Effect of Two Doses of Interval Training on Maximal Fat Oxidation in Sedentary Women

TODD A. ASTORINO[1], MATTHEW M. SCHUBERT[1,2], ELYSE PALUMBO[1], DOUGLAS STIRLING[1], and DAVID W. MCMILLAN[1]

[1]Department of Kinesiology, California State University—San Marcos, San Marcos, CA; and [2]Griffith University, Gold Coast, AUSTRALIA

ABSTRACT

ASTORINO, T. A., M. M. SCHUBERT, E. PALUMBO, D. STIRLING, and D. MCMILLAN. Effect of Two Doses of Interval Training on Maximal Fat Oxidation in Sedentary Women. *Med. Sci. Sports Exerc.*, Vol. 45, No. 10, pp. 1878–1886, 2013. **Introduction**: The primary aim of the current study was to determine the effect of two doses of chronic high-intensity interval training (HIT) on changes in maximal fat oxidation (MFO) and body composition. **Methods**: Sedentary women ($N = 23$, age and $\dot{V}O_{2max} = 24.2 \pm 6.2$ yr and 30.3 ± 5.2 mL·kg^{-1}·min^{-1}, respectively) completed either high (HI) (80%–90% maximal workload) or moderate (MOD) intensity (60%–80% maximal workload) HIT on a cycle ergometer 3 d·wk^{-1} for 12 wk consisting of 6–10 sixty-second bouts interspersed with active recovery. Seven women of similar age and fitness level served as controls. Every 3 wk, substrate oxidation was assessed during progressive exercise via indirect calorimetry to determine MFO and minimum fat oxidation, and body composition was assessed every 6 wk. Repeated-measures ANOVA was used to examine changes in substrate oxidation in response to training, with training group used as a between-subjects variable. **Results**: Results revealed improved MFO ($P = 0.04$, 19%–25%) and minimum fat oxidation ($P = 0.001$, 22–24 W) in response to HIT, yet the magnitude of improvement was similar ($P > 0.05$) between training paradigms. No change ($P > 0.05$) in body weight, percent body fat, or waist–hip circumference was revealed with training. **Conclusion**: These data suggest that 12 wk of either moderate or more strenuous interval training similarly enhance fat oxidation in sedentary women but do not alter body weight or body composition. **Key Words**: EXERCISE, SUBSTRATE USE, RESPIRATORY EXCHANGE RATIO, INTERVAL TRAINING, CYCLE ERGOMETRY

Recently, short-term high-intensity interval training (HIT) consisting of repeated Wingate tests interspersed with a 4- to 5-min recovery has been identified as a time-efficient alternative to traditional endurance training. Data from studies in predominantly healthy young men and women reveal training-mediated improvements in maximal oxygen uptake ($\dot{V}O_{2max}$) (3,14), power output (3), insulin sensitivity (4), and mitochondrial function represented by increased activity of citrate synthase (7), pyruvate dehydrogenase (7,8), and 3-hydroxyacyl–CoA dehydrogenase (8,31). However, the duration of these studies typically ranged from 2 to 6 wk, so it is relatively unknown whether these adaptations ensue with HIT performed over longer periods of time.

An additional parameter modified with HIT is fat oxidation. For example, whole-body fat oxidation as represented by respiratory exchange ratio is increased both at rest (36) and during submaximal exercise (3,7) after 2 wk of HIT. Further increases in fat oxidation were revealed in women performing 2 wk of HIT at 90% $\dot{V}O_{2max}$ (32). Greater lipid oxidation not only potentially improves endurance performance by sparing carbohydrate but also has dramatic effects on health risks. Marra et al. (23) reported that a higher respiratory quotient predicted weight gain in nonobese women. Moreover, impaired lipid oxidation may induce insulin resistance (15). Consequently, the potential of HIT to enhance lipid oxidation seems paramount to reducing health risks in various populations.

Only a few studies have described adaptations to chronic HIT. Tremblay et al. (34) required healthy, inactive men and women ($\dot{V}O_{2max} = 36.7$–38.8 mL·kg^{-1}·min^{-1}) to perform endurance or interval training for 15–20 wk. Results demonstrated significant increases in activities of 3-hydroxyacyl–CoA dehydrogenase and malate dehydrogenase with HIT. In addition, fat loss tended to be greater with interval training compared with endurance training. However, in this study, no dietary controls were described, which may markedly affect resultant changes in body fat and muscle metabolism. In a more recent study (33), inactive young women ($\dot{V}O_{2max} = 28.8$–30.9 mL·kg^{-1}·min^{-1}) performed 15 wk of endurance training or HIT, which consisted of repeated 8-s maximal sprints on a cycle ergometer interspersed with 12 s of light pedaling. Training progression was instituted based

on a blunting of the HR response to training, with up to 20 min of exercise eventually performed per session. Results revealed significant increases ($P < 0.05$) in $\dot{V}O_{2max}$ as well as attenuated fasting insulin concentration, body mass, and abdominal, trunk, and leg fat, which did not occur in the aerobic exercise group. Together, these studies seem to indicate that long-term interval training promotes fat loss in inactive young women, although whether this is due to increased fat oxidation is unknown because no study has examined alterations in fat oxidation in response to prolonged HIT.

One characteristic of most studies examining efficacy of HIT is that primarily supramaximal (250% $\dot{V}O_{2max}$ = the Wingate test) and near-maximal intensities (~90%–100% $\dot{V}O_{2max}$) have been investigated. Nevertheless, Hood et al. (19) demonstrated that 6 d of HIT at 60% peak power output improved oxidative capacity and insulin sensitivity in sedentary adults (age = 45.0 yr). To our knowledge, the efficacy of chronic HIT at relatively moderate intensities such as this has yet to be elucidated. Moreover, Helgerud et al. (16) reported that higher intensity aerobic exercise elicits greater adaptation than moderate exercise, but whether this is true for interval training is unknown.

Consequently, the primary aim of the present study was to compare the effects of moderate versus more intense interval training on maximal fat oxidation (MFO), the maximal rate of whole-body fat utilization, in sedentary women. Change in body composition was also examined, as previous data are equivocal regarding effects of HIT on weight and/or fat loss (25,33,34). Ultimately, results may reveal if adaptations to HIT exhibit a dose–response pattern in that more intense training elicits greater adaptation and consequently improvements in health status. In addition, repeated measures of MFO will describe the time course of changes in fat oxidation in response to interval training, which is of interest to clinicians seeking to optimize fat utilization in their clientele.

METHODS

Subjects. Thirty healthy but sedentary women were recruited to participate in this study. Fifteen were Caucasian, eight were Hispanic, four were Asian/Filipino, and three additional women were African American, Middle Eastern, and Native American, respectively. Sedentary was defined as less than 1 h·wk^{-1} of regular physical activity in the preceding year, which was confirmed with a validated survey (Past Year Total Physical Activity Questionnaire) completed at study initiation. Women were free of known disease as well as musculoskeletal problems such as severe knee or back pain, which would reduce their ability to complete intense cycling, and had body mass index (BMI) <35 kg·m^{-2}. Participants did not take any medications or supplements other than oral contraceptives ($n = 9$) during the study, yet it is evident that oral contraceptive use does not alter fat metabolism (22). They provided written informed consent, and all procedures were approved by the university institutional review board.

Design. Women completed 3 d·wk^{-1} (Monday/Wednesday/Friday, Tuesday/Thursday/Friday, etc.) of supervised HIT for 12 wk at workloads equal to either 80%–90% maximal workload (W_{max}) or 60%–80% W_{max}. Work was identical across groups. For women completing interval training, group assignment was randomized according to a Latin squares design, whereas controls were recruited from a convenience sample of women meeting the inclusion criteria who desired knowledge of various indices of health status but lacked the time to initiate training. At baseline, all physical characteristics including body fat, BMI, fat oxidation, and $\dot{V}O_{2max}$ were similar ($P > 0.05$) between groups. Their physical characteristics are revealed in Table 1. At baseline and every 3 wk, body mass, $\dot{V}O_{2max}$, and substrate oxidation were measured; and at baseline and every 6 wk, body composition was determined. Women were instructed to refrain from regular exercise outside the study and to maintain their dietary intake, which was examined via completion of a 4-d food log required every 6 wk. Time of day across all training sessions and assessments was standardized within subjects during the study.

Baseline testing. On day 1, height and body mass were measured to determine BMI. Body composition was determined using a sum of three skinfold model (21). Following standardized procedures (17), subcutaneous fat was measured twice in rotational order at the triceps, suprailiac, and thigh, and these values were used to calculate body density, which was used to estimate percent body fat (%BF). In addition, circumferences were measured twice in rotational order at the waist (narrowest portion of the trunk),

TABLE 1. Baseline physical characteristics (mean ± SD) of women performing 12 wk of interval training.

Variable	HI ($n = 11$)	Range	MOD ($n = 12$)	Range	CON ($n = 7$)	Range
Age (yr)	22.7 ± 5.4	18–38	24.7 ± 7.0	18–39	20.4 ± 1.7	19–24
Height (cm)	164.4 ± 6.3	155.0–172.0	162.8 ± 8.7	150.0–175.0	161.9 ± 8.4	147.0–172.0
Mass (kg)	68.4 ± 13.7	51.5–94.0	57.6 ± 11.1	44.5–86.9	57.5 ± 11.0	44.5–71.8
BMI (kg·m^{-2})	25.3 ± 4.3	20.6–33.0	22.5 ± 4.5	17.0–31.9	21.2 ± 3.1	17.1–25.5
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	29.8 ± 5.8	18.4–36.8	30.2 ± 3.9	22.2–36.2	30.5 ± 3.3	26.4–35.0
Body fat (%)	25.5 ± 6.1	16.2–35.4	22.3 ± 6.2	10.9–34.3	25.2 ± 4.1	20.8–30.7
Initial workload (W)	144.2 ± 24.6*	116.0–206.0	93.5 ± 14.6	68.0–116.4	NA	NA

Note that this table includes baseline data for all women who initiated the study.
$^*P < 0.05$ versus MOD.
HI, women training at 80%–90% W_{max}; MOD, women training at 60%–80% W_{max}; CON, control group.

umbilicus, and hips to determine waist-to-hip ratio and central adiposity.

Then, an HR monitor (Polar, Woodbury, NY) was placed on the trunk, and women were prepared for incremental exercise to fatigue on an electrically braked cycle ergometer (Velotron Dynafit Pro, Racermate, Seattle, WA) during which pulmonary gas exchange data were obtained to determine $\dot{V}O_{2max}$. Subjects initiated exercise at 40 W for 2 min, after which workload was increased by 20 $W·min^{-1}$ using a ramp protocol (1 $W·3$ s^{-1}) until volitional fatigue (cadence < 50 rpm) was attained. Pedal cadence was maintained at 60–90 rpm, and participants were encouraged to exercise "all out." The metabolic cart (Parvomedics True One, Sandy, UT) was calibrated before exercise according to the manufacturer. Variables obtained from this test included maximal determinations of $\dot{V}O_2$ ($L·min^{-1}$ and $mL·kg^{-1}·min^{-1}$), HR, RER, $\dot{V}CO_2$, and ventilation ($\dot{V}_E$). The attainment of $\dot{V}O_{2max}$ was confirmed by the incidence of a plateau in $\dot{V}O_2$ at $\dot{V}O_{2max}$ as well as $RER_{max} > 1.10$ (2). The coefficient of variation for $\dot{V}O_{2max}$ and W_{max} for inactive populations in our laboratory is equal to 3.2% and 3.7%, respectively. At volitional fatigue, maximal workload (in watts) was noted and used to set training intensities.

Subjects returned a minimum of 24–48 h later at the same time of day after an overnight (12 h) fast to determine substrate oxidation. Before this trial, they were required to denote all food and drink ingested in the previous 24 h on a dietary log and to confirm that they were indeed in a fasted state. This form was photocopied and returned to each subject, and they were required to replicate this dietary intake before subsequent assessments of substrate oxidation. Before exercise, resting gas exchange data were acquired for several minutes to ensure that subjects were not hyperventilating. Exercise consisted of 4 min of cycling at 40 W followed by 20-W increases in intensity every 3 min until RER remained higher than 1.0 for at least 60 s, a protocol similar to that of Achten et al. (1). Cadence was maintained between 60 and 80 rpm. Gas exchange data and HR were continuously obtained every 15 s, and the last 2 min of gas exchange data from each stage was averaged to calculate $\dot{V}O_2$ and $\dot{V}CO_2$, which were used to determine RER. The Frayn (12) equations were used to calculate rates of fat/CHO oxidation in grams per minute, respectively. MFO was identified as the highest rate of fat oxidation in grams per minute and also expressed in watts. The FAT_{max} zone (1) was also determined by averaging fat oxidation ($g·min^{-1}$) from three intensities expressing highest rates of FO inclusive of the workload commensurate with MFO. Minimum fat oxidation (FAT_{min}) was identified as the workload (in watts) at which mean RER was equal to 1.0, and thus fat utilization was negligible.

Interval training. Table 2 summarizes progression of HIT in both the high intensity (HI) and moderate intensity (MOD) groups, with training typically eliciting HR equal to 85%–100% HR_{max} for HI and 75%–95% for MOD, respectively. Each week, intensity was increased by 5% W_{max},

TABLE 2. Summary of interval training paradigms completed by sedentary women.

Week	HI Group	MOD Group
1	6 bouts at 80% W_{max}	6 bouts at 60% W_{max}
2	8 bouts at 85% W_{max}	8 bouts at 65% W_{max}
3	10 bouts at 90% W_{max}	10 bouts at 70% W_{max}
4[*]	6 bouts at 80% W_{max}	6 bouts at 65% W_{max}
5	8 bouts at 85% W_{max}	8 bouts at 70% W_{max}
6	10 bouts at 90% W_{max}	10 bouts at 75% W_{max}
7[*]	6 bouts at 80% W_{max}	6 bouts at 70% W_{max}
8	8 bouts at 85% W_{max}	8 bouts at 75% W_{max}
9	10 bouts at 90% W_{max}	10 bouts at 80% W_{max}
10[*]	6 bouts at 80% W_{max}	6 bouts at 70% W_{max}
11	8 bouts at 85% W_{max}	8 bouts at 75% W_{max}
12	10 bouts at 90% W_{max}	10 bouts at 80% W_{max}

[*] Note that workloads for the subsequent 3 wk were developed from the $\dot{V}O_{2max}$ test completed at the end of the preceding week of training.
HI, women training at 80%–90% W_{max}; MOD, women training at 60%–80% W_{max}; W_{max}, maximal workload (in watts).

and two additional bouts were completed because our goal was to ensure overload. Training sessions began with a 4-min warm-up at 40 W followed by 6–10 sixty-second bouts of interval training. Between bouts, active recovery at 40 W was provided (60 s in the MOD group and 75 s in the HI group), although participants were allotted additional time if needed. A 2- to 4-min cooldown at 40 W was completed after each session. This paradigm of HIT is similar to that used by Hood et al. (19) and Talanian et al. (32) and is more practical for untrained populations than repeated Wingate tests. Furthermore, Hofmann and Tschakert (18) reported that fractions of HR_{max} may be unsuitable for prescribing intensity during HIT, which is why intensity was prescribed based on percentages of W_{max}.

Assessment of dietary intake. Participants completed a 4-d food log at study initiation and at 6 and 12 wk of training. They were instructed to list everything they ate and drank during each of the 4 d (including two weekend days) and to list brand names and methods of preparation and to be as thorough as possible. Using the US Department of Agriculture Nutrient Database (http://ndb.nal.usda.gov/ndb/foods/list), grams and total calories of fat, carbohydrate, and protein were calculated. Subjects were advised to maintain their lifestyle during the study, which included the maintenance of dietary intake and sedentary status, and were told that this was an exercise study and not a weight loss study.

Data analyses. Data are expressed as mean ± SD and were analyzed using the Statistical Package for the Social Sciences (version 20.0; SPSS Inc., Chicago, IL). Multiple two-way repeated-measures ANOVA were used to examine differences in substrate oxidation parameters across intensity and training (0, 3, 6, 9, and 12 wk), with group used as a between-subjects factor. One-way repeated-measures ANOVA was used to examine changes in body weight and body composition across training (0, 6, and 12 wk), with group used as a between-subjects factor. If a significant F-ratio was obtained, Tukey's *post hoc* test was used to identify differences between means. Partial eta-squared (η^2) was determined to estimate effect size. Statistical significance was established as $P < 0.05$.

BASIC SCIENCES

RESULTS

Adherence to training in both groups was high, with completion of 694 out 720 possible sessions, for a total compliance rate equal to 96.4%. One woman in the HI group dropped out at 3 wk because of personal reasons, and two in the MOD group withdrew because of transportation issues ($n = 1$, after 1 wk) and unwillingness to abstain from regular exercise outside the study ($n = 1$, after 6 wk), resulting in 10 women completing 12 wk of HI, 10 women completing 12 wk of MOD, and 7 controls. Compared with baseline, training increased ($P < 0.001$) relative $\dot{V}O_{2max}$ and W_{max} by 22.3% and 20.5%, respectively, in MOD and 21.9% and 17.0%, respectively, in HI, which was not different ($P > 0.05$) between groups. Control subjects revealed no change in $\dot{V}O_{2max}$ from baseline (30.4 ± 3.6 mL·kg^{-1}·min^{-1}) to 6 wk (30.5 ± 4.4 mL·kg^{-1}·min^{-1}) and 12 wk (29.5 ± 4.0 mL·kg^{-1}·min^{-1}) as well as maintenance in all other gas exchange variables (data not reported).

Changes in parameters of substrate oxidation. Across all participants, change in RER in response to training was only examined up to 100 W ($n = 7$ in MOD and HI and $n = 5$ in CON), as many women early in the study did not attain work rates greater than 100 W during the progressive exercise test. During exercise, RER increased ($P = 0.001$, $\eta^2 = 0.94$) and was significantly different ($P = 0.006$, $\eta^2 = 0.30$) in response to HIT. The greatest increase in lipid oxidation ranged from 16% in MOD (from 0 to 3 wk at 40 W; RER decreasing from 0.85 ± 0.06 to 0.80 ± 0.04) to 26% in HI (from 0 to 6 wk at 60 W; RER decreasing from 0.89 ± 0.03 to 0.81 ± 0.03). These data are demonstrated in Figures 1A and B. RER
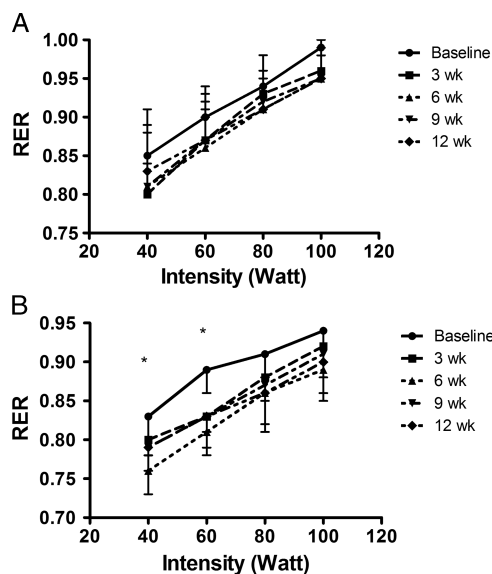

FIGURE 1—Change in RER (mean ± SD) in response to 12 wk of chronic interval training in (A) women in the MOD group and (B) women in the HI group; lower error bars were removed to enhance clarity. RER = respiratory exchange ratio. *$P < 0.05$ versus corresponding value at 0 wk in HI.

tended to be lower in HI versus MOD, but it was not significant (between-subjects effect, $P = 0.27$). *Post hoc* analyses revealed that RER at 80 and 100 W differed from 40 W at all time points of the study. In HI, *post hoc* analyses demonstrated that RER at 40 and 60 W (6 wk only) was reduced ($P < 0.05$) compared with that recorded at baseline but was unaltered during the last 6 wk of the study. Data revealed significant ($P = 0.02$, $\eta^2 = 0.25$) increases in fat oxidation and decreases in CHO oxidation ($P = 0.001$, $\mu^2 = 0.35$) with HIT (Figs. 2A–D), with these values significantly different across all intensities in both groups. CHO oxidation was lower in HI at 6 wk (1.31 ± 0.24 g·min^{-1}) versus baseline (1.86 ± 0.36 g·min^{-1}) at 100 W. There was no difference in fat or CHO oxidation from 0 to 12 wk in CON (data not reported).

Compared with baseline (range = 0.05–0.53 g·min^{-1}), training increased ($P = 0.04$, $\eta^2 = 0.14$) MFO in both MOD (25%) and HI (19%) (range = 0.12–0.48 g·min^{-1} across all subjects). Univariate ANOVA revealed no difference ($P = 0.37$) in MFO between groups. *Post hoc* analyses revealed that MFO at week 3 was higher than baseline values in MOD, yet it did not change during the last 9 wk of training or across the entire study in HI. These data are demonstrated in Table 3. When MFO was expressed in watts, 17 of 21 women revealed maximum fat oxidation at 40 W at baseline, and this was slightly altered with training (15/21). Women in the control group showed maintenance in MFO across the study (0.20 ± 0.14 to 0.20 ± 0.13 and 0.17 ± 0.12 g·min^{-1}, respectively).

FAT_{min} increased ($P = 0.001$, $\eta^2 = 0.39$) in response to training (Table 3). The change in FAT_{min} was similar in MOD (+24 W) versus HI (+22 W), and univariate ANOVA revealed no difference ($P = 0.06$) in FAT_{min} at baseline between groups. Across all subjects, FAT_{min} ranged from 60 to 200 W. Within HI, FAT_{min} recorded at 6, 9, and 12 wk was different ($P < 0.05$) versus baseline. However, *post hoc* analyses revealed no significant difference between any mean values in the MOD group. There was no change in FAT_{min} from 0 to 12 wk in CON (93.3 ± 41.0 W to 86.6 ± 30.1 W).

FAT_{zone} was enhanced with training ($P = 0.001$, $\eta^2 = 0.28$, range = 0.11–0.45 g·min^{-1}). The magnitude of increase in FAT_{zone} was similar in MOD (41%) versus HI (45%). Although this variable was consistently higher in HI versus MOD, this difference was not significant ($P = 0.22$). *Post hoc* analyses revealed that in MOD, baseline values were different than those at 3 and 6 wk. In HI, baseline FAT_{zone} differed from all other scores except the 3-wk values (Table 3). This variable did not change after 6 wk of training in either group. FAT_{zone} did not change in the control group from 0 to 12 wk (0.14 ± 0.12 to 0.14 ± 0.13 and 0.12 ± 0.11 g·min^{-1}, respectively).

Change in body weight and body composition. There was no change ($P > 0.05$) in body weight, %BF, or hip or abdominal circumference in response to training. However, changes in these variables were quite discrepant. Body weight was maintained in response to MOD (58.6 ± 11.9 to
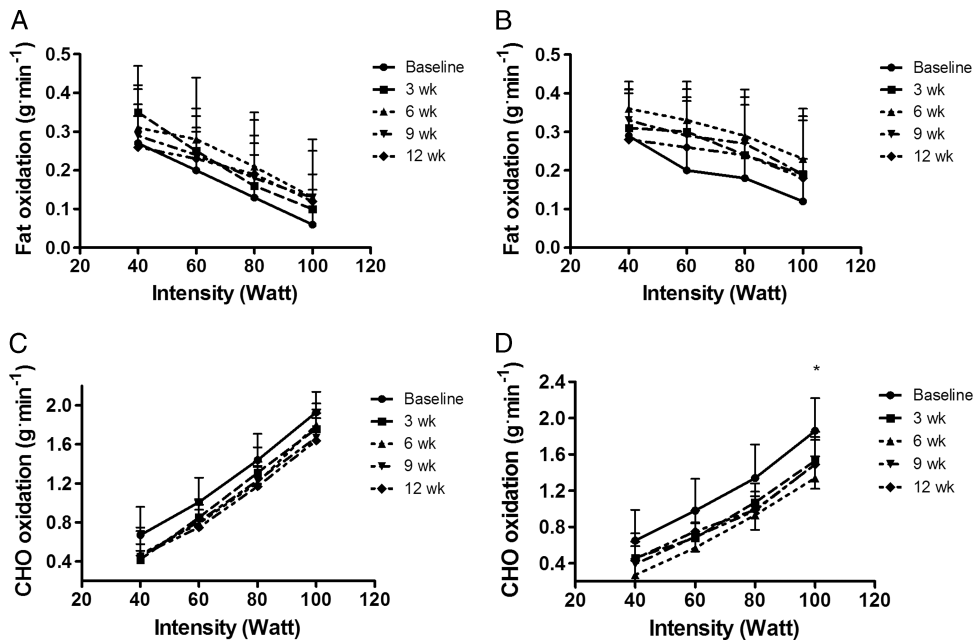
FIGURE 2—Change in carbohydrate oxidation (mean ± SD) in response to 12 wk of chronic interval training in (A) women in the MOD group and (B) women in the HI group. Lower error bars were removed to enhance clarity. *$P < 0.05$ between 6 wk and baseline values in HI. Change in fat oxidation (mean ± SD) in response to 12 wk of chronic interval training in (C) women in the MOD group and (D) women in the HI group. Lower error bars were removed to enhance clarity.

58.4 ± 12.1 kg) and HI (69.6 ± 15.0 to 69.0 ± 15.0 kg) yet increased in CON (55.8 ± 11.0 to 57.3 ± 12.2 kg). Moreover, eight women (four in each group) revealed greater than 1% decrements in %BF, four revealed no change, and eight (five in MOD and three in HI) exhibited greater than 1% increases in %BF. For WC, there was no main effect of training ($P = 0.51$), yet a significant training–group interaction ($P = 0.04$, $\mu^2 = 0.19$) occurred, and *post hoc* analyses showed that WC declined in MOD from 0 to 12 wk (71.5 ± 11.2 to 70.2 ± 10.4 cm) yet did not change in HI (75.9 ± 11.3 to 75.5 ± 10.8 cm) or CON (68.6 ± 8.0 to 69.2 ± 8.6 cm).

Changes in dietary intake. Results revealed no effect of training ($P = 0.19$) or interaction ($P = 0.25$) for total calorie intake, although calorie intake declined in the HI group during the study. Dietary intake was also similar ($P > 0.05$) across all groups at baseline. Similar nonsignificant findings were revealed for grams and percentage of macronutrient intake, which are revealed in Table 4. Women in CON showed no change in total caloric intake (1642 ± 264 kcal to

1463 ± 210 kcal and 1622 ± 256 kcal) or any other dietary variable during the study.

DISCUSSION

The primary aim of this study was to compare changes in MFO in response to two doses of chronic interval training in sedentary women. To our knowledge, this is the first study to examine changes in MFO in response to chronic interval training; and with the documented relationship between fat oxidation and insulin action (26), increased fat oxidation in response to a rather time-efficient paradigm of exercise seems paramount to improve health status in previously sedentary adults. Moreover, data may be used to develop specific guidelines to identify the best intensity of interval training to augment fat oxidation.

Many studies document increased lipid oxidation in response to interval training. However, most of these studies were short-term training interventions (2 wk) with the longest

TABLE 3. Change in MFO, FAT$_{zone}$, and FAT$_{min}$ in response to 12 wk of interval training.

	MFO (g·min^{-1})		FAT$_{zone}$ (g·min^{-1})		FAT$_{min}$ (W)	
Week	**MOD**	**HI**	**MOD**	**HI**	**MOD**	**HI**
0	0.23 ± 0.12	0.27 ± 0.12	0.14 ± 0.10	0.20 ± 0.09	94.0 ± 25.2	115.6 ± 32.8
3	0.31 ± 0.07**	0.28 ± 0.12	0.22 ± 0.08**	0.24 ± 0.13	95.0 ± 39.8	124.4 ± 29.6
6	0.30 ± 0.12	0.34 ± 0.10	0.22 ± 0.12**	0.29 ± 0.12*	112.0 ± 21.5	140.0 ± 33.2*
9	0.26 ± 0.06	0.32 ± 0.10	0.21 ± 0.07	0.28 ± 0.11*	118.0 ± 19.9	144.4 ± 31.2*
12	0.25 ± 0.10	0.30 ± 0.12	0.20 ± 0.11	0.27 ± 0.12*	118.0 ± 22.0	145.0 ± 31.3*

Data are presented as mean ± SD.
*$P < 0.05$ versus 0 wk in HI.
**$P < 0.05$ versus 0 wk in MOD.
HI, women training at 80%–90% W_{max}; MOD, women training at 60%–80% W_{max}.

TABLE 4. Change in macronutrient intake in response to 12 wk of interval training.

Week	Nutrient intake (kcal)		CHO (%)		Fat (%)		Protein (%)	
	MOD	HI	MOD	HI	MOD	HI	MOD	HI
0	1679.4 ± 279.4	1789.1 ± 214.6	46.2 ± 5.7	54.0 ± 7.8	35.1 ± 9.1	31.7 ± 8.4	15.4 ± 3.6	17.3 ± 3.5
6	1783.0 ± 210.4	1557.1 ± 313.9	50.3 ± 6.1	51.0 ± 5.0	34.3 ± 4.9	31.8 ± 3.1	18.6 ± 6.5	15.2 ± 2.4
12	1677.5 ± 233.2	1514.2 ± 280.2	50.1 ± 5.6	52.2 ± 2.7	33.0 ± 6.3	32.0 ± 3.2	17.0 ± 4.2	16.2 ± 3.5

HI, women training at 80%–90% W_{max}; MOD, women training at 60%–80% W_{max}; CHO, carbohydrate.

one lasting only 6 wk. All were characterized by pre- and posttraining assessments of substrate oxidation. For example, an 18.2% improvement in resting lipid oxidation was demonstrated in sedentary men completing 2 wk of Wingate-based interval training (36). In response to 2 wk of interval training performed by active women, Talanian et al. (32) revealed a lower RER (13%–20% improvement in fat oxidation) and 36% increase in whole-body fat oxidation during 1 h of cycling at 60% $\dot{V}O_{2peak}$. Astorino et al. (3) demonstrated lower RER (equivalent to 4%–16% greater lipid oxidation) during cycling at intensities from 50% to 70% W_{max} after 2 wk of Wingate-based HIT. In response to 6 wk of Wingate-based HIT, a 4%–8% increase in lipid oxidation was demonstrated during prolonged cycling at 65% $\dot{V}O_{2peak}$ (8). Discrepancies between studies can be attributed to different HIT paradigms performed, fitness level of participants, and specific protocols used to determine changes in lipid oxidation.

This is the first study to examine changes in RER-derived lipid oxidation as well as MFO, FAT_{min}, and FAT_{zone} in response to interval training. Data showed different magnitude of changes in these latter parameters versus the RER response (Figs. 1 and 2; Table 3). For example, the percent change in lipid oxidation via RER was lower than that revealed for MFO, FAT_{min}, and FAT_{zone}. In addition, lipid oxidation was improved in response to the initial 3–6 wk of either modality of HIT, but no further improvements in this parameter were noted during the final 6 wk of training despite increasing workloads. Perhaps a greater number of bouts, higher relative intensities, or addition of continuous aerobic exercise is needed to sustain these gains in lipid oxidation, which requires additional study. Because of the strong association between lipid oxidation and health risks (26), this is an important topic to further research.

Mechanisms explaining training-induced enhancements in fat oxidation are relatively well understood. In a cross-sectional study, Stisen et al. (31) compared rates of MFO in endurance trained and untrained young women. Despite no differences in MFO, trained women revealed greater fat oxidation at moderate and high exercise intensities. In addition, significant positive correlations were demonstrated between MFO and 3-hydroxyacyl–CoA dehydrogenase activity as well as between citrate synthase and MFO expressed as a percentage of $\dot{V}O_{2max}$. In response to 2 wk of HIT, improvements in maximal activity of 3-hydroxyacyl–CoA dehydrogenase (32%), citrate synthase (20%), and muscle fatty acid binding protein content (25%) were reported (32). It remains to be determined, however, if more prolonged regimens of HIT are capable of sustaining this relatively short-term improvement in capacity to oxidize lipids.

There are clear interindividual differences in adaptation to chronic exercise training. For example, marked differences in $\dot{V}O_{2max}$ in response to training were demonstrated in the HERITAGE study (6), and a recent study (30) furthered this phenomenon by showing that only 55% of subjects revealed favorable changes in $\dot{V}O_{2max}$, resting/exercise HR, and anaerobic threshold after 1 yr of endurance training. However, the literature does not include data regarding the incidence of nonresponders to interval training in regard to changes in substrate oxidation. In the current study, mean data revealed augmented fat oxidation in response to chronic HIT that was similar whether intensities were moderate or relatively high. However, closer examination of individual data reveals markedly variable responses across subjects. Subject RG, a 23-yr-old woman performing MOD with baseline $\dot{V}O_{2max}$ and %BF of 30.85 mL·kg^{-1}·min^{-1} and 22.1%, respectively, revealed less than a 10% increase in MFO during the study, yet FAT_{min} was markedly improved (+60 W) as well as FAT_{zone} (0.15–0.24 g·min^{-1}). In contrast, subject AV, a 22-yr-old woman with baseline $\dot{V}O_{2max}$ and %BF of 28.80 mL·kg^{-1}·min^{-1} and 19.2%, respectively, demonstrated minimal change in FAT_{min} (0 W) or FAT_{zone} (0.21–0.25 g·min^{-1}) in response to HI yet a marked increase (from 0.23 to 0.37 g·min^{-1}) in MFO in the first 6 wk after which it declined. RER has been shown to widely vary across individuals, which may explain some of these discrepant responses (13). In this study, large variability in RER (0.82–0.98) was observed during moderate exercise in cyclists, which supports data from a heterogeneous population (35). At 25% and 50% W_{max}, significant correlates of RER were serum-free fatty acid levels, plasma [lactate concentration], and muscle enzyme activities (13). Intraclass correlations for RER at low (0.85) and moderate intensities (0.66) were similar to data from our laboratory during progressive cycling by moderately active men and women (ICC = 0.71), which suggest marked dissimilarity in RER across days in the same subjects. Because of this large variation in RER, it may be practical to combine this measurement with determinations of MFO, FAT_{min}, and FAT_{zone} to better assess changes in capacity for lipid oxidation in response to training because there may be less day-to-day variation in these variables. Ultimately, training intensities and/or modalities may need to be individualized in participants who show little adaptation to training to promote the greatest health-related benefits, including increased fat oxidation, obtained from regular physical activity.

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The MFO values demonstrated in the present study aligned with previously reported values obtained in a variety of individuals. In untrained adults with $\dot{V}O_{2max}$ equal to 28.0 mL·kg^{-1}·min^{-1} (29), MFO occurred at a workload of 35% $\dot{V}O_{2max}$ and an RPE of 9, which is similar to the present study showing MFO at low (~25% W_{max}) exercise intensities. In active men and women ($\dot{V}O_{2max}$ = 46–57 mL·kg^{-1}·min^{-1}), MFO was equal to 0.39–0.41 g·min^{-1} during treadmill walking (10), comparable with values demonstrated by active subjects during graded cycling (9). Stisin et al. (31) reported similar values of MFO in untrained (0.32 g·min^{-1}) and trained women (0.40 g·min^{-1}) with similar body fat (25%–27%) as those in the present study, although their participants' $\dot{V}O_{2max}$ was dramatically higher (41–53 mL·kg^{-1}·min^{-1}). However, these values are surpassed by those reported in male cyclists (1) equal to 0.56–0.66 g·min^{-1}. Overall, MFO tends to be greater in subjects with greater cardiorespiratory fitness and is typically higher during walking versus cycling due to a greater exercising muscle mass.

At higher exercise intensities, there is enhanced glycolytic flux that reduces muscle pH and concomitantly increases nonmetabolic CO_2 production through bicarbonate buffering. This additional source of CO_2 would increase RER and thus diminish fat oxidation. However, the metabolic cart is unable to account for this discrepancy, so the average RER value determined for each stage at the higher intensities may not be a true reflection of fat oxidation. In fact, Rowlands (28) cited a 3%–12% underestimation of fat oxidation when compartmental CO_2 stores were accounted for, which should increase fat oxidation versus noncorrected values as reported in the present study.

Three previous studies have examined changes in body composition in response to chronic HIT. In one study (34), healthy nonobese men and women performed 15 wk of intermittent exercise. No change ($P > 0.05$) in body weight was revealed with training, although significant ($P < 0.05$) decrements in skinfold-derived estimates of limb and truncal fat were demonstrated. However, participants also performed chronic endurance training, and dietary intake was not assessed, so it is unknown if these changes in body fat were potentiated by the inclusion of endurance training or modifications in food intake. In a study by Trapp et al. (33), inactive young women performed 15 wk of interval training in which DEXA-derived estimates of body mass, fat mass, and %BF were lower ($P < 0.05$) compared with baseline. In contrast, Nybo et al. (25) reported no change ($P > 0.05$) in body weight or body fat in untrained men performing 12 wk of treadmill interval running, which supports our data. As dietary intake did not significantly change in response to training in either group (Table 4), data suggest that the initiation of prolonged interval training without modification of dietary intake does not induce lower body fat or change in body weight, which opposes previous findings (37). Nevertheless, a negative energy balance would be anticipated with the initiation of exercise with no change in dietary intake, so the lack of change in body weight or body fat is rather unexpected. One explanation for this result is underreporting of food intake, which has been documented in women (24). In addition, the implementation of intense training in previously sedentary individuals may elicit compensatory responses to enhance appetite and food intake, which may slow potential for weight loss. Recently, repeated Wingate tests resulted in greater appetite perception yet similar food intake versus endurance exercise, which was attributed to the negative energy balance incurred with prolonged exercise (11). In overweight sedentary men, Rosenkilde et al. (27) showed similar weight loss whether the exercise dose was moderate (300 kcal·d^{-1}) or high (600 kcal·d^{-1}), and the less than expected weight loss in the high dose group was attributed to food intake-related compensation. A threshold intensity or dose of exercise may exist that optimizes weight or body fat loss, although additional study is needed to identify this.

In the current study, we used the widely used exercise protocol of Achten et al. (1) to describe changes in substrate oxidation in response to interval training. This protocol was also used to identify fat oxidation kinetics in a heterogeneous sample of men and women (8). From this protocol, MFO and FAT$_{min}$ can be identified. However, one limitation of this protocol is its initial work rate equal to 40 W, which was used in the present study. For many of our participants, this workload elicited MFO and moreover was equivalent to intensities higher than 60%–65% HR/$\dot{V}O_{2max}$. In untrained individuals, scientists should consider a lower initial workload equal to 20 or 30 W to better portray the change in fat oxidation during graded exercise. Some scientists (5) have speculated that graded exercise consisting of 3 min stages is inaccurate for assessing substrate use in untrained populations who may need additional time to attain a steady state. However, our participants were younger, nonobese, and had a higher $\dot{V}O_{2max}$ than subjects in this study, and reexamination of all gas exchange data showed little variation in $\dot{V}O_2$ and $\dot{V}CO_2$ during the last minute of each stage, so we are confident that a steady state was attained at the end of each 3-min stage.

This study faces several limitations. Participants were a rather small sample of sedentary women heterogeneous in age, ethnicity, and BMI. Yet, $\dot{V}O_{2max}$, %BF, and fat oxidation were similar at baseline across all training groups. Nevertheless, data demonstrated significant improvements in fat oxidation in response to either moderate or intense interval training, which has application to exercise prescription promoting fat utilization. The menstrual cycle was not considered in the current study, which could have affected some of the findings, yet no influence of menstrual phase on substrate oxidation has been reported (20). Concentrations of insulin as well as lactate, glucose, and/or free fatty acid were not assessed, which would have provided greater insight into potential mechanisms explaining the observed findings. In addition, changes in insulin action, which have been shown (15) to be related to capacity for fat

oxidation, were not determined. Nevertheless, this study is strengthened by a high rate of compliance to training, strict consideration of dietary habits, and precise determination of work rates implemented during training, which was supervised. A nonexercising control group composed of young, sedentary women showed no change in $\dot{V}O_{2max}$ or lipid oxidation similar to previous studies (3,21). Moreover, training-induced changes in fat oxidation were examined over a range of intensities rather than one (8,32) or three workloads (3) as previously performed. However, additional studies are needed in larger populations to corroborate these results in sedentary adults and to determine whether chronic interval training can promote weight loss and enhance fat oxidation in these individuals.

In conclusion, data show similar improvements in lipid oxidation whether 18–30 min·wk^{-1} of interval training was performed at moderate or more intense workloads. Fat oxidation peaked approximately 6 wk into training after which it was maintained, which suggests that changes in intensity, number of bouts, or frequency should be implemented to promote continued improvements in fat oxidation. Body composition did not change with training, suggesting that interval training alone without reductions in dietary intake may not elicit weight loss.

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