Influence of dietary fatty acid composition and exercise on changes in fat oxidation from a high-fat diet

J. A. Cooper,1 A. C. Watras,2 T. Shriver,2 A. K. Adams,3 and D. A. Schoeller2

1Department of Nutrition, Hospitality, and Retailing, Texas Tech University, Lubbock, Texas; 2Departments of Nutritional Sciences, and 3Family Practice, University of Wisconsin-Madison, Madison, Wisconsin

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Cooper JA, Watras AC, Shriver T, Adams AK, Schoeller DA. Influence of dietary fatty acid composition and exercise on changes in fat oxidation from a high-fat diet. J Appl Physiol 109: 1011–1018, 2010. First published July 22, 2010; doi:10.1152/japplphysiol.01025.2009.—Acute high-fat (HF) diets can lead to short-term positive fat balances until the body increases fat oxidation to match intake. The purpose of this study was to examine the effects of a HF diet, rich in either monounsaturated or saturated fatty acids (FAs) and exercise, on the rate at which the body adapts to a HF diet.13C-labeled oleate and 2H-labeled palmitate were also given to determine the contribution of exogenous vs. global fat oxidation. Eight healthy men (age of 18–45 yr; body mass index of 22 ± 3 kg/m2) were randomized in a 2 × 2 crossover design. The four treatments were a high saturated fat diet with exercise (SE) or sedentary (SS) conditions and a high monounsaturated fat diet with exercise (UE) or sedentary (US) conditions. Subjects stayed for 5 days in a metabolic chamber. All meals were provided. On day 1, 30% of energy intake was from fat, whereas days 2–5 had 50% of energy as fat. Subjects exercised on a stationary cycle at 45% of maximal oxygen uptake for 2 h each day. Respiratory gases and urinary nitrogen were collected to calculate fat oxidation. Change from day 1 to day 5 showed both exercise treatments increased fat oxidation (SE: 76 ± 30 g, P = 0.001; UE: 118 ± 31 g, P < 0.001), whereas neither sedentary condition changed fat oxidation (SS: −10 ± 33 g, P = not significant; US: 41 ± 14 g, P = 0.07). No differences for dietary FA composition were found. Exercise led to a faster adaptation to a HF diet by increasing fat oxidation and achieving fat balance by day 5. Dietary FA composition did not differentially affect 24-h fat oxidation.

THE PREVALENCE OF OBESITY continues to be a leading public health concern in the United States with approximately two-thirds of the adult population being classified as overweight or obese (18). The development of obesity is by definition an accumulation of excess body fat due to positive energy balance. This is generally assumed to be caused by periods of positive energy balance resulting in the storage of fat but can also occur from short periods of positive fat balance even under eucaloric feeding conditions if, as hypothesized by Flatt, the weak human leptin system is overpowered by the stronger carbohydrate balance system leading to positive energy balance during the days following the period of positive fat balance (5). We and others have studied conditions that lead to a short-term positive fat balance and have shown that a shift to a high-fat (HF) diet from an average fat diet is not accompanied by an immediate increase in fat oxidation that would match the rate of fat intake even when the diets are eucaloric (6, 27, 28). The rate of fat oxidation increases only slowly with time, and thus it can be 5–7 days before fat balance is established on the HF diet. Investigators have previously studied the body’s response to a HF diet [by assessing fat oxidation, nonprotein respiratory exchange ratio (NPRER), and 24-h energy expenditure (24hEE)] during exercise and sedentary conditions (6, 14, 26, 29). Smith et al. (29) reported that when six lean, healthy men switched from a standard American diet (37% energy as fat) to a HF diet (50% of energy as fat), NPRER was decreased while fat oxidation increased during a 5-day study. In a subsequent report, Hansen et al. (6) examined the effects of a HF diet in female subjects during sedentary or exercise conditions. They found that exercise allowed the body to adjust more quickly to a HF diet by increasing 24-h fat oxidation more than during sedentary conditions.

Neither of these studies, however, examined the composition of the dietary fats. Literature suggests that the type of fat being consumed may differentially affect excess energy storage and metabolism (4, 13, 17, 19). Oleic acid, a mono-unsaturated fatty acid (FA), and palmitic acid, a saturated FA, are commonly used to study the effects of different FAs on the body’s metabolism. The ratio of unsaturated fats to saturated fats in the diet has been shown to influence energy substrate utilization in humans (14). Furthermore, acute exercise has been shown to increase dietary oleate oxidation but not palmitate oxidation (35). Therefore, the purpose of this study was to examine the effects of a high saturated (primarily palmitic acid) vs. high mono-unsaturated (primarily oleic acid) fat diet on how quickly the body can adapt to a HF diet by increasing fat oxidation (and decreasing NPRER) during both exercise and sedentary conditions. These two FAs were selected because they are the most common FAs in the diet and because they are also the primary FAs that differ between the Western and Mediterranean diets. Based on previous work on fasting or postprandial fat oxidation that show that oleic acid is oxidized more rapidly that palmitic acid (3) and because we found oleic acid oxidation is upregulated to a greater degree following a bout of exercise, we hypothesized that the high mono-unsaturated fat diet would facilitate a faster increase in fat oxidation during the HF diet and that the effects with exercise would be additive.

MATERIALS AND METHODS

Subjects. Eight healthy men between the ages of 18 and 45 yr were recruited to participate in a research study at the University of Wisconsin (UW) hospital’s Clinical and Translational Research Core (CTRC). This study was reviewed and approved by the Institutional Review Board at UW-Madison, and informed, written consent was obtained from each participant. For this randomized crossover study, inclusion criteria were men with a body mass index (BMI) between 18 and 30kg/m2 and a moderately sedentary lifestyle (<3 h/wk of low-
moderate-intensity exercise and no vigorous exercise). Exclusion criteria included a history of metabolic or pulmonary disease, implanted electrical devices, and claustrophobia. All eight participants completed all four of the study visits.

Protocol. This study involved four treatment conditions that each participant completed in a random order. Treatment conditions included high saturated fat diet and sedentary (SS), high saturated fat diet and exercise (SE), high mono-unsaturated fat diet and sedentary (US), and high mono-unsaturated fat diet and exercise (UE). Each study visit was separated by at least 2 wk. Before participation in the four study visits, subjects underwent a physical exam and screening, which has been described in detail previously (6). Briefly, resting metabolic rate (RMR; kcal/day) was measured for 30 min on the Deltatrac II Metabolic Monitor (Viasys Healthcare, SensorMedics, Yorba Linda, CA) following an overnight fast. The 24-h total energy intake for each participant was calculated to equal predicted energy expenditures of 1.35 kcal/day for each participant was calculated to equal predicted energy intake for each participant was calculated to equal predicted energy expenditures of 1.35 × RMR during sedentary visits and 1.8 × RMR during exercise study visits. Following RMR measurements, subjects underwent a bicycle maximal oxygen uptake (Vo$_2$max, maximal aerobic capacity) test on an Ergoline 800 cycle ergometer (SensorMedics) at the University of Wisconsin Pulmonary Function Laboratory using a CPX-D rapid gas analyzer (Medical Graphics, St. Paul, MN). Achievement of Vo$_2$max included an oxygen consumption (Vo$_2$) plateau or RER of >1.1 and heart rate (HR) of >90% of age-predicted maximal HR.

After completion of the screening visit, participants began the inpatient study visits. To match expenditure with intake to maintain energy balance, the four inpatient visits differed only in the type of dietary fat consumed and energy intake and expenditure between the sedentary and exercise treatments. For 4 days before their inpatient stay, participants were provided with a lead-in diet that was representative of the standard American diet. All foods contained roughly 55% carbohydrate (CHO), 15% protein, and exactly 30% fat. Total caloric intake for the lead-in diet was based on the participant’s RMR*1.65 (1.65 is an average U.S. physical activity value) (11). Additionally, participants were asked to refrain from any moderate physical activity for 24 h before their inpatient visit.

For each study visit, participants arrived at the CTRC at 1800 to begin the 5-day, 6-night inpatient visit in the metabolic chamber. A urine sample was taken at baseline as subjects were given a dose of $^{18}$O (0.025 g/kg body wt) for measurement of total body water (TBW) and body composition from urinary $^{18}$O. Participants slept from 2300–0645 each night, and sleeping during the day was prohibited. On waking at 0645, participants exited the chamber, and RMR was measured on mornings 2, 4, and 6. Subjects then had 30–45 min for personal hygiene before re-entering the chamber at 0815. On day 4 (D4) of each study visit (third day of HF diet), no respiratory gases were collected because participants were out of the chamber to allow for a break from the confinement; however, this day was identical to the rest of the days with respect to diet, activity, and sampling. Discharge occurred at 0800 on the sixth morning of each visit.

Diet. During the study visits, meals were provided by the UW Hospitals and Clinics kitchen. All items were weighed and consumed entirely by subjects. During the first full day of each visit (D1), participants remained on the standard diet consisting of 55% CHO, 15% protein, and 30% fat. On days 2 through 5 (D2–5), participants were either on a high saturated fat or high mono-unsaturated fat diet. Both HF diets provided 35% of energy as CHO, 15% protein, and 50% fat. For the high saturated fat treatments, 25% of total energy consumed was saturated fat, 22% was mono-unsaturated fat, and 10% was poly-unsaturated fat. For the high mono-unsaturated fat treatment, 30% of total energy was mono-unsaturated fat, 10% of energy was saturated, and 10% was poly-unsaturated fat. Subjects received three meals each day (breakfast at 0830, lunch at 1200, and dinner at 1900) along with an afternoon (1600) and evening snack (2200). Individual daily meals consisted of 25%, 25%, 40% minus 50 kcals, and 10% of daily caloric needs for breakfast, lunch, dinner, and afternoon snack, respectively. Additionally, subjects received a 50-kcal evening snack. Total caloric intake was prescribed to equal 24hEE. A caloric intake of RMR*1.35 for sedentary visits was determined from the average of previous sedentary studies in our chamber. The caloric intake of RMR*1.8 was calculated from the sum of sedentary plus the exercise volume, which was based on a study by Smith et al. (28).

Exercise. During the exercise visits, participants rode a stationary bike at 45% of their Vo$_2$max twice within each day including the D1 (baseline) day (from roughly 1000–1100 and 2100–2200). The prescribed exercise was designed to raise 24hEE to 1.8*RMR. To calculate duration of exercise, the energy cost of cycling at 45% of Vo$_2$max was estimated from the individual linear equation between Vo$_2$ and work (watts) generated during each subject’s Vo$_2$max test. Relative Vo$_2$ (ml·kg$^{-1}$·min$^{-1}$) at 45% of Vo$_2$max was used to calculate the rate of energy expenditure (kcal/min) at that intensity (watts), which was used in combination with RMR to estimate minutes of cycling needed to raise 24hEE during the exercise study visits. The total number of minutes was then split into the morning and evening sessions of exercise.

Respiratory chamber. The design of the metabolic chamber at the CTRC was similar in design to the chamber in the Department of Human Biology at Maastricht University in Maastricht, The Netherlands (25), and the specifications and diagnostics have been previously described (34). Additionally, information on chamber temperature, humidity, airflow, pressure, and data collection instrumentation has been described elsewhere (6). Briefly, the composition of air is measured with carbon dioxide (Hartman and Braun Uras-4) and oxygen (Magnos-6) gas analyzers (Applied Automation, Bartlesville, OK).

The chamber was calibrated against methanol burns (25) throughout the duration of the study. The percentage recoveries from each burn were used to develop correction factors for the corresponding chamber data from each study visit. The precisions of the corrections were 5.1% and 1.7% for O$_2$ and CO$_2$, respectively.

Calculation of energy and macronutrient oxidation. Energy expenditure (EE) and substrate oxidation were calculated from CO$_2$ uptake (VCO$_2$), Vo$_2$, and urinary nitrogen (12). Protein oxidation (from urinary nitrogen) was measured for each day and night. Substrate oxidations and EE were then calculated on the basis of day and night and were summed for 24hEE. Thus macronutrients oxidized were converted to nutrients and calculated in grams oxidized over the 24-h period. Grams of nitrogen oxidized were subtracted in equations by Jequier et al. (12) to determine the non-protein VCO$_2$ and Vo$_2$ values and their subsequent NPRER. Because participants were not in the chamber 24 h each day, EE data from 0830–0645 (22.25 h) was extrapolated to 24 h to calculate 24hEE.

Sample collection and analysis. Urine samples (5 ml) were collected each day of all study visits at 0645, 1200, 1600, 2000, and 2300 for H and nitrogen analysis. Additionally, all other urine was acidified with 250 mg/20ml of citric acid (Acros Organics/Fisher, Chicago, IL) to prevent the volatilization of the nitrogen compounds and pooled into waking hours (day) and sleeping hours (night). Samples were then frozen at ~80°C until their dilution for nitrogen analysis (Antek 900 series Nitrogen Analyzer).

For $^{18}$O analysis, 1 ml of urine was equilibrated with CO$_2$ at 25°C for 48 h. The $^{18}$O enrichment was then measured using continuous-flow isotope ratio mass spectrometry (IRMS), as reported elsewhere (6). Both water and urine were analyzed in duplicate, and the results were averaged. The oxygen dilution space was 1.007 × total body weight (TBW) (23), and fat-free mass was calculated as TBW divided by 0.73.

All eight subjects were randomized to receive [1-13C]99% atom rich-labeled oleic acid (Cambridge Isotope Laboratories, Andover, MA) and [d$_{17}$-H]98% atom rich-palmitic acid (Isotec-Sigma Aldrich, St. Louis, MO) on either day 1 or day 2, and all subjects were given the same dose again on day 5 of each treatment visit. In this way, we could investigate both acute and longer term effects but still maintain adequate separation between dosings to prevent interference...
from a previous dose. We incorporated dosing on day 1 for half of the subjects and on day 2 for the other half of the subjects to test whether any increase in dietary FA oxidation was due to the increase in fat intake per se, in which case the change from day 1 to day 2 would be larger than the change from day 2 to day 5. The \(^{13}\text{C}\) FA dose was 0.010g/kg body wt and the \(^{2}\text{H}\) FA dose was 0.015g/kg body wt. Both labeled fats were mixed in with a fruit smoothie that was given at breakfast (0830). For \(^{13}\text{C}\) recovery, hourly breath samples were obtained at baseline (0800) and hourly from 0900 to 2300 as well as at 0800 the following morning (22). Breath (15 ml) was collected in a no-additive Vacutainer (Becton Dickinson, Franklin Lakes, NJ), and the \(^{13}\text{C}-\text{to-}\ ^{12}\text{C}\) ratio of the CO\(_2\) was measured with continuous-flow IRMS (Delta S; Finnigan MAT, Bremen, Germany). For isotope ratio analysis, each sample was injected twice and averaged. \(^{13}\text{C}\) recovery was calculated hourly after ingestion of the label. The average baseline value was subtracted from each post-dose value so that data was expressed as a permit (parts per thousand, ‰) increase relative to the subjects’ own baseline. The \(^{13}\text{C}/^{12}\text{C}\) enrichments above baseline were corrected for background based on our laboratory’s previous study (6). Additionally, \(^{13}\text{C}\) FAs were corrected for acetate sequestration (correction factor of 0.51 for sedentary treatments and 0.57 for exercise visits). Acetate correction factors were determined by previous work in sedentary (1) and exercising (21) subjects and used accordingly. The cumulative \(^{13}\text{C}\) recovery was calculated from enrichment and measured CO\(_2\) production using the trapezoid rule, which has been previously described (34).

For \(^{2}\text{H}\) analysis, urine samples were collected at baseline (0645) and at 1200, 1600, 2000, and 2300, which corresponded to 3.5, 7.5, 11.5, and 14.5 hours post-dose. Additionally, a 24- and 48-hour sample was also obtained. Decolorized urine samples were reduced over chromium and analyzed for deuterium content as a ratio of \(^{2}\text{H}/^{1}\text{H}\) using the Delta Plus IRMS (Finnigan MAT, Bremen, Germany). Data was corrected for \(^{1}\text{H}^{16}\) and expressed relative to standard mean ocean water and enrichment calculated above baseline (24). \(^{2}\text{H}\) recovery was calculated as excess \(^{2}\text{H}\) multiplied by total body water and divided by the dose of \(^{2}\text{H}\) administered, as previously described (34).

The \(^{13}\text{C}\) and \(^{2}\text{H}\) recoveries were used to estimate exogenous fat oxidation on the assumption that the stable isotope oxidation was representative of all dietary FA oxidation. Therefore, the percent recovery for each FA was multiplied by the actual intake of unsaturated fat (for \(^{13}\text{C}\)) and saturated fat (for \(^{2}\text{H}\)) to get grams of unsaturated and saturated dietary fat that were oxidized. These two values were added together to get total dietary (exogenous) FA oxidation.

**Statistical analysis.** The SAS version 8.2 statistical package (SAS Institute, Cary, NC) was used for all data analysis with subjects added together to get total dietary (exogenous) FA oxidation. These two values were also calculated for exogenous fat oxidation on the assumption that the stable isotope oxidation was representative of all dietary FA oxidation. Therefore, the percent recovery for each FA was multiplied by the actual intake of unsaturated fat (for \(^{13}\text{C}\)) and saturated fat (for \(^{2}\text{H}\)) to get grams of unsaturated and saturated dietary fat that were oxidized. These two values were added together to get total dietary (exogenous) FA oxidation.

**Results.** Eight healthy male participants completed all four study visits. Table 1 shows baseline subject characteristics, whereas Table 2 shows body weight and body composition data for each of the four treatments. Per the eligibility criteria, all subjects were sedentary (<3 h/wk of low to moderate exercise) with a BMI between 18 and 30 kg/m\(^2\) and were free from metabolic disease. Fasting blood lipids, glucose, and insulin levels were all in the normal range (Table 1).

**Energy balance.** Total energy intake and expenditure were recorded during every day of each study visit and have been reported elsewhere (2). Briefly, average 24hEE for all days for each of the study visits was 2,240 ± 82, 3,202 ± 146, 2,270 ± 104, and 3,208 ± 151 kcal/day for SS, SE, US, and UE, respectively. Mean 24-h energy intakes were 2,236 ± 129 (SS), 3,007 ± 143 (SE), 2,243 ± 122 (US), and 3,004 ± 144 kcal/day (UE). Energy balance was achieved for the sedentary visits, whereas 24hEE was significantly higher than energy intake during the exercise visits. Importantly, no differences existed in energy balance with regard to FA composition.

**Macronutrient oxidation.** The results for macronutrient oxidation are shown in Fig. 1. A one-way ANOVA revealed a significant difference (P < 0.01) between the UE and SE treatments on D1 for fat oxidation and carbohydrate oxidation. Since this was a potentially confounding baseline from which to measure changes, D1 was used as a covariate in the statistical model. Fat oxidation displayed a main effect for treatment (P < 0.001), time (P < 0.001), and the treatment × time interaction (P < 0.003). The significant treatment effect was that exercise, compared with sedentary conditions, led to a significant increase in fat oxidation over the course of the 4 days of the HF diet. No effect of dietary FA composition was detected. Comparing the final day of the HF diet (D5) to D1 showed that exercise treatments increased fat oxidation for both FA treatments (SE: 76 ± 30 g, P = 0.001; UE: 118 ± 31 g, P < 0.001), whereas neither sedentary condition significantly changed fat oxidation, although there was a trend with the US condition (SS: −10 ± 33 g, P = not significant; US: 41 ± 14 g, P = 0.07). Fat oxidation was also subtracted from fat intake to determine fat balance in grams (see Table 4). Similar to what was shown for fat oxidation above, fat balance was achieved (even a slightly negative fat balance) for both exercise conditions by day 5, whereas both sedentary conditions were still in a positive fat balance at the end of the study.

Not surprisingly, carbohydrate oxidation mimicked the results to that of fat oxidation, albeit in the opposite direction (Fig. 1). There was a significant treatment (P < 0.001), time (P < 0.001), and treatment × time interaction (P < 0.04) for carbohydrate oxidation. The treatment effect was the result of both exercise treatments significantly decreasing carbohydrate oxidation over the course of the HF diet compared with sedentary conditions. No effect of dietary FA composition was found. Protein oxidation did not change during the course of the 5-day study. Statistical analysis revealed no significant effects of treatment, time, or the treatment × time interaction (Fig. 1).

**Table 1. Baseline subject characteristics**

| Age, yr | 25 | 8 |
| Height, cm | 184.82 | 6.16 |
| Weight, kg | 76.5 | 6.6 |
| \(V_{O2\max}\), ml · kg\(^{-1}\) · min\(^{-1}\) | 40.5 | 5.1 |
| BMI, kg/m\(^2\) | 22.5 | 3.3 |
| Fasting total cholesterol, mg/ml | 177 | 5 |
| Fasting glucose, mg/dl | 93.8 | 0.4 |
| Fasting insulin, µU/ml | 6.1 | 0.6 |

BMI, body mass index; \(V_{O2\max}\), maximal aerobic capacity; SD, standard deviation.

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Protein oxidation was calculated from urinary nitrogen analysis and was then used to calculate NPRER. For the standard fat diet day (D1), 24-h NPRER averaged 0.88 ± 0.02, 0.94 ± 0.02, 0.86 ± 0.03, and 0.88 ± 0.02 for SE, UE, SS, and US, respectively, compared with the calculated food quotient (FQ) of 0.885. The FQ describes the ratio of CO2 produced to O2 consumed during complete oxidation of a representative sample of the diet. A one-way ANOVA revealed a significant difference ($P < 0.01$) between the UE treatment and all other treatments on D1. This was a potentially confounding baseline from which to measure changes, so D1 was used as a covariate in the statistical model. The absolute values and change in NPRER from the standard to each HF diet day is shown in Fig. 2. Overall, there was a significant main effect for treatment ($P < 0.01$) and time ($P < 0.001$), and a significant treatment $\times$ time interaction ($P < 0.04$) with both exercise treatments significantly decreasing NPRER over the course of the HF diet compared with sedentary conditions. There was no effect of dietary FA composition. Since the FQ during the HF diet did not differ from day to day or from each treatment, HF D2–5 from each treatment was averaged to yield one HF FQ of 0.826. There were no effects of dietary FA composition both within each day and across days. Within each day, no treatment differences were detected for 13C percent recovery. To compare across days, there was no significant statistical difference between D1 and D2 13C recoveries (SE: 80 ± 12% vs. 110 ± 4%; UE: 104 ± 16% vs. 95 ± 11%; SS: 79 ± 12% vs. 88 ± 12%; US: 86 ± 8% vs. 80 ± 13% for D1 vs. D2, respectively), so these days were combined and then compared with D5 data. Total 24-h 13C percent dose recovery from D1 and D2 vs. D5 showed no significant main effect for treatment or time and no treatment $\times$ time interaction. Similarly to 13C data, [2H] PA 24-h dose recovery showed no treatment differences within each day. For across-day comparisons, there was a significant time effect ($P = 0.02$), so D1 and D2 data were not combined. The significant time effect was an increase in dose recovery from D1 and D2 for the SE treatment ($P < 0.001$). There was also a trend for an increase from D1 to D5 dose recoveries for the SE treatment ($P = 0.07$), but no difference between D2 and D5.

**Stable isotope (exogenous fat oxidation).** The 24-h [13C]oleate and [2H]palmitate percent recoveries for each day of all four treatments can be found in Fig. 3, A and B, respectively. Analyses were done using a repeated-measures ANOVA with post hoc comparisons done using Tukey’s test. We looked at the comparisons both within each day and across days. Within each day, no treatment differences were detected for 13C percent recovery. To compare across days, there was no statistical difference between D1 and D2 13C recoveries (SE: 80 ± 12% vs. 110 ± 4%; UE: 104 ± 16% vs. 95 ± 11%; SS: 79 ± 12% vs. 88 ± 12%; US: 86 ± 8% vs. 80 ± 13% for D1 vs. D2, respectively), so these days were combined and then compared with D5 data. Total 24-h 13C percent dose recovery from D1 and D2 vs. D5 showed no significant main effect for treatment or time and no treatment $\times$ time interaction. Similarly to 13C data, [2H] PA 24-h dose recovery showed no treatment differences within each day. For across-day comparisons, there was a significant time effect ($P = 0.02$), so D1 and D2 data were not combined. The significant time effect was an increase in dose recovery from D1 and D2 for the SE treatment ($P < 0.001$). There was also a trend for an increase from D1 to D5 dose recoveries for the SE treatment ($P = 0.07$), but no difference between D2 and D5.
time required to reach fat balance when the percent of energy from fat in the diet is increased regardless of the FA composition under eucaloric conditions. This provides further evidence that exercise facilitates the process of achieving fat balance in the body more quickly while on a HF diet. This is clinically relevant since repeated periods of a short-term positive fat balance can lead to a significant amount of fat mass gain over time and may be detrimental to health and increase obesity risk. Additionally, switching to a HF diet did not result in an increase in $^{13}$C percent dose recovery but did increase $^2$H percent dose recovery from D1 to D2 for the SE treatment only. There was no effect of exercise or FA composition $^{13}$C or $^2$H percent dose recoveries for exogenous fat oxidation.

The results we observed with total fat oxidation during exercise and sedentary conditions are in agreement with previous results in male (28) and female (6) participants. In humans, consuming a HF diet may decrease mitochondrial oxidative capacity in skeletal muscles. It has been shown that mRNA expression is decreased for enzymes and for transcription factors that are involved in oxidative phosphorylation in response to a HF diet (31). However, not all studies indicate a decreased mitochondrial capacity from a HF diet. More recent studies in rodents actually show increased mitochondrial biogenesis that is induced by high serum FA concentrations and is mediated by an increase in PGC1α protein (8). These studies are often done in rodent models that are insulin resistant, however, and can be several weeks in length (33), so those results may not be applicable to acute HF diets that do not cause insulin resistance. If, in humans, there is in fact a

dose recoveries. Although all four treatments appeared to show an increase in dose recovery at D2 and D5 vs. D1, no other significant differences were detected.

Total percent recovery from $^{13}$C and $^2$H were then used to calculate unsaturated and saturated exogenous fat oxidation from the dietary intake data, respectively. To determine endogenous fat oxidation, exogenous (dietary) fat oxidation was subtracted from the total fat oxidation obtained from the metabolic chamber. The results of total, exogenous, and endogenous fat oxidation can be found in Table 3. Exogenous fat oxidation (in grams) increased significantly from D1 to D2 and from D1 to D5 in all treatments but did not change from D2 to D5 for any treatment condition. Conversely, endogenous fat oxidation did not change from D1 to D2 in any treatment but did increase significantly in both exercise treatments from D1 to D5 and D2 to D5. A nonsignificant increase resulted from D2 to D5 in the US treatment, whereas a significant decrease from D2 to D5 was found in the SS treatment. Importantly, the large increase in exogenous fat oxidation from D1 to D2, which is calculated from the product of fat intake and percent dose recovery of the labeled fat, was due to an increase in dietary fat intake and percent dose recovery.

**DISCUSSION**

The main finding of this study is that dietary FA composition does not influence the rate at which 24-h fat oxidation increases and NPRER decreases following a switch from a standard to a HF diet. We did, however, confirm that exercise shortens the

Table 3. Within treatment changes in exogenous, total, and endogenous fat oxidation for all four treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SE</th>
<th>UE</th>
<th>SS</th>
<th>US</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D5</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D5</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D5</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA</td>
<td>2.9 ± 0.8</td>
<td>10.6 ± 2.5</td>
<td>12.8 ± 2.3</td>
<td>3.8 ± 0.7</td>
<td>6.0 ± 1.5</td>
<td>5.0 ± 0.9</td>
<td>2.0 ± 0.5</td>
<td>5.5 ± 1.9</td>
<td>8.1 ± 1.9</td>
<td>2.5 ± 0.7</td>
<td>3.5 ± 0.9</td>
<td>2.8 ± 0.7</td>
<td>45.0 ± 7.6</td>
<td>80.4 ± 4.7</td>
<td>69.8 ± 6.5</td>
<td>52.8 ± 7.1</td>
</tr>
<tr>
<td>UFA</td>
<td>45.0 ± 7.6</td>
<td>80.4 ± 4.7</td>
<td>69.8 ± 6.5</td>
<td>52.8 ± 7.1</td>
<td>105.0 ± 12.8</td>
<td>102.6 ± 6.2</td>
<td>33.0 ± 6.8</td>
<td>49.0 ± 6.7</td>
<td>49.1 ± 3.4</td>
<td>36.5 ± 5.0</td>
<td>35.3 ± 10.2</td>
<td>68.2 ± 11.0</td>
<td>48 ± 8</td>
<td>91 ± 5*</td>
<td>83 ± 7*</td>
<td>57 ± 7</td>
</tr>
<tr>
<td>Total</td>
<td>47.9 ± 8.4</td>
<td>86.4 ± 5.2</td>
<td>72.7 ± 7.2</td>
<td>59.7 ± 7.7</td>
<td>107.5 ± 13.3</td>
<td>112.6 ± 6.8</td>
<td>40.7 ± 6.3</td>
<td>54.3 ± 7.4</td>
<td>57.9 ± 4.3</td>
<td>42.1 ± 4.8</td>
<td>41.6 ± 10.2</td>
<td>79 ± 6</td>
<td>112 ± 15</td>
<td>158 ± 13</td>
<td>188 ± 21</td>
<td>57 ± 14</td>
</tr>
<tr>
<td>Exogenous</td>
<td>2.9 ± 0.8</td>
<td>10.6 ± 2.5</td>
<td>12.8 ± 2.3</td>
<td>3.8 ± 0.7</td>
<td>6.0 ± 1.5</td>
<td>5.0 ± 0.9</td>
<td>2.0 ± 0.5</td>
<td>5.5 ± 1.9</td>
<td>8.1 ± 1.9</td>
<td>2.5 ± 0.7</td>
<td>3.5 ± 0.9</td>
<td>2.8 ± 0.7</td>
<td>2.5 ± 0.8</td>
<td>52.8 ± 7.1</td>
<td>105.0 ± 12.8</td>
<td>102.6 ± 6.2</td>
</tr>
<tr>
<td>Total</td>
<td>47.9 ± 8.4</td>
<td>86.4 ± 5.2</td>
<td>72.7 ± 7.2</td>
<td>59.7 ± 7.7</td>
<td>107.5 ± 13.3</td>
<td>112.6 ± 6.8</td>
<td>40.7 ± 6.3</td>
<td>54.3 ± 7.4</td>
<td>57.9 ± 4.3</td>
<td>42.1 ± 4.8</td>
<td>41.6 ± 10.2</td>
<td>79 ± 6</td>
<td>112 ± 15</td>
<td>158 ± 13</td>
<td>188 ± 21</td>
<td>57 ± 14</td>
</tr>
<tr>
<td>Endogenous</td>
<td>2.9 ± 0.8</td>
<td>10.6 ± 2.5</td>
<td>12.8 ± 2.3</td>
<td>3.8 ± 0.7</td>
<td>6.0 ± 1.5</td>
<td>5.0 ± 0.9</td>
<td>2.0 ± 0.5</td>
<td>5.5 ± 1.9</td>
<td>8.1 ± 1.9</td>
<td>2.5 ± 0.7</td>
<td>3.5 ± 0.9</td>
<td>2.8 ± 0.7</td>
<td>2.3 ± 0.4</td>
<td>2.2 ± 0.4</td>
<td>2.2 ± 0.4</td>
<td>2.2 ± 0.4</td>
</tr>
</tbody>
</table>

Data are means ± SE. SFA, saturated fatty acid; UFA, mono-unsaturated and poly-unsaturated fatty acid; D, day. *Significant difference from day 1 (P < 0.01). †Significant difference from day 2 (P < 0.05).

Table 4. Fat intake and oxidation data in grams

<table>
<thead>
<tr>
<th>Fat Intake, g</th>
<th>Fat Oxidation, g</th>
<th>Fat Balance, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>D2</td>
<td>D3</td>
</tr>
<tr>
<td>SE</td>
<td>103 ± 5</td>
<td>171 ± 8</td>
</tr>
<tr>
<td>UE</td>
<td>102 ± 5</td>
<td>172 ± 8</td>
</tr>
<tr>
<td>SS</td>
<td>75 ± 4</td>
<td>126 ± 7</td>
</tr>
<tr>
<td>US</td>
<td>76 ± 4</td>
<td>129 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SE. Fat balance is calculated as fat intake − fat oxidation.
decreased capacity of cells to perform oxidative phosphorylation, it could help to explain why, during sedentary conditions, our subjects did not increase fat oxidation to the extent that was needed to achieve fat balance. Conversely, exercise may be able to prevent some of the downregulation of these genes important for fat oxidation. Although the mechanisms of how exercise increases fat oxidation were not examined in this study, other researchers have demonstrated metabolic changes that occur with exercise treatment. Both enzyme levels and gene expression of enzymes that increase fat oxidation in skeletal muscle, such as lipoprotein lipase, triglyceride lipase, CPT-1, mitochondrial number, PDH kinase, fatty acid translocate/CD36, and fatty acid binding protein in the plasma membrane, have all been shown to increase with exercise (7, 9, 10, 20, 32).

To our knowledge, this is the first study to examine the effect of dietary FAs on macronutrient metabolism in a controlled metabolic chamber environment over several consecutive days. However, diets rich in either mono-unsaturated (oleic acid) or saturated (palmitic acid) FAs have been previously compared in other human studies under different experimental conditions (13, 15–17, 19). Kien et al. (16) compared a high oleic acid vs. high palmitic acid diet over a 28-day period and found an increased post-prandial, but not fasting, fat oxidation (and lowered NPRER) with a high oleic acid diet. Conversely, a study by Piers et al. (19) on a 4-wk diet rich in either saturated or mono-unsaturated FAs showed no differences in fasting or post-prandial fat oxidation during the 5-h measurement period. Similarly, 8-h fat oxidation and RER measurements in adults after a 4-wk moderate fat diet (30% of total energy) differing in FA composition showed no significant differences between a high mono-unsaturated FA and high saturated FA diet (17). Further support for a lack of dietary FA differences was found by Fasching et al. (4) when they found no differences in fat oxidation in the post-absorptive state after a 1-wk diet enriched in either saturated or mono-unsaturated FA. Our study extends those findings to whole day fat oxidation and, because we had total dietary control, obviates any criticism that the absence of difference might be due to non-compliance. Combining our results with previous research provides further evidence for a lack of difference in 24-h fat oxidation from diets rich in saturated vs. mono-unsaturated FAs.

With regard to dietary FA composition, we noted a trend for increased fat oxidation (and decreased carbohydrate oxidation) by D5 for the high mono-unsaturated fat diet during sedentary conditions, which was not found in the SS condition. However,
the change by D5 in this US treatment was still smaller than the change in the calculated FQ, suggesting that these individuals were still in a positive fat balance. Furthermore, NPRER from D1 to D5 was not significantly different. It is still possible, however, that without exercise to enhance fat oxidation, a differential effect of dietary FAs may exist but could require a treatment of longer than 5 days or that it is small and thus more subjects would be needed to detect that potential difference. This could possibly explain why Kien et al. (16) found an increase in body mass (with a trend for an increase in fat mass) for subjects on a high mono-unsaturated fat diet vs. a high saturated fat diet after a 28-day period. Although HF diets are also typically high in caloric content, if small differences between FA content do in fact exist, this could also have an impact on changes in fat mass and potentially body mass.

Much like the total fat oxidation results, $^{13}$C-labeled oleic acid and $^2$H-labeled palmitic acid dose recoveries showed no differences in dietary fat oxidation with respect to FA composition of the diet but also did not show a difference between exercise and sedentary treatments. Because we were interested in studying 24-h fat oxidation, we used the summary percent dose recovery variable for our statistical analyses of exogenous fat oxidation. Of note, however, when all time-points of dose recovery were incorporated into the statistical analysis, there was a significant increase in dose recovery of dietary oleate and palmitate with exercise compared with sedentary conditions. That statistical difference was due to increases in dose recovery in the morning and evening postexercise periods, which is in agreement with previous reports (35). In the hours following the time when the exercise treatment was performed, dose recovery increased more for sedentary conditions, and the dose recovery “catches up” with that of the exercise conditions leading to the same 24-h dose recovery for both exercise and sedentary conditions.

Total fat oxidation increased when switching from a standard diet to a HF diet during both exercise conditions but not during the sedentary conditions. By including exogenous (dietary) fat tracers, we were able to determine whether that increase in total oxidation was the result of increased exogenous or endogenous fat oxidation, or both. The lack of change in total fat oxidation over the 5-day study period in the SS and US treatments was the result of no change or decreases in endogenous oxidation as exogenous fat oxidation increased significantly from D1 to D5. Therefore, it appears that the overall increase in total fat oxidation during exercise conditions across the 5-day study period was due to increases in both exogenous and endogenous fat oxidation, with increases in exogenous fat oxidation occurring much more quickly than those in endogenous fat oxidation.

Importantly, the large gram increase in exogenous fat oxidation from D1 to D2 across treatments is the result of the large increase in dietary fat intake. As shown above, the percent dose recoveries did not differ between D1 and D2 for the $^{13}$C-oleate, and only the SE treatment showed a significant increase in percent dose recovery for $^2$H-palmitate. Therefore, whether on a standard diet or HF diet, it appears that a similar percentage of exogenous fat is being oxidized. This is also in agreement with a study by Sonko et al. (30), who showed that, with increasing amounts of fat load above 50-g doses, $^{13}$C percent dose recovery did not significantly change.

There are limitations to our study. It is difficult to find subjects who can perform four 5-day inpatient stays over a 6-mo period and to schedule large numbers of subjects when they do volunteer. Although we found differences between exercise and sedentary conditions, we did not find significant differences with FA composition. Since there was a trend for a change with the US treatment, it is possible that we did not have sufficient power to detect a difference between saturated and mono-unsaturated FAs. With 80% power, we would need 12 subjects total (4 additional subjects) to detect a significant difference with an alpha of 0.05. Also, our subjects were in a slight negative energy balance for both exercise visits, which could potentially impact whole body fat oxidation. However, since the negative energy balance was similar between the two exercise visits, it likely did not adversely affect the results with regard to FA composition. Furthermore, no significant changes in body weight were detected for any of the treatments. Additionally, fat oxidation was very low on day 1 for the UE treatment and differed significantly from the other three treatments. This day was physiologically unusual but could not be traced to any cause. We, therefore, accounted for this in our statistical analysis by using day 1 as a covariate in our statistical model so as not to affect the change in fat oxidation from day 1 to subsequent days. The use of stable isotopes to determine exogenous fat oxidation is also not perfect in that they include only two specific fatty acids out of a larger number of dietary fatty acids. Finally, the subjects in this study were on a HF diet for 4 days and thus cannot address chronic diet effects.

In summary, exercise confirmed that there is a more rapid increase in fat oxidation when exposed to a HF diet compared with sedentary conditions. Furthermore, only the exercise treatments showed a change in NPRER that was equal to or greater than the change in the calculated FQ. This and the fat balance data indicate that only the two exercise treatments achieved fat balance by the conclusion of each study visit. Dietary FA composition, on the other hand, had no effect on total fat oxidation or fat balance. Interestingly, the high mono-unsaturated FA treatment during sedentary conditions trended for a difference with total fat oxidation and showed a smaller positive fat balance, indicating that under sedentary conditions there may be differences in the metabolism of dietary FAs, which warrants further examination. Finally, unlike the exercise effect that was detected for total fat oxidation, 24-h exogenous fat oxidation (as measured by $^{13}$C and $^2$H stable isotope dose recoveries) showed no differences with exercise or dietary FA composition.

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

REFERENCES


