Effects of exercise intensity on 24-h energy expenditure and substrate oxidation

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Submitted for publication November 1995.

Accepted for publication March 1996.

We would like to thank the women who participated in this study, and Bob Petri for technical assistance.

This study was supported by a grant to M. Treuth and G. Hunter (UAB School of Education Grant).

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ABSTRACT TOP

The purpose of this study was to determine: 1) the reliability of 24-h respiratory calorimetry measurements, and 2) the effects of low- versus high-intensity exercise on energy expenditure (EE) and substrate oxidation over a 24-h period. Eight women (age 28 ± 4.3 yr) were measured for body composition, maximal oxygen consumption while cycling, and EE in three, 24-h calorimeter tests, with identical work output but differing intensity during a 60-min exercise session. Low-intensity (LI) exercise involved continuous cycling at 50% \( \dot{VO}_{2\max} \); whereas high-intensity (HI) exercise involved interval cycling (2 min exercise/recovery) at 100% \( \dot{VO}_{2\max} \). Subjects were randomly assigned to the first two tests at LI or HI, with the third test at the alternate intensity. No differences in EE or respiratory quotient (RQ) during rest, sleep, exercise, or over the 24 h were found between the first two tests (C.V. = 6.0%), demonstrating the reliability of the measurements. The HI protocol elicited significantly higher EE than LI during rest, exercise, and over 24 h, whereas sleeping EE approached significance. No significant differences in RQ during rest, sleep, or over 24 h were found, but 24-h lipid and carbohydrate oxidation were similar in the two protocols. The HI exercise RQ was significantly higher than LI. These findings demonstrated higher 24-h EE in the HI than LI protocol, but similar 24-h substrate oxidation rates.

Different exercise intensities (low vs high) differentially affect the type of fuel oxidized (6,7). At low-intensity (LI) exercise below 40-50% of maximal oxygen consumption (\( \dot{VO}_{2\max} \)), the energy supplied is primarily from oxidation of plasma free fatty acids. As exercise intensity increases, the additional energy is obtained by utilization of muscle glycogen, blood glucose, and intramuscular triglyceride (6). During high-intensity (HI) exercise, fat oxidation gradually decreases until 100% carbohydrate oxidation occurs at about 100% of \( \dot{VO}_{2\max} \). The shift from fat to carbohydrate oxidation allows the muscle to obtain more energy from each liter of oxygen consumed from carbohydrate than from fat (23). This allows for greater energy output during HI exercise when oxygen uptake may be a limiting factor. This has led to the commonly held view that LI exercise may be better than HI exercise for increasing fat oxidation and thus weight loss (15).

To understand the impact of various exercise regimens on energy balance, the long-term effect of exercise must be examined. Studies that investigate the exercise energy cost typically measure the energy expenditure (EE) during and immediately after an acute exercise session (excess post-exercise oxygen consumption or EPOC) or measure resting metabolic rate (RMR) the morning after the exercise session. One study that examined different exercise intensities reported that the highest EPOC (up to 3 h after the exercise) in terms of both time and energy utilized was at 70% \( \dot{VO}_{2\max} \) in moderately trained men and women (27). In contrast, no differences were observed between 40% and 60%
VO2max for the magnitude or duration of EPOC in moderately active women (25). Several studies have reported that the energy cost of exercise may be higher during and immediately after HI aerobic (24) or resistance exercise (19). Bahr et al. (3) found an increased EPOC up to 4 h after exhaustive supramaximal (108% VO2max) exercise. Few researchers have examined the effects of different exercise intensities on EE for longer durations, i.e., into sleep or over an entire 24-h period. Laboratories with 24-h calorimeters are best suited for these types of studies.

A respiratory calorimeter is also ideal for studying the long-term effects of exercise on substrate oxidation. Little is known about the amount or type of fuel oxidized for many hours post-exercise. This is of interest because the effects of exercise on fuel utilization may be long lasting and may differ depending on the intensity of the exercise (5). Several studies have reported that carbohydrate oxidation or glycogen synthesis increases post-exercise (13,20,21,26). In one study using a calorimeter, an increase in fat oxidation post-exercise has been reported in subjects who exercised at 50% VO2max for 3 h (4). The impact of different exercise intensities on substrate oxidation over a 24-h period can be examined using a calorimeter.

Thus, the effects of either acute LI or HI exercise on EE and substrate oxidation for an extended time after the exercise are unclear. In this study, we first wished to determine the reliability of our 24-h calorimetry measures in young adult women by repeat testing. Second, we sought to determine the effects of different exercise intensities (LI vs HI) on EE and substrate oxidation in these same healthy women over a 24-h period.

**METHODS**

**Subjects.** Eight females (seven Caucasian and one Asian) between 23 and 35 yr of age participated in the study. Subjects were excluded from the study if they had evidence of cardiovascular disease, anemia, diabetes, significant renal or hepatic disease, hypothyroidism, musculoskeletal problems, if they took medications on a regular basis, or if they were on special diets. Three of the eight women were taking oral contraceptives. In addition, all subjects were weight-stable, with weight not varying more than 1 kg during the previous 6 months. All volunteers provided written informed consent to participate in this study, which was approved by the Institutional Review Board of the University of Alabama at Birmingham.

**Body composition.** Body composition was assessed by dual-energy x-ray absorptiometry or DEXA (DPX-L, Lunar Radiation Corp., Madison, WI). The scan was analyzed using the Adult Software (Version 3.6z). DEXA allows for determination of total and regional lean tissue mass, fat tissue mass (FM), and bone mineral content. Fat-free mass (FFM) was defined as the sum of lean tissue mass and bone mineral content. The subject was asked to lie motionless on a table for approximately 25 min.

VO2max. Maximal oxygen uptake (VO2max) was measured on a cycle ergometer using a Sensormedics 2900 metabolic cart (Loma Linda, CA). The subjects exercised on a Pedalmate cycle ergometer (Warren E Collins Inc, Braintree, MA) for this test and all subsequent exercise in the study. A factory calibration of the ergometer demonstrated its accuracy to measure work to the nearest 1 W. The cycle protocol involved a 2-min warm-up at 50 W. Every 1 min thereafter, the workload increased by 20 W until exhaustion. Heart rate was measured using a POLAR Vantage XL heart rate monitor (Gays Mills, WI). Standard criteria for heart rate, respiratory quotient, and plateauing were used to ensure achievement of VO2max (17). The two workloads, 50% VO2max (LI) and 100% VO2max (HI), were determined from this maximal test utilizing the American College of Sports Medicine metabolic equations for cycle ergometers (2).

**Energy intake.** On the 3 d preceding each of the calorimetry tests, subjects recorded their energy intake. On the day before the first and third tests, each subject consumed a diet the same as the diet she had consumed the day before the first test, so that her diet was identical the day before each of the three calorimetry tests. The main variables of interest were total caloric intake, and percent of calories from carbohydrate, protein, and fat. Food records were analyzed using the USDA Dietary Analysis Program for microcomputers (NTIS Federal Computer Products Center, Springfield, VA).

The diet for the food consumed in the calorimeter was designed to approximate 65% carbohydrate, 20% fat, and 15% protein. The subjects were given identical diets for the 3 d in the calorimeter. All the food was weighed and the subjects were required to consume all the food given to them. To estimate the energy intake requirements of the subjects during their stay in the calorimeter, a resting metabolic rate (RMR) test was conducted in the morning after an overnight fast. After reporting to the laboratory at 7 a.m., the subject rested quietly for approximately 15 min. RMR then was determined using a Sensormedics 2900 system (Sensormedics, Loma Linda, CA) for 30 min. RMR was calculated using the equation of Weir (8). Energy intake then was calculated as 1.3 times the RMR.
**Calorimetry measurements and substrate oxidation.** Measurements of 24-h EE and substrate oxidation were taken in a whole-room respiration calorimeter (3.38 m long, 2.11 m wide, and 2.58 m high). The calorimeter design characteristics and calibration have been previously described in detail (28). Briefly, the room was equipped with a foldout bed, desk, chair, lamp, refrigerator, toilet, sink, television/VCR, cycle ergometer, and telephone. Oxygen consumption (\(\dot{V}O_2\)) and carbon dioxide production (\(VCO_2\)) were continuously measured by the magnetopneumatic differential \(O_2\) analyzer (Magnos 4G) and the NDIR industrial photometer differential \(CO_2\) analyzer (Uras 3G, both Hartmann & Braun, Frankfurt, Germany). The calorimeter was calibrated before each subject entered the chamber. The zero calibration was carried out simultaneously for both analyzers. The full scale was set for 0-1% for the \(CO_2\) analyzer and for 0-2% for the \(O_2\) analyzer.

All subjects spent 1 d in the calorimeter on each of the three visits, which were separated by at least 4 d. Subjects were randomly assigned to exercise at either high or low intensity on the first visit. On the second visit, the subject exercised at the same intensity level as the first visit (reproducibility), such that four subjects exercised twice at HI and four subjects exercised twice at LI. For the third visit, the subjects exercised at the alternate intensity. The subjects were all tested in the time frame of the follicular phase of the menstrual cycle, determined to begin 14 d past the onset of menstruation. On the day before the calorimeter test, the subjects were allowed to exercise, but were requested to repeat the same mode, duration, and intensity on the day before subsequent visits to the laboratory. Exercise had to be completed at least 12 h prior to entry to the calorimeter.

Each subject entered the calorimeter at 8 a.m. and began exercising approximately 1 h later. By electronically controlling the ergometer, the investigator outside the calorimeter precisely controlled the workload and continuously monitored the work output to the nearest Watt. The interface between the cycle in the calorimeter and the control box outside the calorimeter was wired through the water-filled u-tubes. Both LI and HI protocols involved a 5-min warm-up at 50 W. The LI protocol consisted of exercising at 50% \(\dot{VO}_{2\max}\) continuously for 60 min, followed immediately by a 5-min cool-down at 50 W. The HI protocol consisted of interval cycling (2-min cycle, 2-min recovery) at 100% \(\dot{VO}_{2\max}\) for 60 min (15 trials). This was also followed immediately by a 5-min cool-down at 50 W. The work intensity for HI was exactly twice the LI so that the total amount of work accomplished for the protocols was identical. Heart rate was measured continuously by a POLAR Vantage XL heart rate monitor. The heart rate during the exercise was also recorded by the investigator every 6 min during the LI exercise session, and at 1 min 45 s into the exercise and into the recovery for the HI exercise. The subject was not allowed to exercise at any other time in the calorimeter. Meals were provided at the same time for all tests (within 15 min) and the subjects were requested to consume all the food given. Energy intake was constant across all three tests. The remainder of the day was spent in sedentary activities (reading, studying, watching television, etc.). The subjects went to sleep and were awakened at the same time in all three tests so that sleep duration was similar. The onset of sleep was determined by when the lights were turned off. Sleep may have included some resting awake time while the subject was falling asleep. The respiratory calorimeter was not equipped with sensors to detect spontaneous physical activity.

The subject was awakened at 6:30 a.m. on the second morning in the calorimeter. RMR was measured for 30 min. The subject left the room after having spent 23 h in the calorimeter, allowing time for calibration before the next subject entered. RMR, sleeping metabolic rate (SMR), and 24-h EE were calculated by the Weir equation (8). These measures of EE were extrapolated over 24 h and expressed as kJ d\(^{-1}\). For the purposes of this study, SMR was determined by averaging EE for the time from when the subject went to sleep until she was awakened. Protein oxidation was determined from 24-h urinary urea nitrogen excretion. Carbohydrate and fat oxidations were calculated from the 24-h nonprotein respiratory quotient (npRQ) and expressed as percentages of nonprotein EE (NPEE) (11).

**Statistical analyses.** Paired \(t\)-tests and coefficients of variation were determined between the first and second calorimeter tests to determine the reliability of our measurements. Comparisons between the second and third calorimetry tests were done by paired \(t\)-tests to determine any exercise intensity differences. All data was analyzed by SAS for Windows (Cary, NC) with significance set at \(P < 0.05\).

**RESULTS**

**Reliability.** The first and second calorimetry tests were conducted to determine the reliability of the calorimeter and to familiarize the subject with the protocol and testing procedures. The paired-\(t\)-tests between the first and second tests revealed no significant differences in any of the variables measured, including RMR, SMR, exercise EE, and 24-h EE. There were also no differences in rest, sleep, exercise, or 24-h RQ. The coefficient of variation (CV) between test 1 and test 2 was 6% for 24-h EE, 11% for resting EE, 6% for exercise EE, 5% for sleeping EE, and 2.5% for 24-h RQ.
Subject characteristics. The subject characteristics (body composition and \(\dot{\text{VO}}_2\text{max}\) results) are reported in Table 1. There was a large variation in percent body fat. The wide range in \(\dot{\text{VO}}_2\text{max}\) values allowed examination of a range of exercise intensities. The workload for the HI protocol was 145 ± 34 W (range 100-200 W), whereas the workload for the LI protocol was 72.5 ± 17 W (range 50-100 W). The work for the 60 min of exercise (for both the LI and HI protocols) was 4350 ± 1039 W, and was 500 W greater (4850± 1039 W total) with the warm-up and cool-down periods included. Using standard ACSM equations, this calculates to be a predicted exercise energy cost of 1238 ± 264 kJ.

| Age (yr) | 28.1 ± 4.3 | (23–35) |
| Height (cm) | 165.8 ± 6.1 | (160–178) |
| Weight (kg) | 56.3 ± 6.7 | (48–65) |
| Percent fat (%) | 19.9 ± 7.7 | (7.9–31.5) |
| Fat mass (kg) | 11.3 ± 5.4 | (3.6–20.1) |
| Fat-free mass (kg) | 34.2 ± 3.1 | (39.5–50.2) |
| \(\dot{\text{VO}}_2\text{max} \text{ (l} \cdot \text{min}^{-1})\) | 2.17 ± 0.43 | (1.65–2.85) |
| \(\dot{\text{VO}}_2\text{max} \text{ (ml} \cdot \text{kgmin}^{-1})\) | 39.3 ± 10.4 | (25.4–54.8) |
| \(\text{RQ}_{\text{max}}\) | 1.3 ± 0.15 | (1.1–1.5) |
| \(\text{HR}_{\text{max}} \text{ (bpm)}\) | 181 ± 9 | (171–200) |

\(\dot{\text{VO}}_2\text{max}\), maximal oxygen consumption; RQ, respiratory quotient; HR, heart rate.

TABLE 1. Subject characteristics.

Energy intake. The RMR (determined prior to the 24-h calorimetry tests) was 5828 ± 619 kJ·d\(^{-1}\), with a respiratory quotient(RQ) of 0.87 ± 0.09. The energy intake was 7456 ± 1280 kJ·d\(^{-1}\) for the calorimeter tests, with a nutrient intake of 15± 3% protein, 70 ± 3% carbohydrate, and 15 ± 3% fat. The calorimeter energy intake was not significantly different than the energy intake calculated from the dietary records for the 3 d preceding the calorimetry tests (7665 ± 1535 kJ·d\(^{-1}\)). When testing for energy balance, the calorimeter energy intake was not significantly different than 24-h EE for the LI protocol, but was significantly lower than 24-h EE for the HI protocol, due to the higher EE during the HI exercise.

Energy expenditure. The heart rates during the LI and HI exercise are shown in Table 2. The heart rate during the HI was significantly higher \((P < 0.001)\) than the LI exercise. There was no difference between protocols in the heart rates during warm-up; however, the heart rates during the cool-down remained significantly higher after the HI exercise than the LI exercise.
The comparisons between the LI and HI protocols for the components of 24-h EE are shown in Table 3. There was a significantly higher RMR by 418 kJ·d⁻¹ during the HI protocol (P < 0.05). The exercise EE was significantly higher by 267 kJ (P < 0.001) for the HI protocol. The EE for the 2 h immediately after the exercise was significantly greater with the HI protocol (713 ± 138 kJ) than the LI protocol (623 ± 96 kJ, P < 0.01). Although significance was approached, no significant differences were observed between the LI and HI protocols for SMR (P = 0.07). The HI protocol elicited a 669 kJ·d⁻¹ higher 24-h EE than the LI protocol (P < 0.01). Figure 1 illustrates the comparison of the mean of the eight women for exercise, sleep, rest, and 24-h EE between the two exercise intensities.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Warm-up</th>
<th>Exercise</th>
<th>Recovery</th>
<th>Cool-down</th>
<th>%HR_max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low intensity</td>
<td>100 ± 17</td>
<td>111 ± 14</td>
<td>105 ± 20</td>
<td>62 ± 7</td>
<td></td>
</tr>
<tr>
<td>High intensity</td>
<td>103 ± 19</td>
<td>161 ± 11*</td>
<td>99 ± 24</td>
<td>127 ± 21**</td>
<td>89 ± 5*</td>
</tr>
</tbody>
</table>

Significantly different than the low-intensity protocol at *P < 0.001 and **P < 0.01. %HR_max, heart rate during the exercise as a percentage of maximum reached during the VO2max test.

<table>
<thead>
<tr>
<th>Low Intensity</th>
<th>High Intensity</th>
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<tbody>
<tr>
<td><strong>Rest</strong></td>
<td></td>
</tr>
<tr>
<td>kJ·min⁻¹</td>
<td>3.90 ± 0.52</td>
</tr>
<tr>
<td>kJ·d⁻¹</td>
<td>5607 ± 753</td>
</tr>
<tr>
<td>RQ</td>
<td>0.86 ± 0.03</td>
</tr>
<tr>
<td><strong>Exercise</strong></td>
<td></td>
</tr>
<tr>
<td>kJ·min⁻¹</td>
<td>17.09 ± 2.31</td>
</tr>
<tr>
<td>kJ·session⁻¹</td>
<td>1197 ± 163</td>
</tr>
<tr>
<td>RQ</td>
<td>0.85 ± 0.03</td>
</tr>
<tr>
<td><strong>Sleep</strong></td>
<td></td>
</tr>
<tr>
<td>kJ·min⁻¹</td>
<td>3.72 ± 0.44</td>
</tr>
<tr>
<td>kJ·d⁻¹</td>
<td>5351 ± 623</td>
</tr>
<tr>
<td>RQ</td>
<td>0.84 ± 0.03</td>
</tr>
<tr>
<td><strong>24 h</strong></td>
<td></td>
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<tr>
<td>kJ·min⁻¹</td>
<td>5.48 ± 0.53</td>
</tr>
<tr>
<td>kJ·d⁻¹</td>
<td>7883 ± 770</td>
</tr>
<tr>
<td>RQ</td>
<td>0.86 ± 0.03</td>
</tr>
</tbody>
</table>

*Significantly different than low intensity, †P < 0.05, *P < 0.001, and **P < 0.01.

TABLE 3. Energy expenditure and respiratory quotient at low versus high exercise intensity.
Substrate oxidation. The HI and LI comparisons for RQ revealed no significant differences during rest, sleep, or over 24 h (Table 3). During exercise, there was a significantly higher RQ during the HI protocol (Table 3). There were no significant differences in 24-h carbohydrate or fat oxidation rates between the protocols. The carbohydrate oxidation values were 53.7 ± 11.1 versus 57.7 ± 9.4% NPEE for LI versus HI exercise, respectively. The corresponding values for fat oxidation were 46.3 ± 11.1 versus 42.3± 9.4% NPEE for LI versus HI exercise, respectively. The amount of substrate oxidized was 61 ± 11 versus 67 ± 18 g protein, 204± 44 versus 237 ± 41 g carbohydrate, and 79 ± 21 versus 79 ± 24 g fat for the LI versus HI protocols, respectively. There were no significant correlations between body fatness (expressed as% body fat and absolute fat mass) and either resting or exercise RQ.

DISCUSSION

This study demonstrated that total 24-h energy expenditure, including exercise EE and RMR measured 21 h after the exercise, was greater with HI interval exercise (100% $\dot{V}O_{2\text{max}}$) than with LI continuous exercise(50% $\dot{V}O_{2\text{max}}$). Second, we found that even though the RQ was lower during the LI exercise, similar amounts of lipid were oxidized for the two different intensity protocols over the 24-h study.

This investigation was designed to test each subject three times. The similarity of results from the first and second calorimeter tests demonstrated their reproducibility. The first test allowed the subject to become familiar with the calorimeter and the testing situation, thereby ensuring that the comparisons of the LI and HI protocols were unbiased by the novelty of the calorimeter.

Although the total amount of work was constant between the LI and HI protocols, the EE during the exercise was significantly greater with the HI protocol by approximately 267 kJ. The exercise EE accounted for 40% of the increase in 24-h EE with the HI protocol. Consistent with these results, investigations that compared exercise economy during walking, bicycling, and strength training reported that as intensity increases exercise economy decreases.(9,10,12,14,18,19) Possible reasons for this decrease in economy include an increased dependency on inefficient fast-twitch muscle fibers (12,16) and/or an increase in metabolism not directly related to measured work output(for example, statically contracting stabilization muscles).
We also observed an increase in RMR by 418 kJ·d⁻¹ approximately 21 h after the HI exercise. Other investigators have also reported an elevated RMR up to 24 h after an acute HI exercise session (4,22). In addition, aerobic exercise training at an intensity of at least 70% \( \dot{V}O_{2\text{max}} \) is associated with an increase in RMR (24).

Since we found elevations in exercise EE and RMR with the HI protocol, the 24-h EE was therefore also higher (by 669 kJ) with the HI protocol. Within the 24-h period, we also examined the EE for the 2 h immediately after the exercise (EPOC). The EE was significantly higher after the HI versus the LI exercise. This is in agreement with another aerobic exercise study that reported a greater energy cost during and immediately after HI exercise (24). The SMR was not significantly different between the protocols. A previous study (4) reported no elevation in SMR, 9-17 h post-exercise. In our study, sleep was measured approximately 12-21 h post-exercise. Thus, the exercise intensity appears to be affecting the awake component of metabolism more than the sleep component. The explanation for the higher RMR and exercise EE with the HI protocol and no significant increase in SMR may involve the sympathetic nervous system. If sympathetic arousal is responsible for the increase in RMR following HI exercise (24), perhaps the sympathetic arousal is blunted during sleep.

The similarities in 24-h lipid oxidation between the two protocols may be explained by the different fuels used during and post-exercise. Lipid oxidation was probably higher during the LI exercise. Yet, more lipid was probably used as a fuel after HI exercise, since carbohydrate would have been used to replace the greater amounts of glycogen depleted with the HI exercise.

Only one study (4) of which we are aware has specifically investigated substrate oxidation in a calorimeter after an acute exercise session. Bielinski et al. (4) examined subjects over 17 h in a calorimeter under a control, sedentary condition and after a 3-h exercise session at 50% \( \dot{V}O_{2\text{max}} \). Resting RQ was 14% lower after the exercise session. We were not able to directly compare studies since Bielinski et al. (4) used only one exercise intensity compared with control, and the exercise session was substantially longer in duration. Exercise duration may be an important factor which affects oxidation, since it has long been established that exercise duration affects lipid oxidation rates (1). Perhaps a greater exercise duration is required to produce measurable changes in oxidation following exercise.

Significantly higher heart rates and RQ, as well as reported perceptions of difficulty during HI exercise, indicated that the HI exercise protocol was harder for the subjects. The HR data showed a tendency to increase during the exercise for the last few trials and the subjects tended to maintain a higher heart rate during the 2-min recovery (heart rate data for each trial not shown). The heart rate was also significantly higher during the cool-down phase of HI than during LI exercise. Since some individuals may be less likely to adhere to more strenuous exercise programs, LI exercise may be advantageous for them despite greater energy expenditure with the HI exercise. Adherence to a program, as well as caloric expenditure, must be considered when prescribing an exercise program.

Our results show that 24-h indirect calorimetry can provide repeatable measures for EE and substrate oxidation. Second, 24-h EE was higher in the HI protocol; however, there were no differences between protocols in substrate oxidation rates over a 24-h period. Future studies are necessary to determine whether these same effects occur in different subject populations and what the optimal exercise prescription (in terms of intensity and duration) is for increasing both energy expenditure and lipid oxidation.

REFERENCES

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**CALORIMETRY:** *′VO_{2max}′*, **METABOLISM**, **RESPIRATORY QUOTIENT**