Metabolic response of trained and untrained women during high-intensity intermittent cycle exercise

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Submitted 8 November 2006; accepted in final form 19 September 2007

Trapp EG, Chisholm D, Boutcher SH. Metabolic response of trained and untrained women during high-intensity intermittent cycle exercise. Am J Physiol Regul Integr Comp Physiol 293: R2370–R2375, 2007. First published September 26, 2007; doi:10.1152/ajpregu.00780.2006.—The metabolic response to two different forms of high-intensity intermittent cycle exercise was investigated in young women. Subjects (8 trained and 8 untrained) performed two bouts of high-intensity intermittent exercise: short sprint (SS) (8-s sprint, 12-s recovery) and long sprint (LS) (24-s sprint, 36-s recovery) for 20 min on two separate occasions. Both workload and oxygen uptake were greater in the trained subjects but were not significantly different for SS and LS. Plasma glycerol concentrations significantly increased during exercise. Lactate concentrations rose over the 20 min and were higher for the trained women. Catecholamine concentration was also higher postexercise compared with preexercise for both groups. Both SS and LS produced similar metabolic response although both lactate and catecholamines were higher after the 24-s sprint. In conclusion, these results show that high-intensity intermittent exercise resulted in significant elevations in catecholamines that appear to be related to increased venous glycerol concentrations. The trained compared with the untrained women tended to show an earlier increase in plasma glycerol concentrations during high-intensity exercise.

intermittent exercise; catecholamines; glycerol; lactate

STEADY-STATE, MODERATE-INTENSITY aerobic exercise has been recommended for fat loss because the proportion of lipid in the fuel oxidized during low-intensity physical activity is greater than during high-intensity exercise (25). However, it has been shown that individuals engaging in vigorous exercise were leaner than those participating in less intense exercise (31). The ability of high-intensity exercise to cause negative energy balance was also shown by a 15-wk high-intensity intermittent exercise (HIIE) program that resulted in a greater decrease in skinfold thickness relative to energy expenditure compared with 20 wk of endurance exercise (32). The HIIE in this study consisted of short (15–30 s) and long (60–90 s) sprints separated by recovery periods (1–2 min) allowing the heart rate (HR) to return to 120–130 beats/min. The energy cost of the HIIE was less than half of that of the endurance program. Thus, despite using half the energy, the impact of the HIIE program, compared with steady-state exercise, on subcutaneous adiposity was significantly greater. However, the effects of HIIE on the components of energy balance were undetermined. Thus how HIIE may cause significant reductions in adiposity is unknown. Possible mechanisms include factors affecting energy intake (24) and postexercise energy expenditure (32).

It is feasible that participating in repeated bouts of HIIE could influence postexercise energy expenditure. For example, HIIE is likely to result in a significant elevation of catecholamines (35), which have been shown to elevate postexercise energy expenditure. However, the metabolic response during one bout of HIIE is unclear. For example, when and if free fatty acids (FFAs) are utilized during HIIE is undetermined. Also the catecholamine response and the time course of the lactate response has not been established. Finally, whether the metabolic response to HIIE is influenced by regular cycle training is also undetermined. Therefore, the purpose of this study was to compare the oxygen uptake (VO2), catecholamine, lactate, and glycerol responses of trained and untrained women to short-sprint (SS; 8-s sprint, 12-s recovery) and long-sprint (LS; 24-s sprint, 36-s recovery) 20-min bouts of HIIE. We hypothesized that an acute 20-min bout of HIIE would result in significant increases in plasma glycerol and catecholamine levels.

METHODS

Subjects. Subjects in the untrained (U) group (n = 8) were healthy, active young students in a Health and Exercise Science degree who engaged in recreational sport. The trained (T) subjects (n = 8) were athletes recruited from cycling and triathlon clubs. Women were chosen for this study to avoid any confounding effects of sex on study outcome. Approval was granted from a University Ethics Committee and informed consent was obtained from each subject. All subjects were healthy and none were obese (Table 1.) T subjects possessed significantly greater peak VO2 (VO2 peak), but there were no significant differences in mass, height, Σ skinfolds, percent body fat, or blood lipids.

Study design. Each subject visited the laboratory on three separate occasions. On the first visit VO2 peak, mass, height, anthropometric measures, resting lactate, HR, and blood pressure were recorded. On the second and third occasions subjects performed an HIIE bout (SS or LS) in counterbalanced order.

Peak physical work test. This test was a ramp test on an electrically braked, computer-controlled Monark 839 cycle ergometer with 3-min stages following a 3-min warm-up. Work increments for each stage were 15 W (U) and 25 W (T), at 90 rpm. Capillary blood samples were taken in the last 30 s of each stage of the test and at the end of a 5-min cool down. VO2 (l/min), ventilation (l/min), carbon dioxide production (VCO2, l/min), and respiratory exchange ratio (VCO2/VO2) were measured via an open-circuit indirect calorimetry system (ParvoMedics, 2003).

High-intensity intermittent cycle exercise protocol. The HIIE bouts were completed early in the morning after an overnight fast. Subjects were instructed to eat a meal that was 60–70% carbohydrate the night before and to arrive at the laboratory having consumed nothing other
than water that morning. The subjects were given examples of meals that complied with these requirements and were asked to have the same meal on both nights before the testing sessions. Tests were completed within a week of each other but separated by at least 2 days to avoid any carryover effect. All women were tested in the follicular phase of the menstrual cycle. On arrival at the laboratory, each subject had a small cannula inserted in an antecubital vein and then rested in a supine position for 30 min to allow catecholamine concentrations to stabilize. After resting for 30 min, baseline blood samples were taken. Samples were assayed immediately for lactate and glucose using a YSI analyzer (Yellow Springs, 2300 STAT). Blood lipid profiles (Cholestech LDLX) were also obtained immediately. Whole blood in EDTA tubes was spun immediately in a chilled centrifuge at 4°C, 3,000 rpm for 10 min, and then aliquots of plasma were placed in 1.5 ml microtubes and frozen at −86°C for later analysis. The power output for each subject was set at the power output achieved at 70% VO₂peak. The ergometer phase of the menstrual cycle.

RESULTS

Power output. T cyclists produced a significantly higher (P < 0.0001) power output than U for both SS and LS. The mean power output across both exercise modalities was 153.2 ± 6.9 W for T and 96.1 ± 7.1 W for U. When power output between the SS and LS bouts was compared there was a significantly higher power output for LS (P < 0.03). T and U produced a mean power output of 167.1 ± 3.8 and 105.4 ± 4.2 W, respectively, for the LS exercise and a mean power output of 139.4 ± 1.7 and 86.6 ± 2.6 W, respectively, for the SS exercise.

Heart rate. The only significant finding for HR was that it increased over the 20 min of exercise so that it was significantly higher at the end of exercise compared with the beginning in both the SS and LS (P < 0.0001). There was no difference in HR when T and U were compared, for either SS or LS (Fig. 1).

VO₂ response. There were no significant differences in VO₂ between the two exercise protocols. There was a significant difference (P < 0.0001) between baseline measures, the VO₂ of the work bouts, and recovery (Fig. 2). The absolute VO₂ for both exercise protocols was significantly higher (P < 0.0001) in T than U (Fig. 2). However, when examined as a percentage of VO₂peak, mean oxygen cost was not significantly different for T (69% VO₂peak) and U (65% VO₂peak).

Glycerol. T subjects tended to increase glycerol concentrations earlier than U and the values tended to be higher (Fig. 3),

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Untrained (n = 8)</th>
<th>Mean</th>
<th>SE</th>
<th>Range</th>
<th>Mean</th>
<th>SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>21.2</td>
<td>± 0.1</td>
<td>19–24</td>
<td>25.3</td>
<td>± 1.5</td>
<td>20–30</td>
</tr>
<tr>
<td>Mass, kg</td>
<td>62.2</td>
<td>± 2.2</td>
<td>56.6–66.6</td>
<td>68.2</td>
<td>± 2.5</td>
<td>52.9–72.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>167.8</td>
<td>± 2.1</td>
<td>163–174.5</td>
<td>171.5</td>
<td>± 2.3</td>
<td>163–182.5</td>
</tr>
<tr>
<td>VO₂peak,l/min</td>
<td>2.8</td>
<td>± 0.2</td>
<td>2.1–3.3</td>
<td>3.7</td>
<td>± 0.1</td>
<td>3.2–4.2</td>
</tr>
<tr>
<td>BMI</td>
<td>22.0</td>
<td>± 0.9</td>
<td>18.5–26.7</td>
<td>23.2</td>
<td>± 0.8</td>
<td>19.9–26.3</td>
</tr>
<tr>
<td>BMI</td>
<td>115.5</td>
<td>± 9.0</td>
<td>80.7–164</td>
<td>101</td>
<td>± 7.7</td>
<td>67.4–127.4</td>
</tr>
<tr>
<td>%Body fat</td>
<td>22.6</td>
<td>± 1.1</td>
<td>18.8–28.3</td>
<td>21.1</td>
<td>± 1.2</td>
<td>15.4–24.9</td>
</tr>
</tbody>
</table>

VO₂peak, peak oxygen uptake; BMI, body mass index.

Biochemical assays. For the glycerol analyses, triglyceride reagent (GPO-Trinder Reagent A, Sigma Laboratories) was reconstituted with distilled water. Serial dilutions for a standard curve were then prepared using a triglyceride 250 mg/dl standard (Sigma Laboratories). Frozen samples were thawed and immediately reacted with the triglyceride reagent and mixed, then incubated for 20 min at 37°C. This mixture was then placed in a microplate reader to measure absorbances at 550 nm. Samples were analyzed in duplicate as internal controls.

Catecholamine analysis was performed by mass spectrometry. Instrumentation included a 5973N mass selective detector, coupled to a 6890N gas chromatograph, and a SGE Forte BPX5 × 0.25 ID × 0.25 μm column. The catecholamines were derivatized in a number of steps, and single ion monitoring was used to determine the concentrations of the substrate. Internal standards were used to create a standard curve for the assay.

Statistical analyses. Data analysis was completed using SPSS 14.0 software to perform a two-way between-group repeated-measures ANOVA. All values are means ± SE and a significance level of P < 0.05 was used. The Bonferroni adjustment was used to further examine significant main effects between groups. There were no significant group-by-treatment interactions.

Fig. 1. Heart rate (HR) response of trained (T) and untrained (U) subjects during short (SS) and long (LS) bouts of high-intensity intermittent exercise.
however, these differences did not achieve significance. There was a significant difference ($P < 0.0001$) between baseline and exercise glycerol levels for both groups during both exercise bouts (Fig. 3).

**Lactate.** There was a significant increase ($P < 0.0001$) in lactate production from baseline to exercise for both exercise protocols and both groups (Fig. 4). In U there was no significant difference between SS and LS exercise with regard to lactate levels (Fig. 4). In T, however, there were significantly greater lactate levels ($P < 0.01$) in the LS compared with the SS exercise (Fig. 4).

**Catecholamines.** Epinephrine and norepinephrine levels were significantly higher ($P < 0.001$) postexercise compared with baseline (Figs. 5 and 6). In both exercise protocols the trained tended toward higher postexercise epinephrine and norepinephrine levels, but this difference was not significant.

**RPE.** For both T and U, RPE gradually increased over the 20 min of exercise in both cycling protocols so that there was a significant difference ($P < 0.0001$) in RPE between the first 5 min of exercise and the last 5 min. In U subjects, the RPE for both protocols was not different. Nor was there a difference when RPE for the SS exercise was compared between groups. RPE was significantly higher ($P < 0.001$) in LS compared with SS exercise for T subjects.

**DISCUSSION**

The major finding of this study was that for both groups plasma glycerol concentrations increased over the 20 min of HIIE. Both SS and LS forms of intermittent exercise produced similar metabolic responses although both lactate and catecholamines were higher after the LS condition. Catecholamine concentrations were also higher postexercise compared with preexercise for both groups. T differed from U women by displaying a tendency for peak glycerol concentrations earlier during exercise and higher lactate concentrations throughout both exercise conditions.

An increase in glycerol concentration suggested an increasing reliance on fats as a fuel despite increased lactate concentrations. There are limitations associated with using glycerol concentrations to estimate fat oxidation, but Børsheim et al. (5) have suggested that it is marker of lipolysis and Greer et al.
to be resaturated during the rest periods (12). Astrand et al. (1) have suggested that myoglobin plays a contributory role as an oxygen store, which supports aerobic metabolism and, presumably, the use of FFAs as a substrate. The source of these FFAs is undetermined, but it has been suggested that intramuscular triacylglycerols (IMTG) stores provide some substrate (32, 34). Other possible sources of FFAs are plasma FFAs, triglycerides, low-density lipoprotein, very-low-density lipoprotein, chylomicrons, and adipocytes (13, 17, 30, 33).

Catecholamine response to these exercise protocols was typical of high-intensity exercise (16, 27) in that postexercise levels were significantly higher than preexercise values. However, the epinephrine response was greater than levels reported by Christmass et al. (6), who measured catecholamine response to long- and short-bout intermittent treadmill exercise and found that norepinephrine was elevated postexercise but epinephrine was unchanged. Also typical of HIIE was the trend for the LS exercise, which produced higher lactate concentrations, to have higher catecholamine concentrations (7, 27). In the Christmass et al. study, fat oxidation decreased over time in the LS exercise, which may be a function of the higher lactate concentrations and lower epinephrine concentrations because epinephrine is known to stimulate lipolysis (18, 21). In the present study, glycerol concentrations were maintained above basal levels in the LS exercise (although it was lower than the SS exercise), and this may be the result of higher epinephrine concentrations. Catecholamines were collected before and after exercise, whereas glycerol was assessed during exercise, creating a temporal mismatch between these variables. However, Greer et al. (14), examining repeat Wingate tests, did assess both catecholamines and glycerol levels at the same time and showed that they increased simultaneously.

It is feasible that HIIE creates a "substrate shuttle" whereby there are repeated shifts from anaerobic to aerobic energy sources. As discussed previously, there is evidence that ATP, creatine phosphate, glycogen, and myoglobin oxygen are partly depleted during the high-intensity work phases. During the rest periods, there appears to be a resynthesis of ATP and creatine phosphate, a resaturation of myoglobin oxygen, and a recycling of lactate (12). Glycogen does not appear to be resynthesized during the rest periods, and ongoing diminution has been shown to occur over the exercise session (4). Also, it has been reported that repeated exercise impairs glycolysis because of cytosolic citrate accumulation that inhibits phosphofructokinase activity (11). These factors permit repeated bouts of exercise at high-intensity and reduce the amount of lactate accumulation. The lower lactate accumulation accompanied by aerobic metabolism may allow the use of fats as a fuel. This hypothesis is supported by the present data showing an increase in plasma glycerol over the 20 min of exercise in both bouts that suggests increasing release of FFAs. Why high lactate levels do not prevent the ability of the exercising muscle to utilize FFA in HIIE appears to be unknown.

Chronic HIIE appears to impact on adipose tissue stores and has led to greater fat loss (19, 23, 28, 36). What is unknown is the source of the FFAs during acute HIIE. If IMTGs were utilized during HIIE, they would likely be restored postexercise along with glycogen. So, restoration of glycogen and IMTG combined with this "shuttle" effect may have metabolic consequences in the recovery from exercise and be partially responsible for an elevated postexercise oxygen con-
sumption. Studies assessing actual muscle measurements need to be performed to identify the mechanisms of the above mentioned effects.

T participants had greater aerobic power and power output, and this was reflected by the differences in lactate concentrations between the two exercise bouts. For U, there was no difference, metabolically or perceptually, between the LS and SS exercise. Although U were able to work significantly harder in the long-bout exercise, it was not sufficiently intense to alter the metabolic responses to the exercise protocols. All the measured metabolic parameters (\(\text{VO}_2\), HR, glycerol, lactate, glucose, and catecholamines) and RPE were not significantly different for U when the two bouts were compared. This finding probably reflects the lack of experience of U subjects with cycling exercise and their perception that they were working as hard as they could in both bouts. The situation was different for the T group. During the LS exercise they were able to generate sufficiently greater power outputs to alter the metabolic responses to the activity. The LS exercise produced significantly higher lactate concentrations as expected and, after the first 5 min of exercise, the increased lactate was associated with a lower level of glycerol production. These results are supported by the perceived exertion response that showed for T the LS exercise resulted in higher RPE. However, that \(\text{VO}_2\) was not significantly different for T between the two exercise bouts adds support to the notion that, for this group, the energy source for the additional demand was supplied by anaerobic glycolysis.

In conclusion, despite increased lactate levels during HIIE, both groups showed an increase in plasma glycerol levels. HIIE resulted in significant elevations in catecholamines. Trained compared with untrained women showed a trend for an earlier increase in plasma glycerol during high-intensity exercise.

Perspectives and significance. Repeated bouts of high-intensity, intermittent exercise led to increased lactate and catecholamine levels. The accompanying increase in glycerol levels suggests that fat stores may supply a significant amount of energy during this form of exercise. Consequently, it is possible that high-intensity intermittent training may contribute to programs designed to promote fat loss.

REFERENCES
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