate oxidation at various different workloads (1,47). Briefly the participants started exercising at a speed of 3.5 $km \cdot h^{-1}$ and a gradient of 1%. The speed was increased by 1 $km \cdot h^{-1}$ every 3 min until a speed of either 6.5 $km \cdot h^{-1}$ or 7.5 $km \cdot h^{-1}$ was reached. At this point, the gradient was increased by 2% every 3 min until RER = 1. Finally, the speed was increased every minute until exhaustion. Breath-by-breath measurements were taken throughout exercise, using an Oxycon Pro automated gas-analysis system (Jaeger, Wuerzeburg, Germany). The gas analyzers were calibrated using a 4.95% CO_2–95.05% N_2 gas mixture (BOC Gases, Surrey, UK), and the volume transducer was calibrated with a 3-L calibration syringe. HR was measured continuously by telemetry, using a Polar Vantage HR monitor (Polar Electro Oy, Kempele, Finland).

Steady-state exercise. The participants arrived at the laboratory in the morning (between 7:30 and 9:00 a.m.) after an overnight fast (10 h). On arrival, weight was taken, and then they exercised on a treadmill at 50% of the predetermined $\dot{V}O_{2max}$. During minutes 5–10, 15–20, and 25–30, breath-by-breath measurements were taken, as above. HR was measured continuously by telemetry, using a Polar Vantage HR monitor.

Endurance exercise training. All participants performed two 4-wk blocks of endurance training; 1-TP_{CON}, endurance training at an individually predetermined exercise intensity designed to elicit maximal rates of fat oxidation; and 2-TP_{INT}, interval training that consisted of 5 min each at ± 20% FATmax. Training was carried out 5 $d \cdot wk^{-1}$ for 30 min in week 1, increasing by 10 $min \cdot wk^{-1}$ to 60 $min \cdot wk^{-1}$ at week 4. The training programs were randomly allocated in a two-way crossover design and were separated by a 6-wk block of nonexercise. Volume and energy expenditure for both exercise groups were kept constant, and heart rate (HR) was measured continuously by telemetry, using a Polar Vantage HR monitor (Polar Electro Oy, Kempele, Finland). All exercise sessions were verified by direct supervision of a qualified gym instructor (YMCA), or by use of a heart rate monitor.

Analyses. Blood samples were collected into prechilled EDTA-containing tubes (Becton Dickinson, Plymouth, UK) and centrifuged at 3000*g* at 4°C for 10 min. Aliquots of plasma were immediately frozen in liquid nitrogen and stored at −70°C for later analysis. Where appropriate, glucose (Glucose HK, ABX Diagnostics, UK) and FFA (NEFA-C, Wako Chemicals, Neuss, Germany) were analyzed spectrophotometrically on a COBAS MIRA semiautomatic analyzer (La Roche, Basel, Switzerland). Insulin was analyzed by enzyme-linked immunosorbent assay (ELISA EIA-2935, DRG Instruments GmBH, Germany).

Indirect calorimetry and calculations. During the graded exercise test, $\dot{V}O_2$ and $\dot{V}CO_2$ were averaged for the final 2 min of each stage, and nonprotein substrate oxidation was determined using the following stoichiometric equations of Jeukendrup and Wallis (20):

$$\text{Total fat oxidation} = 1.695\ \dot{V}O_2 - 1.701\ \dot{V}CO_2$$

$$\text{Total CHO oxidation} = 4.210\ \dot{V}CO_2 - 2.9621\ \dot{V}O_2$$

where $\dot{V}O_2$ and $\dot{V}CO_2$ are in liters per minute.

The plasma glucose and insulin concentrations during the OGTT were used to determine the whole-body insulin sensitivity index (ISI) according to the following equation of Matsuda (28):

$$\text{ISI} = \frac{1000}{\sqrt{(\text{FPG} \cdot\ \text{FPI})(\text{mean OGTT insulin concentration})(\text{mean OGTT glucose concentration})}}$$

where FPG is the fasting plasma glucose concentration, FPI is the fasting insulin concentration and 1000 represents a

TABLE 3. Energy expenditure and substrate oxidation during 30 min of steady-state exercise at 50% pretraining $\dot{V}O_{2max}$ before and after training.

	TP_{CON}		TP_{INT}	
	Pre	Post	Pre	Post
HR (bpm)	116 ± 3	113 ± 2	115 ± 4	112 ± 4
$\dot{V}O_2$ ($mL \cdot min^{-1}$)	1580 ± 52	1630 ± 62	1561 ± 110	1552 ± 118
RER	0.91 ± 0.01	0.87 ± 0.01*	0.89 ± 0.00	0.89 ± 0.01
EE ($kcal \cdot min^{-1}$)	8.44 ± 0.27	8.54 ± 0.29	8.25 ± 0.58	8.19 ± 0.62
Fat oxidation ($g \cdot min^{-1}$)	0.25 ± 0.02	0.35 ± 0.02*	0.29 ± 0.02	0.29 ± 0.04

Values are expressed as means ± SE. HR, heart rate; $\dot{V}O_2$, oxygen uptake; RER, respiratory exchange ratio; EE, energy expenditure. * Significantly different from pretraining, $P < 0.01$.

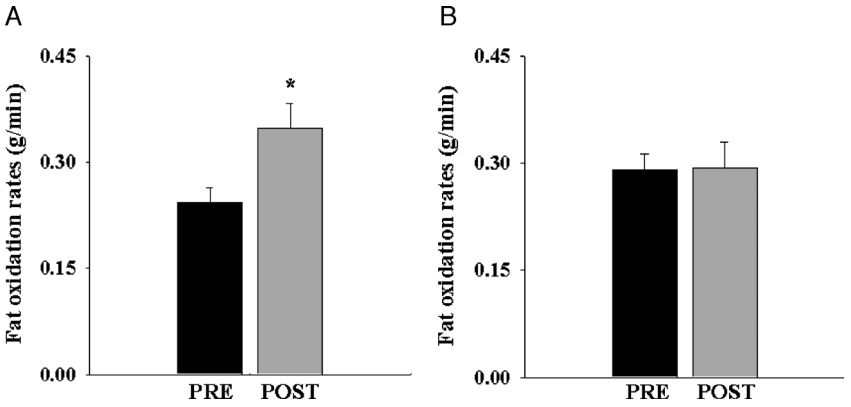

FIGURE 2—Absolute fat oxidation rates during 30 min of steady-state exercise at 50% pretraining $\dot{V}O_{2max}$, before and after training for both TP_{CON} (*A*) and TP_{INT} (*B*). * Significant difference from pretraining program, $P < 0.05$.

constant that allows numbers ranging between 1 and 12 to be obtained. The square root conversion is used to correct the nonlinear distribution of values.

Statistics. Data are expressed as means ± SE unless otherwise stated. Variables were compared using exercise (pre-, posttraining) × type (TP_{CON}, TP_{INT}) repeated-measures analyses of variance. Correlations (Pearson product) were performed between percent changes in selected variables. Statistical significance was accepted if $P < 0.05$.

RESULTS

Participants. There were no significant differences at baseline between the pretraining groups in body weight, waist to hip ratio (WHR), body mass index (BMI), percent body fat (%BF), or $\dot{V}O_{2max}$ (Table 1). All of the characteristics shown remained unaltered after 4 wk of exercise training at either TP_{CON} or TP_{INT}.

Exercise training. Average energy expenditure per training session was not different between TP_{CON} and TP_{INT} during any of the training weeks 1–4 (TP_{CON}; 231 ± 22, 314 ± 28, 391 ± 38, and 477 ± 50 and 258 ± 15, 354 ± 23, 419 ± 28, and 481 ± 28 kcal·wk^{-1}, respectively), whereas average training intensity for TP_{CON} was 44 ± 6% $\dot{V}O_{2max}$, with the low and high intensities for TP_{INT} being 25 ± 6 and 65 ± 6% $\dot{V}O_{2max}$, respectively. Average fat used during the training programs was twofold higher in the TP_{CON} sessions compared with the TP_{INT} sessions (366 ± 87 and 171 ± 76 g, respectively, for the 4-wk duration, $P < 0.001$). All participants fully completed the prescribed duration of 900 min during the 4-wk block for both training programs. Of the eight participants, seven completed all of their training sessions supervised in the laboratory. The remaining participant had two sessions per week supervised in the laboratory.

Graded exercise to exhaustion. Although there was no change in $\dot{V}O_{2max}$ after training, on average, all participants continued exercise for at least one extra stage. This equates to a significant increase in time to exhaustion of 5.2% after TP_{CON} and 6% after TP_{INT} ($P < 0.05$). There was no shift in the exercise intensity at which maximal fat oxidation occurred (Table 2).

Substrate use and energy expenditure during steady-state exercise. There was no difference in average workload or energy expenditure during 30 min of steady-state exercise before and after training for either TP_{CON} or TP_{INT} (Table 3). Although there was a main exercise effect (F (1,7) = 6.618, $P < 0.05$) before and after training for HR, there was no difference between training programs.

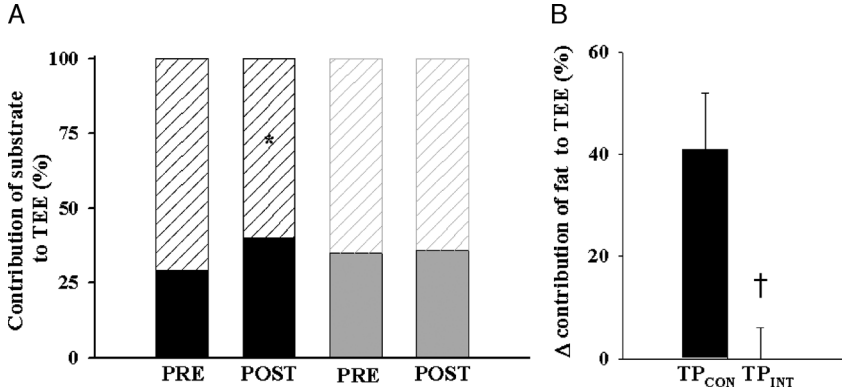

FIGURE 3—Contribution of substrates to total energy expenditure (*A*) and percent change in fat contribution (*B*) during 30 min of steady-state exercise at 50% $\dot{V}O_{2max}$. *Black* and *gray bars* represent TP_{CON} and TP_{INT}, respectively, with *solid* and *hatched bars* representing fat and CHO contribution, respectively. * Significant difference from pretraining program, $P < 0.05$; † significant difference between training programs, $P < 0.05$.

TABLE 4. Effects of exercise training on fasting plasma glucose, insulin, and FFA.

	TP_{CON}		TP_{INT}	
	Pre	Post	Pre	Post
FPG (mM)	5.6 ± 0.2	5.3 ± 0.2	5.8 ± 0.2	5.7 ± 0.2
FPI ($\mu U \cdot mL^{-1}$	20.7 ± 5.1	19.7 ± 5.2	21.2 ± 4.1	20.6 ± 11.2
FFA ($\mu mol \cdot mL^{-1}$)	224 ± 47	197 ± 29	230 ± 22	218 ± 33

Values are means ± SE (N = 8). FPG, fasting plasma glucose; FPI, fasting plasma insulin.

However, there was a significant reduction in RER after TP_{CON} ($P < 0.01$), but not after TP_{INT} (Table 3), with the change in RER after TP_{CON} being significantly different from the change after TP_{INT} ($P < 0.005$). As total energy expenditure remained constant between trials, the reduction in RER during exercise meant that there was a significant increase in the fat oxidation rate during exercise. Rates of fat oxidation increased by 44% after TP_{CON}, but no change was observed after TP_{INT} (Fig. 2). Similar differences were observed when expressed as percentage of the contribution of fat to total energy expenditure (F (1,7) = 19.58, $P < 0.005$) (Fig. 3), with increases seen after TP_{CON} (29 ± 2 vs 40 ± 3%, $P < 0.005$) but not TP_{INT}.

Insulin sensitivity. Neither fasting plasma glucose nor insulin changed significantly after exercise training in either program (Table 4). Although an exercise effect was observed in glucose AUC such that training reduced glucose AUC, there was no significant difference between groups (−13 ± 3 and −7 ± 3% after TP_{CON} and TP_{INT}, respectively) (Fig. 4A). Insulin AUC was significantly reduced after TP_{CON} (8967 ± 635 and 7438 ± 784 $\mu U \cdot mL^{-1} \cdot 120\ min^{-1}$ before and after, respectively; $P < 0.05$) but not TP_{INT} (Fig. 4B).

As shown in Figure 5, there was a 27% increase in the insulin sensitivity index after TP_{CON} (2.47 ± 0.25 and 3.13 ± 0.31 before and after, respectively; $P < 0.001$), but no change was observed after TP_{INT}. The percent change in the insulin sensitivity index was associated with the reduction in insulin AUC ($r = -0.751$, $P < 0.01$).

Relationships between changes in fat oxidation and changes in glucose tolerance. Pearson correlations were carried out between percent change in fat oxidation rate during the 30-min steady-state exercise before and after training and percent changes in glucose and insulin AUC during a 2-h OGTT and the insulin sensitivity index of Matsuda before and after training. It was shown that there was a significant relationship between change in fat oxidation rate during the 30-min steady-state exercise bout and change in insulin AUC ($r = -0.438$, $P < 0.05$), and also between change in fat oxidation rate during the 30-min steady-state exercise bout and change in insulin sensitivity ($r = 0.476$, $P < 0.05$).

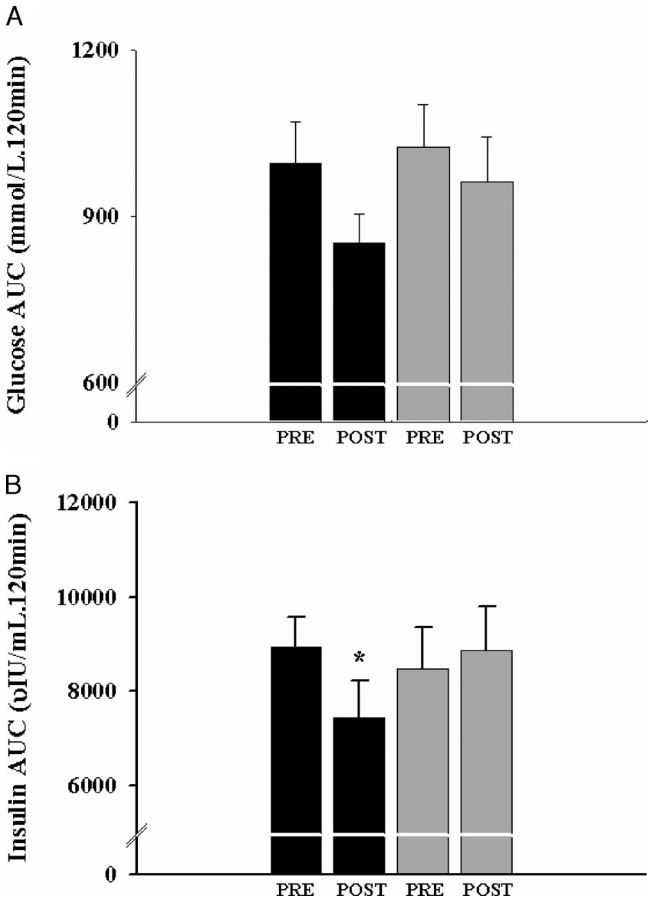

FIGURE 4—Glucose (*A*) and insulin (*B*) AUC before and after training. *Black* and *gray bars* represent TP_{CON} and TP_{INT}, respectively. * Significant difference from pretraining program, $P < 0.05$.

DISCUSSION

Obesity and physical inactivity are associated with a reduced reliance on lipid oxidation and reduced insulin sensitivity, and there is evidence to suggest that the two states

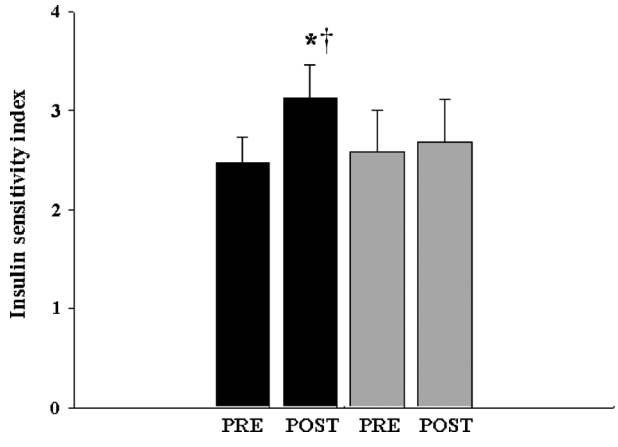

FIGURE 5—Insulin sensitivity index before and after training. *Black* and *gray bars* represent TP_{CON} and TP_{INT}, respectively. * Significant difference from pretraining program, $P < 0.05$; † significant difference between training programs, $P < 0.05$.

are in some way causally related. The major finding of the present study was that when energy expenditure is kept constant during daily exercise sessions, exercise intensity plays an important role in determining improvements in health-related parameters such as fat oxidation and insulin sensitivity in obese, middle-aged men. It was also demonstrated that these changes in fat oxidation and insulin sensitivity after exercise training are correlated, and that the improvements associated with continuous low-intensity exercise can be brought about within 4 wk.

Our findings that continuous, low-intensity exercise training improves fat oxidation by 44% during exercise are in agreement with those of a number of studies that have investigated similar changes in healthy (38), obese men (46), and upper-body obese women (45). It was concluded that the increase in total body fat oxidation observed in the above studies was not brought about by increases in plasma FA oxidation but, rather, by an increase in nonplasma FA oxidation (38,46). These increases observed in fat oxidation after exercise training could be attributable to increased mitochondrial content (14) or to increased activity of carnitine palmitoyltransferase-1 (CPT-1), an important rate-limiting step in mitochondrial FA uptake and subsequent oxidation. Cross-sectional studies have reported that CPT-1 activity is reduced in the skeletal muscle of obese individuals (25) and is increased in the muscle of trained individuals compared with untrained individuals (21,43). Furthermore, it has recently been observed that CPT-1 activity can be elevated in the muscle of obese individuals after endurance training (9) and that this increase is associated with an increased mitochondrial FA oxidation. Interestingly, Bezaire et al. (5) have identified the presence of FAT/CD36 on the mitochondrial membrane within skeletal muscle and have shown that its presence, along with CPT-1, is required for long-chain FA oxidation. In addition, Schenk and Horowitz (37) have reported that endurance exercise training can alter the localization of FAT/CD36 and increase its colocalization with CPT-1 at the mitochondrial membrane. This increase in colocalization of FAT/CD36 and CPT-1 was highly correlated to the increase in whole-body fat oxidation.

However, our findings of constant-intensity exercise of relatively low intensity improving insulin sensitivity is in direct contrast to a number of studies that have found no change in insulin sensitivity during low-intensity exercise in both healthy (8,39) and obese (18,22) groups. Seals et al. (39) investigated the effects of 6 months of low-intensity exercise training (walking 4–5 $d \cdot wk^{-1}$ for 30 min per session at approximately 60% HRmax) followed by 6 months of high-intensity exercise training (cycling, treadmill walking, or jogging 3–4 $d \cdot wk^{-1}$ for 30–45 min per session at 80–90% HRmax) in healthy older individuals. They reported 8% and 23% decreases in insulin AUC after low- and high-intensity exercise, respectively, and they conclude, therefore, that high-intensity exercise was more beneficial in terms of increased insulin sensitivity. However, it must be noted that the 23% decrease in insulin AUC at the end of the 12 months of training was compared with before any training; therefore, it is a combination of both low- and high-intensity training, and there was no significant decrease during the high-intensity period alone in insulin AUC. Similar to these findings are those of Kang et al. (22), who report that insulin AUC was decreased after 7 d of cycling exercise at 70% $\dot{V}O_{2peak}$, yet no change was observed after exercise at 50% $\dot{V}O_{2peak}$.

Independent of either intensity or volume of exercise, Houmard et al. (18) have demonstrated that it is session duration that is the most important determinant of increased insulin sensitivity. However, in this study, one group that displayed improved insulin sensitivity with high-intensity exercise had a significantly reduced body mass. We know that reduced body mass alone without exercise has been shown to increase insulin sensitivity (15) and that this may at least partly explain the improved insulin sensitivity in this group. In a study by Oshida et al. (29) in which body mass was kept constant, a low-intensity training program (40% $\dot{V}O_{2max}$), consisting of 30–40 $min \cdot d^{-1}$, 4 $d \cdot wk^{-1}$, improved insulin sensitivity without any change in $\dot{V}O_{2max}$, a finding consistent with the present study.

After interval training (TP_{INT}), we observed no increase in fat oxidation or improvements in whole-body insulin sensitivity. This somewhat surprising finding may be explained by the different metabolic responses to each intervention—specifically, the amount of fat oxidized during training. During TP_{INT}, 11.4 g of fat was oxidized during a 60-min training session, whereas during TP_{CON} it was more than twice this amount at 24.4 g, and this may go someway to explain differences between the programs. Although there is some evidence within the literature to suggest that high-intensity aerobic interval training can increase whole-body fat oxidation during exercise (44) and, indeed, improve insulin sensitivity (7,10,27), there are many differences between the studies that may explain our conflicting findings. The most obvious difference between the studies is the training intensity chosen; Talanian et al. used 10 × 4-min bouts at 90% $\dot{V}O_{2max}$ (total: 40 min at 90% $\dot{V}O_{2max}$ per session), whereas the present study used six 5-min bouts at 65% $\dot{V}O_{2max}$ (total: 30 min at 65% $\dot{V}O_{2max}$)—a much lower volume, intensity, and, therefore, total energy expenditure. In addition, the participants used in that study were moderately active women, whereas in the present study sedentary obese men were investigated, and there is evidence to suggest that both gender (women) and weekly activity levels are associated with higher rates of fat oxidation (47). As far as application, the intensity used by Talanian et al. would far exceed the tolerance of the obese sedentary male.

With respect to insulin sensitivity, studies that measured whole-body glucose tolerance (7,27) after high-intensity bouts of exercise are actually investigating the acute effects of exercise, which is quite different from those following a period of training, as investigated in the present study. Indeed, Larsen et al. compared glucose tolerance for a period of time either with high-intensity exercise or without (27).

Burgomaster et al. (10) performed their investigations at 72 h after the final training session, but they did not directly measure whole-body insulin sensitivity; instead, they report that GLUT-4 protein content had increased by 25%. This is a much lower increase than the 98% increase observed during the more traditional, moderate-intensity training (17), and it could be argued that an increase of 25% in GLUT-4 protein content after high-intensity exercise is not sufficient to bring about any improvement in whole-body insulin sensitivity.

The present study has also demonstrated that a significant positive correlation exists between improvements in fat oxidation and improvements in insulin sensitivity. It is well documented that exercise intensity is one of the most important factors determining substrate use (1,4,35,41,47). Achten et al. (1) have reported that maximal rates of fat oxidation occur at an exercise intensity of 65% $\dot{V}O_{2max}$ in a group of moderately trained cyclists. When a more heterogeneous population group was examined (47), FATmax, on average, was found to occur at 48% $\dot{V}O_{2max}$, with a high positive correlation between FATmax and weekly physical activity ($r = 0.31$, $P = 0.00$). This study also demonstrates that a high level of interindividual variation exists in both maximal rates of fat oxidation and the intensity at which maximal rates of fat oxidation occur (FATmax). We therefore designed individual training programs for our study volunteers at the intensity that would elicit maximal fat oxidation. If we relate this to the findings of the studies above that have demonstrated that endurance training in obese men increases mitochondrial FA oxidation (9) not by increasing plasma FA oxidation, but by means of increasing nonplasma FA oxidation (46), we can connect it to studies in which the accumulation of DAG correlates with the onset of insulin resistance (26,48), and we can speculate that the training program TP_{CON} has brought about improvements in insulin sensitivity through its ability to increase reliance on intramuscular lipids during exercise and, therefore, reduce the accumulation of associated metabolites.

A second mechanism through which improvements in insulin sensitivity could be brought about after continuous, low-intensity endurance training could be the reduction in plasma FA. The decrease we have reported in plasma FA concentrations, although not significant, could partly explain the increase observed in insulin sensitivity. Studies have shown that manipulating plasma FA can alter insulin resistance. Santomauro et al. (36) have shown that lowering FA levels with overnight acipimox administration improves insulin resistance in obese and diabetic subjects who have chronically elevated plasma FA concentrations, whereas Belfort et al. (3) have shown that elevating plasma FA with a lipid infusion can induce insulin resistance in normal, glucose-tolerant individuals. Although we observed a 15% decrease in plasma FA concentrations after TP_{CON}, this did not quite reach statistical significance. This may be attributable to the fact that the pretraining fasted plasma FA concentrations found in the individuals in the present study (261 ± 44 vs 197 ± 14 and 240 ± 23 vs 230 ± 35 μM before and after TP_{CON} and TP_{INT}, respectively) were low compared with the values reported in other studies (12) (493 ± 76 vs 294 ± 24 μM before and after exercise training, respectively).

In summary, our study has shown that a training program encompassing continuous, low-intensity exercise can improve fat oxidation, and that this improvement in fat oxidation is associated with enhanced insulin sensitivity in obese, middle-aged men. This could have important clinical relevance when considering exercise prescription as a means for improving such health-related parameters.

The authors acknowledge Christopher Mann and Guftar Shaikh for giving their time to the health checks and also to Benjamin Heywood for his help with data collection. We would also like to thank all of our participants for their time and commitment.

REFERENCES

1. Achten J, Gleeson M, Jeukendrup AE. Determination of the exercise intensity that elicits maximal fat oxidation. *Med Sci Sports Exerc*. 2002;34(1):92–7.
2. American College of Sports Medicine. *ACSM's Guidelines for Exercise Testing and Prescription*, 6th ed. Philadelphia (PA): Lippincott Williams and Wilkins; 2000.
3. Belfort R, Mandarino L, Kashyap S, et al. Dose-response effect of elevated plasma free fatty acid on insulin signaling. *Diabetes.* 2005;54:1640–8.
4. Bergman BC, Brooks GA. Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *J Appl Physiol*. 1999;86:479–87.
5. Bezaire V, Bruce CR, Heigenhauser GJ, et al. Identification of fatty acid translocase on human skeletal muscle mitochondrial membranes: essential role in fatty acid oxidation. *Am J Physiol Endocrinol Metab*. 2006;290:E509–15.
6. Boden G, Lebed B, Schatz M, Homko C, Lemieux S. Effects of acute changes of plasma free fatty acids on intramyocellular fat content and insulin resistance in healthy subjects. *Diabetes.* 2001;50:1612–7.
7. Bonen A, Ball-Burnett M, Russel C. Glucose tolerance is improved after low- and high-intensity exercise in middle-age men and women. *Can J Appl Physiol*. 1998;23:583–93.
8. Borghouts LB, Backx K, Mensink MF, Keizer HA. Effect of training intensity on insulin sensitivity as evaluated by insulin tolerance test. *Eur J Appl Physiol Occup Physiol*. 1999;80:461–6.
9. Bruce CR, Thrush AB, Mertz VA, et al. Endurance training in obese humans improves glucose tolerance, mitochondrial fatty acid oxidation and alters muscle lipid content. *Am J Physiol Endocrinol Metab*. 2006;291:E99–107.
10. Burgomaster KA, Cermak NM, Phillips SM, Benton CR, Bonen A, Gibala MJ. Divergent response of metabolite transport proteins in human skeletal muscle after sprint interval training and detraining. *Am J Physiol Regul Integr Comp Physiol*. 2007;292:R1970–6.
11. Colberg SR, Simoneau JA, Thaete FL, Kelley DE. Skeletal muscle utilization of free fatty acids in women with visceral obesity. *J Clin Invest*. 1995;95:1846–53.
12. Crampes F, Marion-Latard F, Zakaroff-Girard A, et al. Effects of a longitudinal training program on responses to exercise in overweight men. *Obes Res*. 2003;11:247–56.
13. Durnin JV, Womersley J. Body fat assessed from total body

density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr*. 1974;32:77–97.
14. Gollnick PD, Saltin B. Significance of skeletal muscle oxidative enzyme enhancement with endurance training. *Clin Physiol*. 1982;2: 1–12.
15. Goodpaster BH, Kelley DE, Wing RR, Meier A, Thaete FL. Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. *Diabetes.* 1999;48:839–47.
16. Goodpaster BH, Theriault R, Watkins SC, Kelley DE. Intramuscular lipid content is increased in obesity and decreased by weight loss. *Metabolism.* 2000;49:467–72.
17. Gulve EA, Spina RJ. Effect of 7–10 days of cycle ergometer exercise on skeletal muscle GLUT-4 protein content. *J Appl Physiol*. 1995;79:1562–6.
18. Houmard JA, Tanner CJ, Slentz CA, Duscha BD, McCartney JS, Kraus WE. Effect of the volume and intensity of exercise training on insulin sensitivity. *J Appl Physiol*. 2004;96:101–6.
19. Hulver MW, Berggren JR, Cortright RN, et al. Skeletal muscle lipid metabolism with obesity. *Am J Physiol Endocrinol Metab*. 2003;284:E741–7.
20. Jeukendrup AE, Wallis GA. Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int J Sports Med*. 2005;26(Suppl. 1):S28–37.
21. Jong-Yeon K, Hickner RC, Dohm GL, Houmard JA. Long- and medium-chain fatty acid oxidation is increased in exercise-trained human skeletal muscle. *Metabolism.* 2002;51:460–4.
22. Kang J, Robertson RJ, Hagberg JM, et al. Effect of exercise intensity on glucose and insulin metabolism in obese individuals and obese NIDDM patients. *Diabetes Care.* 1996;19:341–9.
23. Kelley DE, Goodpaster B, Wing RR, Simoneau JA. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *Am J Physiol*. 1999;277:E1130–41.
24. Kim JK, Gimeno RE, Higashimori T, et al. Inactivation of fatty acid transport protein 1 prevents fat-induced insulin resistance in skeletal muscle. *J Clin Invest*. 2004;113:756–63.
25. Kim JY, Hickner RC, Cortright RL, Dohm GL, Houmard JA. Lipid oxidation is reduced in obese human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2000;279:E1039–44.
26. Kraegen EW, Saha AK, Preston E, et al. Increased malonyl-CoA and diacylglycerol content and reduced AMPK activity accompany insulin resistance induced by glucose infusion in muscle and liver of rats. *Am J Physiol Endocrinol Metab*. 2006;290:E471–9.
27. Larsen JJ, Dela F, Madsbad S, Galbo H. The effect of intense exercise on postprandial glucose homeostasis in type II diabetic patients. *Diabetologia.* 1999;42:1282–92.
28. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care.* 1999;22:1462–70.
29. Oshida Y, Yamanouchi K, Hayamizu S, Sato Y. Long-term mild jogging increases insulin action despite no influence on body mass index or VO2 max. *J Appl Physiol*. 1989;66:2206–10.
30. Pan DA, Lillioja S, Kriketos AD, et al. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes.* 1997;46:983–8.
31. Perseghin G, Price TB, Petersen KF, et al. Increased glucose transport-phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. *N Engl J Med*. 1996;335:1357–62.
32. Perseghin G, Scifo P, De Cobelli F, et al. Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H-13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes.* 1999;48:1600–6.
33. Phillips DI, Caddy S, Ilic V, et al. Intramuscular triglyceride and muscle insulin sensitivity: evidence for a relationship in nondiabetic subjects. *Metabolism.* 1996;45:947–50.
34. Ranneries C, Bulow J, Buemann B, Christensen NJ, Madsen J, Astrup A. Fat metabolism in formerly obese women. *Am J Physiol*. 1998;274:E155–61.
35. Romijn JA, Coyle EF, Sidossis LS, et al. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am J Physiol*. 1993;265:E380–91.
36. Santomauro AT, Boden G, Silva ME, et al. Overnight lowering of free fatty acids with Acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects. *Diabetes.* 1999;48:1836–41.
37. Schenk S, Horowitz JF. Coimmunoprecipitation of FAT/CD36 and CPT I in skeletal muscle increases proportionally with fat oxidation after endurance exercise training. *Am J Physiol Endocrinol Metab*. 2006;291:E254–60.
38. Schrauwen P, van Aggel-Leijssen DP, Hul G, et al. The effect of a 3-month low-intensity endurance training program on fat oxidation and acetyl-CoA carboxylase-2 expression. *Diabetes.* 2002;51: 2220–6.
39. Seals DR, Hagberg JM, Hurley BF, Ehsani AA, Holloszy JO. Effects of endurance training on glucose tolerance and plasma lipid levels in older men and women. *JAMA.* 1984;252:645–9.
40. Segal KR, Edano A, Abalos A, et al. Effect of exercise training on insulin sensitivity and glucose metabolism in lean, obese, and diabetic men. *J Appl Physiol.* 1991;71:2402–11.
41. Sidossis LS, Gastaldelli A, Klein S, Wolfe RR. Regulation of plasma fatty acid oxidation during low- and high-intensity exercise. *Am J Physiol*. 1997;272:E1065–70.
42. Simoneau JA, Colberg SR, Thaete FL, Kelley DE. Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. *FASEB J*. 1995;9:273–8.
43. Starritt EC, Howlett RA, Heigenhauser GJ, Spriet LL. Sensitivity of CPT I to malonyl-CoA in trained and untrained human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2000;278:E462–8.
44. Talanian JL, Galloway SD, Heigenhauser GJ, Bonen A, Spriet LL. Two weeks of high-intensity aerobic interval training increases the capacity for fat oxidation during exercise in women. *J Appl Physiol.* 2007;102:1439–47.
45. van Aggel-Leijssen DP, Saris WH, Wagenmakers AJ, Hul GB, van Baak MA. The effect of low-intensity exercise training on fat metabolism of obese women. *Obes Res*. 2001;9:86–96.
46. van Aggel-Leijssen DP, Saris WH, Wagenmakers AJ, Senden JM, van Baak MA. Effect of exercise training at different intensities on fat metabolism of obese men. *J Appl Physiol*. 2002;92:1300–9.
47. Venables MC, Achten J, Jeukendrup AE. Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. *J Appl Physiol*. 2005;98:160–7.
48. Yu C, Chen Y, Cline GW, et al. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. *J Biol Chem*. 2002;277:50230–6.