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Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study

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Venables, Michelle C., Juul Achten, and Asker E. Jeukendrup. Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. *J Appl Physiol* 98: 160–167, 2005. First published August 27, 2004; doi:10.1152/japplphysiol.00662.2003.—The aim of the present study was to establish fat oxidation rates over a range of exercise intensities in a large group of healthy men and women. It was hypothesised that exercise intensity is of primary importance to the regulation of fat oxidation and that gender, body composition, physical activity level, and training status are secondary and can explain part of the observed interindividual variation. For this purpose, 300 healthy men and women (157 men and 143 women) performed an incremental exercise test to exhaustion on a treadmill [adapted from a previous protocol (Achten J, Venables MC, and Jeukendrup AE. *Metabolism* 52: 747–752, 2003)]. Substrate oxidation was determined using indirect calorimetry. For each individual, maximal fat oxidation (MFO) and the intensity at which MFO occurred (Fat_{max}) were determined. On average, MFO was 7.8 ± 0.13 mg·kg fat-free mass $(FFM)^{-1}\cdot min^{-1}$ and occurred at 48.3 ± 0.9% maximal oxygen uptake ($\dot{V}O_{2\,max}$), equivalent to 61.5 ± 0.6% maximal heart rate. MFO (7.4 ± 0.2 vs. 8.3 ± 0.2 $mg\cdot kg\cdot FFM^{-1}\cdot min^{-1}$; $P < 0.01$) and Fat_{max} (45 ± 1 vs. 52 ± 1% $\dot{V}O_{2\,max}$; $P < 0.01$) were significantly lower in men compared with women. When corrected for FFM, MFO was predicted by physical activity (self-reported physical activity level), $\dot{V}O_{2\,max}$, and gender ($R^2 = 0.12$) but not with fat mass. Men compared with women had lower rates of fat oxidation and an earlier shift to using carbohydrate as the dominant fuel. Physical activity, $\dot{V}O_{2\,max}$, and gender explained only 12% of the interindividual variation in MFO during exercise, whereas body fatness was not a predictor. The interindividual variation in fat oxidation remains largely unexplained.

indirect calorimetry; substrate crossover; physical activity

IT IS WELL DOCUMENTED THAT carbohydrate (CHO) and fatty acids are the dominant fuels oxidized by the muscle for energy production during exercise and that the absolute and relative contribution of these fuels can be influenced by diet (4, 16, 30), muscle glycogen content (75, 76), exercise intensity (4, 23, 51, 52, 55, 69), duration (51), and training status (29, 34, 72). Some controversy still exists as to whether gender modulates fat oxidation because studies have reported that fat oxidation in women makes a larger contribution to oxidative metabolism than men (66–68), whereas others did not find such an effect (15, 52).

One of the most important regulators of substrate oxidation is exercise intensity, because it has been demonstrated that increases in glycolytic flux will inhibit long-chain fatty acid transport into the mitochondria and therefore reduce long-chain fatty acid oxidation (17, 55). It has been suggested that the reduction in long-chain fatty acid transport into the mitochondria could be a consequence of the reduced pH brought about through an accumulation of H^+ ion during high-intensity exercise because a reduction in pH has been demonstrated to inhibit the activity of carnitine palmitoyl transferase I (61), a key enzyme in fatty acid transport. Such increases in glycolytic flux will also have the effect of increasing the production of pyruvate and ultimately an increase in lactate accumulation. It has been shown both in dogs (22) and humans (5) that lactate can directly inhibit adipose tissue free fatty acid release. More recently, Achten et al. (2) have demonstrated that in healthy trained men the onset of plasma lactate accumulation occurs at the same exercise intensity (Fat_{max}) as for maximal fat oxidation (MFO).

Although the above studies have identified a number of important factors regulating fat oxidation, it is apparent from these studies that a considerable degree of interindividual variability in substrate utilization persists even when these factors have been controlled for. Helge et al. (27) found the respiratory exchange ratio (RER) to range between 0.83 and 0.95 in a group of untrained men exercising at 55% maximal oxygen uptake ($\dot{V}O_{2\,max}$). Goedecke et al. (26) studied 61 trained cyclists and measured RER at rest and during exercise at three different intensities (25, 50, and 70% of peak power output), and they found that resting RER ranged between 0.72 and 0.93 and that this degree of variability remained throughout all exercise intensities.

Studies investigating RER at rest and during exercise give conflicting explanations behind the variability, with body composition being attributed to some of the variance in certain studies (73, 79) but not in all (26, 27). In addition to diet, exercise intensity and duration, and muscle glycogen content, other contributory factors are thought to include proportion of type I muscle fibers, dietary fat intake, training status, and blood metabolites [plasma lactate and serum free fatty acid (FFA) concentrations] (26). Also, there is still controversy behind the importance of gender and body composition on substrate oxidation.

An inability to oxidize lipids appears to be an important factor in the etiology of obesity. A study investigating Pima Indians has shown that an elevated 24-h respiratory quotient, indicative of reduced levels of fat oxidation, is associated with a high rate of weight gain (79). Obesity is a condition associated with increased intramuscular triglycerides and insulin resistance, in which resting levels of fat oxidation is disturbed

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Table 1. *Subjects' characteristics*

Variable	Combined Group (n = 300)	Women (n = 143)	Men (n = 157)
Age, y	31±12 (18–65)	32±12 (18–65)	30±11 (18–65)
BF, %	21.5±7.7 (5.7–40.8)	23.7±7.2 (9.2–40.8)	19.5±7.6*(5.7–39.8)
Body mass, kg	76.2±15.8 (46.2–131.8)	66.9±11.1 (46.2–112.7)	84.6±14.8*(55.1–131.8)
Height, m	1.72±0.10 (1.51–1.95)	1.64±0.06 (1.51–1.83)	1.78±0.07*(1.62–1.95)
BMI, kg/m^2	26±4 (18–47)	25±4 (18–47)	26±4*(18–42)
FM, kg	16.8±8.2 (3.4–46.2)	16.4±7.4 (4.9–46.0)	17.2±8.9 (3.4–46.2)
FFM, kg	59.5±11.6 (35.6–91.9)	50.5±6.1 (35.6–73.0)	67.6±9.1*(50.1–91.9)
$\dot{V}O_{2max}$, ml·kg^{-1}·min^{-1}	46.3±0.7 (20.9–82.4)	41.4±0.9 (20.9–67.6)	50.7±0.9*(22.5–82.4)
$\dot{V}O_{2max}$ ml·kg FFM^{-1}·min^{-1}	58.1±0.6 (30.7–90.6)	53.5±0.9 (30.7–80.1)	62.3±0.8*(37.3–90.6)
SRPAL, kcal·wk	3,665±165 (0–14,321)	3,074±152 (0–11,335)	4,230±171*(0–19,849)
HR_{max} beats/min	190±14 (141–223)	190±14 (141–218)	191±12 (156–223)

Values are means ± SD with range in parentheses, except maximal oxygen consumption ($\dot{V}O_{2max}$) and self-reported physical activity level (SRPAL) are means ± SE. BF, body fat; BMI, body mass index; FM, fat mass; FFM, fat-free mass; HR_{max} maximal heart rate. *$P < 0.001$.

(35). This defect persists after weight loss and may predispose to weight regain (35, 48). A better understanding of factors regulating fat oxidation is important for the development of interventions allowing effective treatment of conditions in which fat oxidation is disturbed.

Therefore, the purpose of the present study was to establish whether the changes in fat oxidation rates associated with increases in exercise intensities observed in healthy trained men also occur in a large group of healthy trained and untrained men and women. Second, we determined the contribution of several factors, including gender, body composition, physical activity level, and training status, on fat oxidation. It was hypothesized that exercise intensity would be the primary determinant of fat oxidation rate with such factors as gender, body composition, physical activity level, and training status partially explaining the interindividual differences.

METHODS

Volunteers. Three hundred volunteers participated in the study, which was approved by the South Birmingham Local Research Ethics Committee, UK. All volunteers were healthy as assessed by a general health questionnaire, and no volunteers were accepted onto the study if they were diabetic or were being medically treated for heart or blood pressure irregularities. All volunteers were informed of the purpose and nature of the study, after which their written, informed consent was given.

General design. The 300 volunteers, the physical characteristics of whom are shown in Tables 1 and 2, performed a graded exercise test to exhaustion on a treadmill (PPS 70sport-I, Woodway, Weil am Rhein, Germany). Fat and CHO oxidation were determined by indirect calorimetry and plotted as a function of exercise intensity.

Experimental design. The volunteers reported to the laboratory after a 4-h fast. They had all been instructed to avoid strenuous exercise and alcohol for the preceding 24 h. Body mass and height were determined, and body fat estimations were made by using the four-site skinfold technique according to the method of Durnin and Womersley (19). The exercise protocol used here was adapted from a previously described and validated protocol (1, 3) in which it was concluded that an incremental exercise test with stages of 3-min duration could be used to determine both MFO and Fat_{max}. Briefly, the volunteers started exercising at a speed of 3.5 km/h and at a gradient of 1%. The speed was increased by 1 km/h every 3 min until a speed of either 6.5 or 7.5 km/h was reached. At this point, the gradient was increased by 2% every 3 min until an RER of 1 was reached. Finally, the speed was increased every minute until exhaustion. The aim of the final section of the exercise test was to obtain a measure of $\dot{V}O_{2\,max}$ within a short time. Breath-by-breath measurements were taken throughout exercise by using an automated gas-analysis system (Oxycon Pro, Jaeger, Wuerzberg, Germany). The gas analyzers were calibrated with a 4.95% CO_2-95.05% N_2 gas mixture (BOC Gases, Surrey, UK), and the volume transducer was calibrated with a 3-liter calibration syringe (Jaeger, Wuerzberg, Germany). Heart rate was recorded continuously by telemetry using a heart rate monitor (Polar Vantage NV, Polar Electro Oy, Kempele, Finland).

Indirect calorimetry and calculations. Oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were averaged over the last 2 min of each exercise stage, during which the RER was <1. For each of these stages, fat and CHO oxidation and energy expenditure were calculated by using stoichiometric equations (21), with the assumption that urinary nitrogen excretion rate was negligible. Substrate oxidation rates were then plotted as a function of exercise intensity, expressed as a percentage of maximal oxygen uptake ($\dot{V}O_{2\,max}$). From each fat oxidation curve, several features were identified according to a previously described procedure (1): *1*) MFO, the peak rate of fat oxidation measured over the entire range of exercise intensities, and *2*) Fat_{max}, the exercise intensity at which the fat oxidation rate was maximal.

Table 2. *Stepwise multiple linear regression analyses for MFO and MFO/FFM*

					Coefficients		Correlations		
Dependent Variable	Independent Variable	R	R^2	Adjusted R^2	β	Significance	Zero order	Partial	Part
MFO	FFM	0.60	0.36	0.35	0.41	0.00	0.47	0.29	0.25
	SRPAL				0.15	0.01	0.32	0.16	0.13
	Gender				−0.29	0.00	0.27	−0.22	−0.18
	$\dot{V}O_{2max}$				0.50	0.00	0.23	0.34	0.29
	FM				0.46	0.00	0.19	0.32	0.27
MFO/FFM	Gender	0.36	0.13	0.12	−0.32	0.00	−0.21	−0.31	−0.30
	$\dot{V}O_{2max}$				0.20	0.00	0.15	0.18	0.18
	SRPAL				0.16	0.01	0.17	0.16	0.15

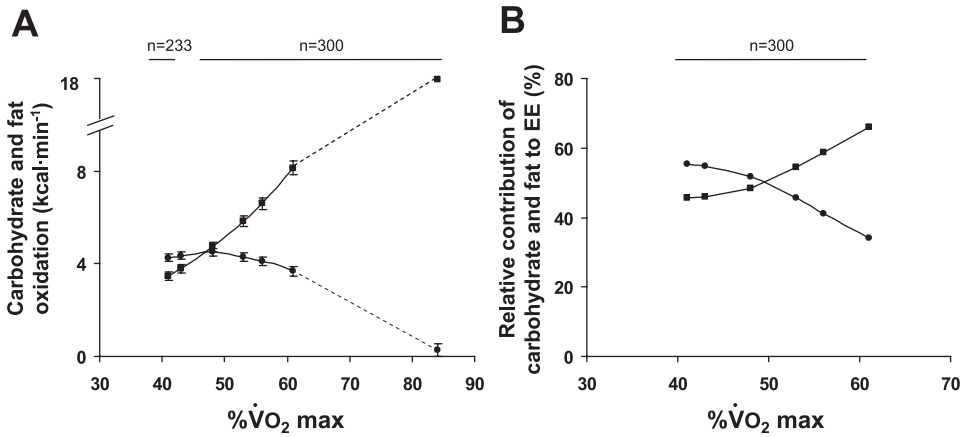

Fig. 1. Mean absolute (*A*) and relative (*B*) substrate energy expenditure at 41, 43, 48, 53, 58, and 61% maximal oxygen uptake ($\dot{V}O_{2\,max}$). Values are means ± SE; *n*, no. of subjects. ▲, Fat; ■, carbohydrate.

In addition, substrate oxidation rates were calculated for 95, 90, and 80% of MFO along with their corresponding exercise intensities. Ventilatory threshold (VT) was calculated for each subject. VT was manually identified as the point at which the plot $\dot{V}O_2$ vs. minute ventilation ($\dot{V}E$) deviated from linearity (41).

Self-reported physical activity levels. All volunteers completed a self-reported physical activity level (SRPAL) questionnaire, adapted from a previously validated questionnaire (44). Physical activity was converted into weekly leisure time energy expenditure by using published tables of energy expenditure for household, recreational, and sports activities (39, 45).

Statistical analysis. Gender differences in substrate utilization across exercise intensities were identified by using repeated-measures ANOVA with post hoc *t*-tests. Differences between the relative exercise intensity ($\%\dot{V}O_{2\,max}$) at VT and MFO were identified by using a *t*-test. Bivariate correlations were carried out between maximal fat oxidation when expressed both in absolute terms (MFO; g/min) and when scaled for fat-free mass (FFM) (MFO/FFM; mg·kg FFM^{-1}·min^{-1}) with the following independent variables: age, body mass, height, body mass index, percent body fat, fat mass, FFM, $\dot{V}O_{2\,max}$ (ml·kg^{-1}·min^{-1}), and SRPAL. Stepwise multiple linear regression analysis was then used to predict MFO and MFO/FFM with all the significant independent variables found in the bivariate analysis. The results were analyzed using the SPSS for Windows version 10.0 (SPSS, Chicago, IL) software package. Values for all descriptives are presented as means ± SD and in all other cases means ± SE.

RESULTS

Figure 1*A* describes the relationship that exists between energy derived from CHO and fat and exercise intensity, expressed as a percentage of $\dot{V}O_{2\,max}$. The initial two data points for both fat and CHO oxidation described in Fig. 1*A* are mean values from 233 subjects; the remainder of points are from all 300 subjects. The data described in the text are for all 300 subjects rather than 233, because using all 300 data sets instead of the 233 only did not change any of our conclusions, nor did it affect the shape of the curve shown in Fig. 1*A*.

Mean substrate oxidation. As exercise intensity increased, absolute fat oxidation rate increased up to a maximum of 0.46 ± 0.01 g/min (range 0.18–1.01 g/min) or 7.8 ± 0.1 mg·kg FFM^{-1}·min^{-1} (range 2.7–15.4 mg·kg FFM^{-1}·min^{-1}). MFO occurred at an intensity of 48 ± 1% $\dot{V}O_{2\,max}$ (range 25–77% $\dot{V}O_{2\,max}$), corresponding to 62 ± 1% maximal heart rate (HR_{max}) (range 41–91 $\%HR_{max}$). This intensity more or less coincides with the "crossover point" of substrate utilization, that is, the point at which CHO becomes the predominant fuel source over fat (Fig. 1*B*). On increasing exercise intensity further, fat oxidation rate started to fall, and RER values of 1 (RER = 1) were reached around 84% $\dot{V}O_{2\,max}$ and 89% HR_{max}. Absolute rates of CHO oxidation, however, continued to rise with increasing exercise intensity. Therefore, when substrate oxidation data were expressed as a percentage of total energy expenditure, at low exercise intensities the relative contribution of fat to total energy expenditure was 55%, decreasing at higher exercise intensities. Concomitantly, the relative contribution of CHO to total enery expenditure increased from 44% at low exercise intensities to become the predominant fuel at higher intensities (Fig. 2).

The VT occurred at an intensity of 65 ± 1% $\dot{V}O_{2\,max}$ (range 45–89% $\dot{V}O_{2\,max}$; 63 ± 1 and 67 ± 1% $\dot{V}O_{2\,max}$ for men and women, respectively), with MFO occurring as reported previously at 48 ± 1% $\dot{V}O_{2\,max}$ (45 ± 1 and 52 ± 1% $\dot{V}O_{2\,max}$ for men and women, respectively). The exercise intensity at VT was found to be significantly higher than the intensity at MFO ($P < 0.01$).

Gender differences. There were no gender differences in mean age or fat mass; however, the men were significantly heavier, were taller, and possessed a higher FFM than women ($P < 0.01$). $\dot{V}O_{2\,max}$ and weekly energy expenditure on the basis of their SRPAL were higher in men than women ($P < 0.01$), with women having a higher percent body fat than men ($P < 0.01$). During exercise, the absolute rate of CHO oxida-

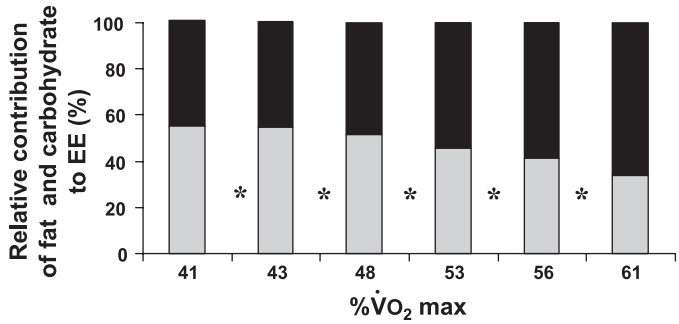

Fig. 2. Mean relative substrate energy expenditure (EE) at 41, 43, 48, 53, 58, and 61% $\dot{V}O_{2\,max}$ Values are means; $n = 300$ subjects. Black bars, carbohydrate; gray bars, fat *Significant difference between adjacent exercise intensities, $P \leq 0.01$.

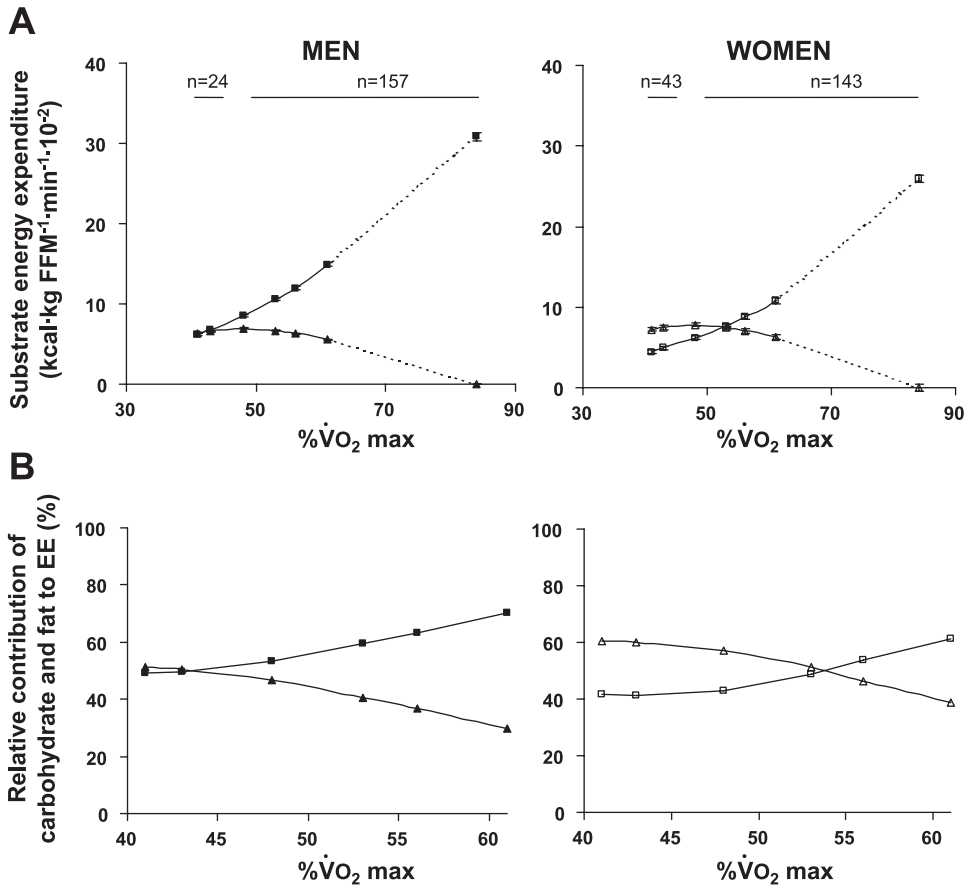

Fig. 3. Gender differences in mean absolute (*A*) and relative (*B*) substrate EE at 41, 43, 48, 58, and 61% $\dot{V}O_{2\,max}$. △, Women fat; ▲, men fat; □, women carbohydrate; ■, men carbohydrate. Values are means ± SE; men $n = 157$ men and $n = 143$ women.

tion was significantly higher in the men, with absolute MFO per kilgram FFM being significantly lower (Fig. 3*A*; $P < 0.01$) compared with the women. Also, the exercise intensity that elicited MFO was significantly lower in the men than the women (Fig. 3*A*: 45 ± 1 vs. 52 ± 1% $\dot{V}O_{2\,max}$; $P < 0.01$). As can be seen from Fig. 3*B*, this is more or less coincident with the lower crossover point of substrate utilization observed in the men. When the oxidation data were expressed as a percentage of total energy expenditure, the contribution of fat oxidation to total energy expenditure was greater in the women than the men with a concomitant lower contribution of CHO oxidation to total ($P < 0.01$). This effect was equal across all exercise intensities (Fig. 4; $P < 0.01$).

Determinants of MFO during exercise. Regression analyses were performed with maximal fat oxidation expressed in absolute (MFO; g/min) terms or when scaled for FFM (MFO/FFM; mg·kg $FFM^{-1}\cdot min^{-1}$) as the dependent variable (Table 2). The predictor variables included were those variables that were significantly correlated with the dependent variable in the bivariate analysis. With MFO as the dependent variable, FFM ($r = 0.47$, $P = 0.00$), height ($r = 0.42$, $P = 0.00$), body mass ($r = 0.42$, $P = 0.00$), SRPAL ($r = 0.31$, $P = 0.00$), gender ($r = 0.28$, $P = 0.00$), $\dot{V}O_{2\,max}$ ($r = 0.26$, $P = 0.00$), body mass index ($r = 0.21$, $P = 0.00$), and fat mass ($r = 0.17$, $P < 0.01$) were entered into the regression model. The results show that only FFM, SRPAL, $\dot{V}O_{2\,max}$, gender, and fat mass are significant predictors of MFO, together accounting for 34% of the variance in MFO. With MFO/FFM as the dependent variable, body mass ($r = -0.13$, $P = 0.03$), $\dot{V}O_{2\,max}$ ($r = 0.17$, $P = 0.003$), SRPAL ($r = 0.17$, $P = 0.003$), and gender ($r = -0.19$, $P = 0.001$) were entered into the regression model. The results show that $\dot{V}O_{2\,max}$, SRPAL, and gender are significant predictors of MFO/FFM, together accounting for 12% of the variance.

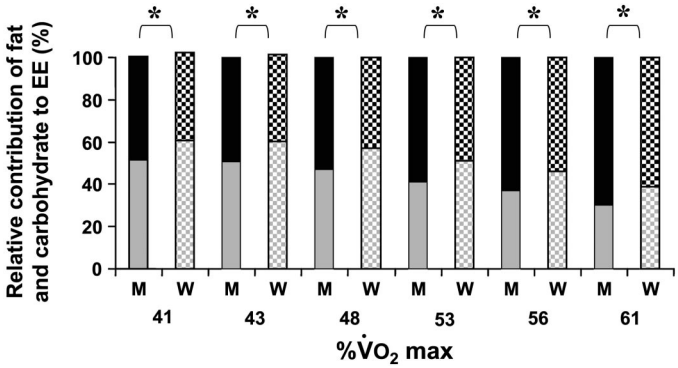

Fig. 4. Gender differences in mean absolute (*A*) and relative (*B*) substrate EE at 41, 43, 48, 58, and 61% $\dot{V}O_{2\,max}$.Values are means; $n = 157$ men and $n = 143$ women. Black bars, carbohydrate; gray bars, fat *Significant difference, $P \leq 0.01$.

DISCUSSION

Exercise intensity has been shown to be one of the most important factors in determining substrate utilization (1, 4, 51, 55, 69). We have demonstrated this to be the case in a wide range of healthy men and women such that, on increasing exercise intensity from low to moderate to high, absolute substrate oxidation can be seen to follow two patterns: CHO oxidation continues to increase, whereas fat oxidation follows an inverted hyperbola. We have shown that fat oxidation increases from ~35% $\dot{V}O_{2\,max}$ to a maximal rate at an intensity of 48 ± 1% $\dot{V}O_{2\,max}$. Further increases in exercise intensity lead to a reduction in fat oxidation. In addition, we have shown that gender and indexes of an individual's level of fitness and activity, such as $\dot{V}O_{2\,max}$ and SRPAL, can account for a small but significant part of the variability in fat oxidation observed in the literature.

Our findings with regard to exercise intensity and substrate oxidation are consistent with the "crossover" concept of fuel utilization discussed by Brooks and colleagues (6–8) in that, during low-intensity exercise, lipids provide slightly more than half of the energy, whereas, as exercise intensity increases, the relative contribution from lipids decreases and that from CHO increases. In our study, we confirmed that the crossover point occurred between 48 and 53% $\dot{V}O_{2\,max}$, consistent with the 50% $\dot{V}O_{2\,max}$ suggested by Brooks and colleagues (6–8, 23, 24). Factors influencing this up- and downregulation of fat oxidation during increases in exercise intensity have been an important area of investigation since 1963 when Randle et al. (47) proposed the classic glucose-fatty acid cycle theory, in which they suggested that an increased availability of FFA to the muscle increases fat oxidation and reduces CHO oxidation. Since this initial investigation of Randle et al., little evidence of the glucose-fatty acid cycle has been found to exist in exercising human skeletal muscle. Moreover, there is an increasing body of evidence to suggest that it is indeed an increase in CHO metabolism that regulates fat metabolism (17, 20, 49, 56, 57) because studies have demonstrated that an increase in glycolytic flux through increases in the exercise intensity (55) or by inducing hyperglycemia and hyperinsulinamia (17) will reduce long-chain fatty acid oxidation. The recent advances and mechanisms behind the fat-CHO interaction are far beyond the scope of this paper, and the readers are directed to pertinent reviews of the area (8, 33, 59, 60).

Primary to the determination of fat and CHO utilization using indirect calorimetry is the assumption that $\dot{V}O_2$ and $\dot{V}CO_2$ measured in expired air will reflect $\dot{V}O_2$ and $\dot{V}CO_2$ at the tissue level. Whereas $\dot{V}O_2$ will reliably reflect tissue $\dot{V}O_2$, $\dot{V}CO_2$ will only be a reliable estimate of tissue $\dot{V}CO_2$ in the presence of a stable bicarbonate pool. During the incremental exercise test used in this study, especially at the higher exercise intensities, it could be argued that acid-base balance has not been reached. Under these circumstances of increased glycolytic flux, lactate will accumulate in the contracting muscle and will begin to move into the extracellular fluid. On the basis of the physicochemical approach to acid-base balance of Stewart (63), in which the acid-base variables such as bicarbonate concentration, H^+ concentration, and the H^+-buffering nonvolatile anion concentration are determined by three independent variables [PCO_2, the total nonvolatile buffer concentration and the strong ion difference (= Σstrong cations − Σstrong anions)], this movement of lactate and other strong ions exchanged between compartments will lead to a decrease in the strong ion difference and an increase in H^+ concentration. The increase in H^+ concentration will be buffered by bicarbonate concentration and ultimately nonoxidative CO_2 produced. This will have the effect of elevating $\dot{V}CO_2$ and therefore overestimating CHO and underestimating fat oxidation. However, Romijn et al. (50) investigated the validity of the indirect calorimetry technique for substrate oxidation determination by comparing it to a breath ^{13}C-to-^{12}C ratio technique, in which absolute substrate oxidation could be determined independently of $\dot{V}CO_2$ and therefore independently of a stable bicarbonate pool. The authors concluded that indirect calorimetry could be used to accurately determine fat and CHO oxidation at exercise intensities up to 80–85% $\dot{V}O_{2\,max}$. To gain an indication of the onset of metabolic acidosis and therefore to validate further our own data, we have calculated VT for each subject because Wasserman et al. (74) described the VT as the work rate just below the onset of metabolic acidosis. We have shown the exercise intensity at VT to be significantly higher than any of the exercise intensities on which we have based our conclusions. Therefore, we believe that the $\dot{V}CO_2$ measured gives us a valid determination of substrate oxidation for the intensities that we have discussed in the present study.

When fat oxidation was scaled for FFM, we confirmed the results of prior gender studies (24, 31, 40, 42, 66–68) in which it was demonstrated that the contribution of lipids to oxidative metabolism is higher in women than in men. In addition, we have shown that women utilize both a higher absolute rate of lipids and a higher relative contribution to total energy expenditure from lipids than men over a wide range of exercise intensities. Men also exhibit a lower crossover point than the women such that they exhibit an earlier dependency on CHO during exercise. It is known that increases in such factors as training history and $\dot{V}O_{2\,max}$ can lead to increased rates of fat oxidation; however, we have shown that, despite the lower $\dot{V}O_{2\,max}$ and activity levels (SRPAL) associated with our study women, lipids still contribute a higher percentage of energy expenditure than the men. There are many mechanisms thought to be involved in the fat oxidation gender dimorphism; briefly, these include levels of circulating hormones (18, 36) and catecholamines (31), muscle fiber type proportion (62), adrenergic regulation of FFA mobilization (28), and activity of hormone-sensitive lipase (38).

The large variation we observed in RER (0.82–0.95) and therefore MFO (0.18–1.01 g/min) compares with previous studies that have shown this trend to be apparent in both trained (26) and untrained (27) subjects. Goedecke et al. (26) investigated the variation in RER in 61 trained cyclists at rest and during exercise at three different workloads (25, 50, and 70% of peak power output). They found RER to vary between 0.72 and 0.93 at rest and that the degree of variation persisted at all exercise intensities. It was concluded that the variability of RER and therefore substrate utilization could be consistently attributed to training volume, dietary fat intake, muscle glycogen content, and circulating substrates. However, these factors could still only account for 58, 45, 42, and 56% of the variability at the respective workloads. A similar degree of variability in RER (0.83–0.95) was found in untrained individuals exercised at 55% $\dot{V}O_{2\,max}$ (27).

In the present study, we have shown that MFO is positively correlated with FFM, gender, $\dot{V}O_{2\,max}$, SRPAL, and fat mass. Schutz et al. (54) demonstrated a positive relationship between body fat and resting levels of fat oxidation in a cross-sectional study of 106 obese women. However, for a given fat mass, there was a large degree of variation in fat oxidation, and so fat mass is not thought to be the major regulatory factor contributing to the increased fat oxidation. Wade et al. (73) also demonstrated a positive relationship between body fat and fat oxidation during exercise, with body fat accounting for 40% of the variation in fat oxidation. However, contradictory studies have shown that both at rest and during exercise body fat shows no correlation with fat oxidation (25–27). The differences in methodologies may partially explain the differences because Wade et al. exercised their subjects at an absolute workload of 100 and not at the same relative workload; also the subjects used in the study were all lean. Our study shows a positive relationship to exist between fat mass and FFM, a relationship also observed by Schutz et al. (54), and a negative relationship between fat mass and both $\dot{V}O_{2\,max}$ and SRPAL. Therefore, the relationship observed by Wade et al. (73), Schutz et al. (54), and ourselves could be attributed to our subjects' fitness and/or activity levels and therefore their FFM and not fat mass. When corrected for FFM, the relationship between MFO and fat mass is lost. These data are also somewhat in line with observations by Nielsen et al. (43), who concluded that FFA release is positively correlated with resting energy expenditure, which in turn is mainly dictated by FFM. However, these authors also found that FFA release was correlated with resting energy expenditure but not with FFM, suggesting that additional factors make resting energy expenditure a better predictor of FFA release than FFM.

Both $\dot{V}O_{2\,max}$ and SRPAL are significant predictors of fat oxidation, regardless of whether we express fat oxidation as a total rate or per kilogram of FFM. This would imply that $\dot{V}O_{2\,max}$ and SRPAL can increase fat oxidation by increasing FFM and also by increasing the capacity of the muscle to oxidize fat. It has been shown in both longitudinal (12, 13, 24, 46) and cross-sectional (14, 34, 37, 58) training studies that trained individuals utilize more fat at the same relative (higher absolute) exercise intensity than untrained individuals. Training has also been shown to be an effective means of increasing fat oxidation during exercise in obese men (71) and women (70) and that the intensity found to induce these changes was low (40% $\dot{V}O_{2\,max}$). Our study found maximal fat oxidation rates at relatively low intensities (44.9 $\pm$ 0.9% $\dot{V}O_{2\,max}$ in men and 51.9 $\pm$ 1.0% $\dot{V}O_{2\,max}$ in women). Schrauwen et al. (53) investigated the effects of a 12-wk low-intensity (40% $\dot{V}O_{2\,max}$) training program on fat oxidation and found that, even without an increase in $\dot{V}O_{2\,max}$, the increase in weekly energy expenditure alone was sufficient to induce changes in fat oxidation during exercise. The data taken from our cross-sectional study shows that a positive correlation exists between SRPAL and MFO.

Unfortunately, because of the nature of the study, we did not control for diet or menstrual phase; therefore, we cannot say how much of the variation in fat oxidation can be attributed to these factors. Although there are studies reporting that neither menstrual phase (64, 78) nor oral contraceptives (32, 65) have any effect on whole body lipid oxidation during moderate-intensity exercise, there are findings that report the opposite (10, 11). However, despite the lack of control for these factors, we have shown that a gender difference does exist in substrate use during exercise. Also it is highly likely that a degree of the variation found in fat oxidation could be accounted for by diet because it has been shown that altering the diet, either to a high-fat/low-CHO (9) or low-fat/high-CHO (16) diet, can increase or decrease fat oxidation, respectively. Therefore, a more carefully controlled study could explain a greater degree of the observed variation in fat oxidation.

In summary, when MFO was corrected for FFM, part of the observed interindividual variability can be attributed to gender and indicators of fitness (SRPAL and $\dot{V}O_{2\,max}$) but not indicators of body fatness (fat mass and percent body fat). Although gender accounts for only a small fraction of the observed total variability in fat oxidation, it is apparent from this study that a gender dimorphism does exist in that women have higher maximal rates of fat oxidation and that lipid remains the dominant fuel at higher exercise intensities than in the men. However, there still remains a large part of the interindividual variation that cannot be explained with the variables investigated in this study.

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