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Effect of Exercise on the Diurnal Variation in Energy Substrate use During a High-Fat Diet

KR Hawkins, MS¹, KC Hansen, PhD², DA Schoeller, PhD³, and JA Cooper, PhD¹

¹Department of Nutrition, Hospitality, and Retailing, Texas Tech University, TX, USA PO Box 41240 Lubbock, TX 79409

²Division of Geriatric Medicine, University of Colorado Denver, CO, USA, 12401 E. 17th Ave, PO BOX 6511, Mail Stop B179, Aurora, CO 80045

³Department of Nutritional Sciences, University of Wisconsin-Madison, WI, USA, 1415 Linden St. Madison, WI 53706

Keywords

obesity; high fat diet; exercise; fat oxidation

Introduction

The prevalence of obesity has become a major health concern in the United States and other countries as one in three Americans is classified as obese based on measured body mass index [7]. A sedentary lifestyle and excessive energy intake, in particular a high-fat (HF) diet, are common behaviors linked to obesity. It has been well demonstrated that aerobic exercise enhances the ability to increase 24 hour fat oxidation in response to increasing dietary fat intake [9;11;28]. Therefore, the incorporation of exercise allows fat balance to be achieved more rapidly on an acute HF diet. What remains to be determined is whether it is the actual exercise bout, or the effects of exercise on the remainder of the day and night, that is eliciting these effects.

Achieving energy balance is the most important factor for maintaining body weight and preventing obesity. It has been hypothesized, however, that energy balance is more easily maintained when the body is able to match fat oxidation to dietary fat intake [9;11]. Without large fluctuations in macronutrient content in the diet, an individual can usually maintain macronutrient balance. If, however, an individual changes the macronutrient composition of their diet, macronutrient oxidation must be altered in order to remain in macronutrient balance. For the purpose of this study, we defined “adaptation” as a change in macronutrient oxidation in response to changes in dietary macronutrient composition. The adaptation process to an acute HF diet is much slower compared to a high carbohydrate diet [9;11]. While the adaptation process may only take 1–2 days when a person consumes a high carbohydrate diet [2], adapting to a HF diet may take as long as 7 days for the amount of fat oxidized to match the higher levels of dietary fat intake [23;24;26;29]. During this adaptation process, the individual will be in a positive fat balance which can lead to weight gain if this occurs frequently over a longer period of time in conjunction with a positive energy balance.

Previous studies have shown that the incorporation of exercise when switching from a moderate fat to a HF diet can increase the rate of adaptation to that diet. This decreases the amount of time that a person is in a positive fat balance by 3–4 days [9;11;28]. Although low intensity exercise increases energy expenditure, and for the most part fat oxidation, it is not clear if the increase in the percentage of energy provided by fat oxidation during exercise is sufficient to match the percentage of energy of fat in the diet. Further, it remains to be determined when, during a 24h period, that enhanced adaptation to a HF diet is occurring with exercise conditions. Therefore, the aim of the current study was to examine the time of day adaptation to a HF diet in exercise versus sedentary conditions from two previous studies in both men and women [9;11]. To study the time of day adaptation, we divided each 24h time period into three time segments: exercise + recovery hours, sleep hours, and all remaining wake hours. We hypothesized that fat oxidation would be greater (and non-protein respiratory exchange ratio (NPRER) lower) for exercise vs. sedentary treatments for all three time segments.

Methods and Procedures

Design

This analysis was performed as a secondary analysis combining data from two previously published studies done at the University of Wisconsin (UW)-Madison [9;11]. Both of these studies examined the adaptation to a HF diet with small differences in study design or protocol. Cooper et al [9] studied 8 male subjects and included an aspect of determining the effects of dietary fatty acid composition on the adaptation to a HF diet. Subjects completed four inpatient visits in order to complete all four of the following treatment conditions: a high mono-unsaturated fat diet with and without exercise and a high saturated fat diet with and without exercise. Since the results did not show any significant effect with regard to dietary fatty acid composition on 24h NPRER, fat oxidation, or energy expenditure, data from both sedentary visits (high mono-unsaturated fat diet and high saturated fat diet) were combined for the high fat feeding portion of the study. The other study by Hansen et al [11], which was done in 10 female participants and included three inpatient visits, compared sedentary conditions with two different exercise volumes (resting metabolic rate (RMR)*1.6 and RMR*1.8) to evaluate if they had a graded effect on the adaptation to a HF diet. For this current data analysis project, only the sedentary visits and highest volume of exercise visits (RMR*1.8) were used since they matched the designed energy expenditure in the sedentary and exercise visits from the male study by Cooper et al [9]. We also analyzed the data based on sex differences between the two studies and found that there were no significant differences in the patterns of change over the course of the 5-day study between men and women for any of the time segments. Therefore, from both studies, the treatments to be compared were sedentary vs. exercise conditions. Both studies were approved by the Institutional Review Board at UW-Madison and informed written consent was obtained from each participant.

Time Segment Analysis

Both studies collected continuous 24-hour data for all days of each study visit. We chose to divide the 24h data into three time segments to determine when during a 24h period the adaptations to a HF diet were occurring. The time segments were: (1) The sleep time segment which included 7.75 hours of data collected between 2300-0645h; (2) The exercise + recovery time period which combined two times during the day and included a total of four hours. The first exercise + recovery time segment began at 1000 and ended at 1200h (1h of exercise and 1h of recovery), and the second was during the hours of 2100 to 2300 (1h of exercise and 1h of recovery); (3) The wake time segment which included a total of

10.5h from 0830 to 1000h and 1200 to 2100h. The subjects were out of the chamber each day between 0645 and 0830h so no data was collected during this time.

Subjects

Eight healthy men and ten healthy women between the ages of 18 and 45 were recruited at the University of Wisconsin (UW)-Madison to participate in two research studies at the UW hospital's Clinical and Translational Research Core (CTRC). For this randomized crossover study, inclusion criteria were males and females between the ages of 18 and 45, with a BMI between 18 and 29kg/m², and a moderately sedentary lifestyle (<3h/wk of low-to-moderate-intensity exercise and no vigorous exercise). Subjects actually reported getting less than 1.5 hour of physical activity per week with little to no structured exercise. Subjects were asked to maintain their regular exercise patterns during the lead in period and in between each study visit. The same questions regarding their physical activity were asked prior to each inpatient study and no differences were found between study visits. Exclusion criteria included a history of metabolic, cardiac, or pulmonary disease and an irregular menstrual cycle for females, as described previously [11]. The female volunteers were allowed to continue taking birth control pills for the duration of the study and were admitted for their inpatient visits during the follicular phase of their menstrual cycle. All participants completed all phases of the study.

Protocol

The two studies had similar designs. Potential subjects first underwent a physical exam and screening. Resting metabolic rate (RMR; kcal/d) was measured for 30 minutes on the Deltatrac II Metabolic Monitor (VIASYS Healthcare, Inc., SensorMedics, Yorba Linda, CA) following an overnight fast. Following RMR measurements, subjects underwent a bicycle VO₂ max test on a Ergoline 800 cycle ergometer (SensorMedics Corp., Yorba Linda, CA) at the University of Wisconsin Pulmonary Function Laboratory using a CPX-D rapid gas analyzer (Medical Graphics Corp., St. Paul, MN). Achievement of VO₂ max included a VO₂ plateau, or RER greater than 1.1 in conjunction with heart rate (HR) greater than 90% of age-predicted HR max.

Participants were asked to refrain from any vigorous physical activity for 24h prior to their inpatient visit. The order of inpatient treatments (exercise vs sedentary) was randomized. Participants were admitted to the CTRC in the evening at 1800h to begin the 5 day, 6 consecutive night inpatient visit in the metabolic chamber. Total body water was used to measure body composition [9]. Body water was calculated by dilution on the assumption that the oxygen dilution space was 1.007 total body weight (TBW), and fat-free mass was calculated as TBW divided by 0.73. The 0.73 is used based on the assumption that 73% of FFM is total body water [21;22]. Participants slept from 2300-0645h each night and sleeping during the day was prohibited. Each morning (0645h) the participants were awakened and exited the chamber and had 30–45 minutes for personal hygiene before re-entering the chamber at 0815h. 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. This day was identical to the rest of the days pertaining to the diet, activity, and sampling. Participants were discharged at 0800h on the sixth morning of each visit.

Diet

For both studies, participants were given prepared meals for 4 days before the inpatient visits and during the stay in the chamber. These lead-in diets provided 30 percent of energy from fat, 55 percent energy from carbohydrates, and 15 percent of energy from protein. The total caloric intake for the lead-in diet were designed to maintain body weight and had an energy content of RMR*1.65, representing the average U.S. physical activity level [14].

During the inpatient study visits, all meals were provided by the UW Hospitals and Clinics kitchen as ordered and monitored by the CTRC dietician. Day one was the same composition as the lead-in diet. Days two through five were the HF diet; which consisted of 50 percent fat, 35 percent carbohydrates, and 15 percent protein. The subjects received 3 meals daily (breakfast at 0830, lunch at 1200, and dinner at 1900). An afternoon snack (1600) and an evening snack (2200) were also given in addition to the meals. Individual daily meals consisted of 25%, 25%, 40% (minus 50kcal), and 10% of daily caloric needs for breakfast, lunch, dinner, and afternoon snack respectively. They also received a 50kcal snack each evening. The meals were designed to match the total caloric intake needed to match their 24 hour energy expenditure. The caloric intake was $\text{RMR} \times 1.4$ for sedentary visits and $\text{RMR} \times 1.8$ for exercise visits [17]. Participants were required to eat all food provided to them during the duration of the study. Total energy intake was recorded for all days during each study visit. No differences were recorded between the dietary intakes on the days of the HF diet and therefore the values were averaged for all HF days. The mean 24h energy intake of the HF diets for the male participants was 3003 ± 144 kcal/d for the exercise visits and 2240 ± 126 kcal/d for the sedentary visits. For the female subjects, average 24h energy intakes were 2547 ± 129 kcal/d for the exercise visits and 2025 ± 96 kcal/d for the sedentary visits.

Exercise

During exercise visits, subjects rode on a stationary bike at 45% of their $\text{VO}_{2\text{max}}$ two times each day (at 1000h and 2100h). Exercise visits were designed to raise the participants' 24 hr energy expenditure to a $\text{RMR} \times 1.8$. To determine duration of exercise, the energy cost of cycling at 45% of $\text{VO}_{2\text{max}}$ was estimated from the linear equation between VO_2 and work (watts) that occurred during their $\text{VO}_{2\text{max}}$ test. To estimate the minutes of cycling required to elevate 24 h energy expenditure during exercise visits, the relative VO_2 (ml/kg/min) at 45% of $\text{VO}_{2\text{max}}$ was used to calculate the rate of energy expenditure (kcal/min) with the intensity (watts) which was used in combination with RMR. The entire number of minutes obtained from this process was then divided into the morning and evening segments of exercise and this equated to approximately 1 hour of exercise in the morning and 1 hour in the evening. The actual energy expended during each exercise bout averaged 357 ± 27 kcal/d.

Sample Collection and Analysis

Specimens for urinary nitrogen were collected at intervals of 4 hours and was acidified with 250mg citric acid as described previously [9]. Briefly, the volume of all urine was recorded and 15mL was saved. The samples were stored at -5°C then diluted 1-to-100 in triple-distilled water for analysis. A chemiluminescence nitrogen analyzer was used for nitrogen analysis. Nitrogen concentration was measured in parts per million against a calibration curve with known concentrations of urea (0–100ppm).

Calculations of Substrate Oxidation

The equations of Jequier and Schutz [17] for VCO_2 produced, VO_2 consumed, and urinary nitrogen were used to calculate NPRER and substrate oxidation. All urine samples collected during the day were pooled to calculate one average value of protein oxidation (grams per minute) for the day. Similarly, all urine samples collected during the night were pooled and used to calculate a night-time protein oxidation (grams per minute). The non-protein VO_2 and VCO_2 values were then used to calculate a NPRER. Finally, macronutrients oxidized were then converted to nutrients and calculated in grams oxidized for each time segment.

Statistical Analysis

The data was compiled from the two previous studies to allow for within subject whole group comparisons for each of the three time segments. Data is reported as mean $\pm$ SE unless otherwise noted. The variables that were included in this data analysis were NPRER and fat, carbohydrate, and protein oxidation. The SAS version 9.2 statistical package (SAS Institute Inc, Cary, NC) was used for all data analyses. A repeated measures ANOVA was used to test for treatment effects, time effects, and treatment by time interactions. If significance was found, a post hoc analysis was conducted using a Tukey's test. The statistical significance was set at $p < 0.05$.

Results

Subjects

Eight healthy male participants and ten healthy female participants completed this study and their demographics can be found in Table 1. We did not find any significant differences in the patterns of change over the course of the 5-day study between males and females with respect to NPRER, so males and females were grouped together in all analyses. There were, however, some sex differences in absolute macronutrient oxidation, and sex specific data is also provided below. All participants were considered sedentary (<3 h per week of low-to-moderate intensity exercise). The $\text{VO}_{2\text{max}}$ values of the subjects were representative of average individuals for males and females, ages 20–29 [1]. The BMI of the subjects ranged from 18 to 29 kg/m^2 and all were free of metabolic disease. No significant changes in body weight occurred during any of the five day stays in the metabolic chamber. Blood, glucose, and insulin were all in normal range (data not shown) [8].

Time Segment NPRER

NPRER data for all three time segments can be found in Figures 1 and 2. Figure 1 shows the results for the two treatment conditions over the course of the 5 day study. There was a significant effect of time for all three time segments ($p < 0.001$ for each segment). The effect of time (days) on the acute HF diet was a decrease in NPRER over the course of 5 days. There were significant treatment effects for the exercise + recovery time segment ($p < 0.001$) and the sleep time segment ($p < 0.001$) but not for the wake time segment. Exercise conditions showed a higher NPRER value compared to sedentary conditions for the exercise + recovery segment. Conversely, the treatment effect observed during sleep hours was a lower NPRER value during exercise vs. sedentary conditions. No treatment by time interaction was found for any of the three time segments. Figure 2 shows the average of the values across the HF diet days (days 2–5) of each treatment condition for all 3 time segments. NPRER values for exercise vs. sedentary conditions were 0.82 ± 0.01 vs. 0.84 ± 0.01 , ns (wake); 0.77 ± 0.01 vs. 0.81 ± 0.01 , $p < 0.05$ (sleep); and 0.88 ± 0.01 vs. 0.85 ± 0.01 , $p < 0.001$ (ex+recov), respectively. While NPRER was significantly lower for the exercise vs. sedentary conditions in the sleep time segment (-0.04 ± 0.01), it was significantly higher for exercise vs. sedentary conditions for the exercise + recovery time segment (0.03 ± 0.01), and was not significantly different between treatments for the wake time segment (-0.02 ± 0.01).

The FQ describes the ratio of CO_2 produced to O_2 consumed calculated for physiologic oxidation of a representative sample of the diet. Since the FQ during the HF diet did not differ from day to day or between each treatment, HF days 2–5 were averaged to yield one HF FQ of 0.826. Comparing NPRER values during the HF diet to the FQ suggests that only the sleep time segment was in negative fat balance and that the exercise conditions produced a much larger negative fat balance compared to sedentary conditions (Figure 2). The exercise + recovery time segment had higher NPRERs for exercise and sedentary conditions

compared to the calculated FQ while sedentary conditions had a higher NPRER and exercise conditions had a slightly lower NPRER than the calculated FQ for the wake time segment.

Time Segment Macronutrient Oxidation

Absolute fat oxidation (grams) for all three time segments can be found in Figures 3 and 4. A significant time effect was found for all three time segments with fat oxidation increasing over the 5-day study period ($p < 0.001$ for each segment, Fig 3). There were also significant treatment effects for exercise vs. sedentary treatments for all three time segments ($p < 0.001$ for each segment, Fig 3). The treatment effect was greater fat oxidation for exercise compared to sedentary conditions. Finally, there was also a significant treatment by time interaction for all three time segments ($p < 0.01$ for each segment). Additionally, we looked at fat oxidation expressed as g/min/fat free mass (FFM) in kg which can be found in Figures 5 & 6. The statistical results were the same as those for fat oxidation expressed as total grams. Therefore, there was a significant time effect ($p < 0.001$ for each segment), a significant treatment effect ($p < 0.001$ for each segment), and a significant treatment by time interaction ($p < 0.01$ for each segment) for all three time segments (Figure 5).

We also averaged fat oxidation values across all HF diet days (days 2–5) for exercise vs. sedentary conditions and these are shown in Figures 4 and 6. Average fat oxidation (total grams for each segment) for exercise vs. sedentary conditions were 62 ± 3 vs. 51 ± 3 g ($p < 0.001$) for wake, 41 ± 2 vs. 31 ± 2 g ($p < 0.001$) for sleep, and 33 ± 3 vs. 16 ± 1 g ($p < 0.001$) for exercise + recovery time segments, respectively. This corresponded to significant treatment differences of 11 ± 3 g for wake, 10 ± 2 g for sleep, and 16 ± 2 g for exercise + recovery. Average fat oxidation expressed as g/min/FFM for exercise vs. sedentary conditions were 0.00207 ± 0.00007 vs. 0.00173 ± 0.00007 g/min/FFM ($p < 0.001$) for wake, 0.00183 ± 0.00009 vs. 0.00138 ± 0.00006 g/min/FFM ($p < 0.001$) for sleep, and 0.00299 ± 0.0003 vs. 0.00147 ± 0.00011 g/min/FFM ($p < 0.001$) for exercise + recovery time segments, respectively. This corresponded to significant treatment differences of 0.00034 ± 0.00002 g/min/FFM for wake, 0.00045 ± 0.00003 g/min/FFM for sleep, and 0.00152 ± 0.00019 g/min/FFM for exercise + recovery. Not surprisingly, carbohydrate oxidation mimicked fat oxidation results albeit in the opposite direction. There was a significant time effect for all three segments as carbohydrate oxidation decreased over the course of each 5-day visit ($p < 0.05$ for each segment). There were also significant treatment effects for the sleep (28 ± 4 vs. 40 ± 3 g, $p < 0.001$), wake (77 ± 5 vs. 90 ± 6 g, $p < 0.001$), and exercise + recovery segments (142 ± 14 vs. 39 ± 3 g, $p < 0.001$) for exercise vs. sedentary conditions, respectively. There were no significant treatment*time interactions. Protein oxidation for all three time segments revealed no significant time effects, treatment effects, or treatment by time interactions.

Sex Comparison

We initially analyzed the data looking at differences between exercise vs. sedentary conditions for males only, females only, and then the combination of males and females. Where we found significant time effects, treatment effects, or treatment by time interactions in males, we also found those same significant differences in females. This data, highlighting treatment differences between exercise vs. sedentary conditions in either males or females can be found in Table 2. We performed additional analyses to compare absolute values between sexes for exercise vs. sedentary conditions for all three time segments (Table 2). For the exercise condition, we found significant treatment differences between males and females for NPRER during the exercise + recovery time segment (0.90 ± 0.01 vs. 0.87 ± 0.01 , $p < 0.001$), for fat oxidation (in total grams) during the wake (69.7 ± 3.5 vs. 56.9 ± 3.3 g, $p < 0.001$) and sleep (0.90 ± 0.01 vs. 0.87 ± 0.01 g, $p < 0.001$) time segments, and for CHO oxidation during the sleep (18.8 ± 5.1 vs. 33.5 ± 3.2 g, $p < 0.001$) and exercise + recovery (193.6 ± 20.7 vs. 100.9 ± 4.4 g, $p < 0.001$) time segments. For sedentary conditions, we found a

significant treatment difference for fat oxidation (in total grams) during the sleep time segment (34.1 ± 2.2 vs. 27.6 ± 1.9 g, $p < 0.001$), but when fat oxidation expressed as g/min/kg of FFM was analyzed, there were differences in the wake (0.00151 ± 0.00013 vs. 0.00191 ± 0.00010 g/min/FFM, $p < 0.001$) and exercise + recovery (0.00167 ± 0.00014 vs. 0.00121 ± 0.00013 g/min/FFM, $p < 0.001$) time segments. There were no treatment differences between genders for NPRER for any of the three time segments during sedentary conditions.

Discussion

The results from the studies by Cooper et al [9] and Hansen et al [11] that exercise increases 24h fat oxidation and lowers 24h NPRER values more quickly compared to sedentary conditions in response to subjects being placed on an acute HF diet. This is indicative that the body is adapting to a HF diet more quickly under exercise vs. sedentary conditions. For this current study, we were interested in determining when, during a 24h period, that adaptation to the HF diet might be occurring. To our knowledge, we are the first to report that the adaptation, which based on results from previous studies has been shown to occur more quickly with exercise conditions, is taking place during sleeping hours. Non-protein RER was significantly higher for the exercise vs. sedentary condition in the exercise + recovery time segment; whereas, NPRER values were significantly lower for the exercise vs. sedentary conditions during the sleep time segment (indicating greater fat oxidation) and did not differ for the wake time segment. We analyzed fat oxidation in two different ways: (1) total fat in grams that was oxidized for each time segment, and (2) fat oxidation expressed as grams per minute per kg of fat free mass. The g/min/FFM was performed to adjust for body size and composition differences between males and females. Fat oxidation, expressed in grams and in g/min/FFM (kg), was significantly higher for exercise vs. sedentary conditions for all three time segments. However, dietary fat intake was also greater during the exercise visits (due to the higher energy expenditure of those visits). Even though fat oxidation was higher for exercise vs. sedentary conditions, it is not relative to the increase in dietary fat intake until the NPRER goes down. Therefore, since the two study conditions (exercise vs. sedentary) had very different caloric intakes as well as different caloric expenditures, by looking at NPRER, which can provide information on relative macronutrient oxidation and is not affected by the rate of energy expended, we can normalize the data to eliminate potential problems with differences in energy intake and expenditure between study conditions.

As mentioned above, we found that the greatest relative response to the HF diet based on NPRER data was occurring during the sleep time segment. The subjects are actually burning less fat during the sleeping hours compared to exercise hours, and while we agree that exercise could have a bigger impact on overall fat oxidation, the subjects are sleeping for several hours vs. 1 hour exercise, so small amounts of fat oxidized during an hour of sleep can amount to something substantial over the 7–8 hour time period for sleep. While the exact physiological mechanisms to explain this remain unknown, there are a few possibilities for why NPRER was different between treatment conditions for sleeping hours. Relative fat oxidation in the liver could have been higher during the sleep hours for the exercise visits due to an increased need for ATP production to fuel hepatic gluconeogenesis during this time of fasting [6]. Another possibility is that the timing of the evening exercise bout affected fat metabolism during sleep. Aerobic exercise stimulates the sympathetic nervous system and increases carbohydrate oxidation, [20] which we observed in our study during the exercise bouts. This leads to an increase in lipid metabolism during the recovery period following exercise in order to spare carbohydrate to protect lower glycogen stores [5]. Our subjects performed one of their exercise bouts, followed by a 50kcal snack, just before going to bed. This was not likely a sufficient amount of calories to fully restore glycogen stores, therefore possibly leaving the individual slightly glycogen depleted during

sleep. This may have elicited enough of an effect to further increase fat metabolism in an effort to spare as much glycogen as possible which would explain why we saw the largest differences in fat metabolism between sedentary and exercise visits during the sleep time segment.

While our previous studies showed that 24h relative fat oxidation increased during exercise conditions, the current analysis shows that the actual exercise bout is not when the adaptation to a HF diet occurred. Although fat oxidation is greater in exercise vs. sedentary conditions for this time segment, energy expenditure for exercise conditions is also greater. Therefore, all macronutrient oxidation was higher compared to sedentary conditions. The NPRER was higher for exercise vs. sedentary conditions which is the best indicator that the adaptation to the HF diet was not occurring during the actual exercise bouts. It remains unclear whether the results we observed were due to having two separate exercise bouts during the day, as opposed to just one longer exercise bout, and if the same results would be observed under different exercise conditions.

The exact mechanisms by which aerobic exercise is leading to a faster adaptation to a HF diet were not explored in this study. However, others have speculated on potential mechanisms. Chronic adaptations to aerobic exercise are initiated acutely following exercise. Some of the adaptations that occur following exercise are increased cardiac output, increased gas exchange in the lungs, increased capillary density in skeletal muscle, and increased blood volume [3;12;15;18;25;28]. There are also several adaptations that occur at the cellular level that help increase energy production and help the body become more efficient at oxidizing fat for energy. These include triacylglycerol (TAG) hydrolysis and fatty acid (FA) release, free fatty acid (FFA) transport and uptake into skeletal muscle, and metabolism of FFA inside cells [12;15;19;25;30]. An increase in available FA in the cell results in an increase in carnitine palmitoyl transferase (CPT) and carnitine levels to allow for increased transport of FA into the mitochondria [3;12;15;18;25;28]. All of these factors together can increase fat oxidation in response to aerobic exercise training and some of these adaptations may be occurring during sleep hours which would help to explain our results found here.

There are some limitations to consider with this study. First there could be a limitation with combining the two previous studies by Cooper et al [9] and Hansen et al [11], especially because dietary fatty acid composition of the diets were not identical. However, our previous study did not show a significant effect of dietary fatty acid composition [8;9;11] which is why we were justified in combining treatment groups from that study in order to include them with the study done on females under sedentary and exercise conditions. Another limitation is that the subjects who participated in this study were untrained normal and overweight individuals, so we cannot speculate as to whether we would get the same response with aerobic trained individuals or obese individuals.

It is hypothesized that many factors can affect fat oxidation such as the fitness levels and obesity status of individuals. Although there was a difference in BMI status between our males and females, by doing a gender analysis, we were also performing a comparison between two BMI groups (normal weight vs. overweight). While we did not show any significant differences in NPRER between BMI groups, absolute values of fat oxidation were higher during wake and sleep hours in our normal weight group because of a greater dietary fat intake and energy expenditure. No differences between normal weight vs. overweight subjects was found in fat oxidation during the exercise+ recovery time segment. These results are different than previous studies. Ara et al,[4] showed that obese individuals exhibited higher maximal fat oxidation during leg exercise compared to overweight subjects. Goodpaster et al,[10] demonstrated that in sedentary men, the obese group had increased

rates of fatty-acid oxidation compared with the lean group under exercise conditions. However, neither of these two studies compared normal weight individuals to overweight individuals, nor were the subjects fed a HF diet.

Although we cannot speculate about the results of this study on aerobically trained individuals, it warrants further research in this area. Our male subjects had a higher VO_2max than the female subjects, but no differences in fat oxidation between males and females were observed during the exercise+ recovery segment. One of the main goals of endurance training is to shift substrate utilization to decrease the reliance on carbohydrate use and increase fat oxidation during exercise [13;16]. The results of a study by Sidossis et al,[27] demonstrated that in trained vs. sedentary individuals, when exposed to exercise of the same intensity, fat oxidation was higher in the endurance trained individuals. They believed this was due to accelerated fatty acid entry into the mitochondria in the trained individuals as compared to the sedentary individuals. This adaptation in substrate utilization could in turn provide different results to a time of day adaptation while exposed to a HF diet as compared to the results of the untrained individuals observed in this study.

As shown previously, aerobic exercise increases 24 h fat oxidation (and lowers NPRER) in subjects following initiation of a HF diet. In this study, we found that the time of day in which there is an increase in the ratio of fat to carbohydrate oxidation under exercise conditions is primarily occurring during sleeping hours. This was the only time segment that showed a lower NPRER for exercise compared to sedentary conditions. NPRER for exercise treatments was actually higher during the exercise + recovery period compared to sedentary treatments, and there was no difference in NPRER during waking hours between the two treatment conditions. Therefore, we can conclude that exercise is important for adaptations to a HF diet both in increased fat oxidation throughout the day and also in regards to RER during sleep. However, it is not the acute exercise bout that is promoting the adaptation to the HF diet; rather, it is the effects of exercise during subsequent sleeping hours that allows a person to oxidize more fat over the entire 24h period. Further research is needed to investigate the exact biochemical mechanisms during sleeping hours that is allowing this adaptation to occur.

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Figure 1.

Non-Protein Respiratory Exchange Ratio (NPRER) for all three time segments NPRER for wake (11h), sleep (7.75h), and exercise + recovery (4h) time segments under sedentary and exercise conditions. These results showed significant time effects for all three time segments and significant treatment effects for the sleep and exercise + recovery segments. There were no significant treatments by time interactions for any of the time segments. The food quotient (FQ) of 0.826 was calculated based on the HF diet. The exercise condition during the sleep time segment had the greatest negative fat balance based on the calculated FQ. When treatment effects were found, post-hoc analyses revealed treatment differences on specific days. Those treatment differences are denoted with an * to be significant at $p < 0.05$.

Figure 2.

Mean Non-Protein Respiratory Exchange Ratio (NPRER) for all three time segments. Average NPRER values for wake, sleep, and exercise + recovery (Ex + Rec) time segments for both sedentary and exercise conditions. Data is the average of the HF diet days (days 2–5). The results show no significant treatment effect for the wake time segment. NPRER for exercise conditions was significantly lower during the sleep segment but significantly higher during the exercise + recovery segment. The food quotient (FQ) of 0.826 was calculated based on the HF diet. Only the sleep time segment had a NPRER lower than the FQ. This suggests a slight negative fat balance for the sedentary condition and a large negative fat balance for the exercise condition.

* denotes significant difference between treatment groups at $p < 0.05$.

Figure 3.

Fat oxidation for all three time segments

Total fat oxidized for wake (11h), sleep (7.75h), and exercise + recovery (4h) time segments under sedentary and exercise conditions. These results showed a significant time effect, a significant treatment effect, and a significant treatment by time interaction for all three time segments. When treatment effects were found, post-hoc analyses revealed treatment differences on specific days. Those treatment differences are denoted with an * to be significant at $p < 0.05$.

Figure 4.

Mean fat oxidation for all three time segments.

Average fat oxidation for wake, sleep, and exercise + recovery (Ex + Rec) time segments for both sedentary and exercise conditions. Data is the average of the HF diet days (days 2–5).

The results showed significant treatment effects for all three time segments.

* denotes significant difference between treatment groups at $p < 0.05$.

Figure 5.

Fat oxidation shown as grams oxidized per minute per kg of fat free mass. Fat oxidized for wake (11h), sleep (7.75h), and exercise + recovery (4h) time segments shown in grams per minute per fat free mass (FFM) under sedentary and exercise conditions. These results showed a significant time effect, a significant treatment effect, and a significant treatment by time interaction for all three time segments. When treatment effects were found, post-hoc analyses revealed treatment differences on specific days. Those treatment differences are denoted with an * to be significant at $p < 0.05$. No differences were found with the data shown this way compared to total fat oxidation (in grams) for each time segment.

Figure 6.

Mean fat oxidation in g/min/FFM for all three time segments.

Average fat oxidation shown in grams per minute per fat free mass (FFM) for wake, sleep, and exercise + recovery (Ex + Rec) time segments for both sedentary and exercise conditions. Data is the average of the HF diet days (days 2–5). The results showed significant treatment effects for all three time segments, which was similar to fat oxidation expressed in total grams.

* denotes significant difference between treatment groups at $p < 0.05$.

Table 1

Subject Characteristics

Men (n=8)	Mean	SD	Women (n=10)	Mean	SD
Age	25	8.0	Age	25	3.5
Height (cm) *	184.8	6.2	Height (cm)	168.6	5.8
Weight (kg)	76.5	6.5	Weight (kg)	73.6	16.9
Body Fat Percentage *	20.3	2.7	Body Fat Percentage	33.7	5.7
BMI (kg/m ²)	22.5	3.3	BMI (kg/m ²)	25.9	4.0
VO ₂ max (ml/kg/min) *	40.5	5.1	VO ₂ max (ml/kg/min)	32.5	7.7

BMI= Body Mass Index

VO₂max = maximal aerobic capacity

* Denotes significant difference at p<0.05 between males and females for either exercise or sedentary conditions.

Table 2

Sex specific comparison of all outcome variables for each time segment

Men (n = 8)	Exercise	Sedentary	Women (n = 10)	Exercise	Sedentary
Wake (D2-5)					
NPRER	0.82 ± 0.02	0.85 ± 0.02	NPRER	0.82 ± 0.01	0.83 ± 0.02
Fat oxidation (total grams)	69.7 ± 3.5 ^{^,*}	51.9 ± 4.5	Fat oxidation (total grams)	56.9 ± 3.3 [^]	50.6 ± 3.4
CHO oxidation (total grams)	78.7 ± 9.0 [^]	99.9 ± 9.8	CHO oxidation (total grams)	75.1 ± 4.8 [^]	82.2 ± 5.7
Fat oxidation (g/min/FFM)	0.00202 ± 0.00011	0.00151 ± 0.00013 [*]	Fat oxidation (g/min/FFM)	0.00211 ± 0.00009	0.00191 ± 0.00010
Sleep (D2-5)					
NPRER	0.75 ± 0.02 [^]	0.81 ± 0.01	NPRER	0.78 ± 0.01 [^]	0.81 ± 0.01
Fat oxidation (total grams)	50.3 ± 1.9 ^{^,*}	34.1 ± 2.2 [*]	Fat oxidation (total grams)	36.8 ± 1.9 [^]	27.6 ± 1.9
CHO oxidation (total grams)	18.8 ± 5.1 ^{^,*}	42.9 ± 5.6	CHO oxidation (total grams)	33.5 ± 3.2 [^]	38.4 ± 2.3
Fat oxidation (g/min/FFM)	0.00200 ± 0.00016	0.00136 ± 0.00012	Fat oxidation (g/min/FFM)	0.00169 ± 0.00009	0.00141 ± 0.00007
Exercise + recovery (D2-5)					
NPRER	0.90 ± 0.01 ^{^,*}	0.87 ± 0.01	NPRER	0.87 ± 0.01 [^]	0.85 ± 0.01
Fat oxidation (total grams)	31.9 ± 5.2 [^]	15.9 ± 1.8	Fat oxidation (total grams)	33.0 ± 2.3 [^]	16.9 ± 1.7
CHO oxidation (total grams)	193.6 ± 20.7 ^{^,*}	44.9 ± 4.1	CHO oxidation (total grams)	100.9 ± 4.4 [^]	38.3 ± 2.3
Fat oxidation (g/min/FFM)	0.00251 ± 0.00050	0.00167 ± 0.00014 [*]	Fat oxidation (g/min/FFM)	0.00337 ± 0.00034	0.00121 ± 0.00013

Data are presented as the averages of the high-fat diet days (days 2-5)

NPRER Non-protein respiratory exchange ratio, FFM fat free mass

[^] Significant difference at $p < 0.05$ between sedentary and exercise conditions within sex^{*} Significant difference at $p < 0.05$ between males and females for either exercise or sedentary conditions