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

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Running Head: High Intensity Intermittent Cycle Exercise
Abstract

The metabolic response to two different forms of high intensity intermittent cycle exercise was investigated in young females. 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 when compared to pre-exercise 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. Trained compared to the untrained women tended to show an earlier increase in plasma glycerol concentrations, during high intensity exercise.

Keywords: intermittent exercise, fat oxidation, RER, catecholamines
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-week high-intensity intermittent exercise (HIIE) program that resulted in a greater decrease in skinfold thickness relative to energy expenditure compared to 20 weeks 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 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 to 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, catecholamine, lactate, and glycerol response of trained and untrained females to SS (8 s sprint, 12 s recovery) and 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. Females were chosen for this study to avoid any confounding effects of gender 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 \( \dot{V}O_{2\text{peak}} \) but there were no significant differences in mass, height, \( \Sigma \) skinfolds, percent body fat, or blood lipids.

Study design. Each subject visited the laboratory on three separate occasions. On the first visit \( \dot{V}O_{2\text{peak}} \), mass, height, anthropometric measures, resting lactate, heart rate (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. \( \dot{V}O_2 \) (l min\(^{-1}\)), ventilation (\( \dot{V}_E \), l min\(^{-1}\)), carbon dioxide production (\( \dot{V}CO_2 \), l min\(^{-1}\)) and RER (\( \dot{V}CO_2 /\dot{V}O_2 \)) were measured using an open circuit indirect calorimetry system (ParvoMedics Inc, 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 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 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 two days to avoid any carry over 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 analyser (Yellow Springs, 2300 STAT). Blood lipid profiles (Cholestech LDX) were also obtained immediately. Whole blood in EDTA tubes was spun immediately in a chilled centrifuge at 4\(^\circ\)C, 3000 rpm for 10 min, and then aliquots of plasma were placed in 1.5 ml microtubes and frozen at minus -86\(^\circ\)C for later analysis. The power output for each subject was set at the power output achieved at 70% \( \dot{V}O_2_{\text{peak}} \). The ergometer was set at 30 W for the between-sprint recovery periods. Subjects were instructed to cycle as hard as
they possibly could for the work phase of the intermittent protocol and to turn over the bike pedals during the rest period as slowly as possible. At the end of 5 min of work/rest intervals, blood samples were taken and dealt with in the same manner as the baseline measures. Plasma catecholamine levels were measured at baseline and immediately postexercise. HR and ratings of perceived exertion (RPE) were recorded every 5 min throughout the work bout. At the end of 20 min of work/rest cycles, the subject recovered by cycling for 5 min at a power output that was comfortable. Repeat blood samples were taken at 5 min post cool down. The long and the short bout protocols varied only in the work:rest ratio.

*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 x 0.25 IDx 0.25 micron column. The catecholamines were derivatized in a number of steps and single ion monitoring (SIM) 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 analyses of variance (ANOVA). All values are mean ± 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.

**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 $153.2 \pm 6.9$ W for T and $96.1 \pm 7.1$ 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 $167.1 \pm 3.8$ W and $105.4 \pm 4.2$ W respectively for the LS exercise and a mean power output of $139.4 \pm 1.7$ W and $86.6 \pm 2.6$ 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 when compared to 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 (Figure 1).

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

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

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

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

Ratings of perceived exertion (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 to 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 when compared to pre-exercise for both groups. Trained differed from untrained 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 (5) has suggested it is marker of lipolysis and Greer (14) has suggested that elevated glycerol levels provide an
indicant of intramuscular triglyceride contribution to the energy requirements of repeated Wingate tests. Also Medbø and Jebens (20) have shown that skeletal muscle releases glycerol during recovery from intense cycle exercise. Thus, the rise of venous blood glycerol levels in the present study could reflect release of fatty acids from adipose and intramuscular triacylglyceride stores during, as well as recovering from, repeated intermittent exercise. High lactate concentrations are thought to inhibit carnitine-acyl CoA transferase which limits the rate at which β-oxidation can occur (29). That lactate was higher throughout LS for the T women supports this notion. However, it has been suggested that the lactate threshold is attained at blood concentrations of 3.5 mmol\textsuperscript{\text{-1}} (8) or 4.0 mmol\textsuperscript{\text{-1}} (15). So, in spite of lactate concentrations elevated above that considered as the lactate threshold, glycerol concentrations in the present study still increased. The effect of high levels of blood lactate on the ability of the exercising muscle to utilize FFA was not examined in this study but is an important issue for future research.

Tremblay et al. (32) have suggested that the ability of exercising muscle to utilize FFAs, despite high lactate levels, is due to the intermittent nature of the HIIE. For example, it has been demonstrated that during the intensive component of HIIE, ATP, and creatine phosphate are broken down to produce energy and are resynthesised during the nonintensive rest periods via the aerobic system (11). Because the rest periods were comparatively short (12 s in the current study), this resynthesis is likely to be incomplete, thus, anaerobic glycolysis has been suggested to provide the remainder of the required energy (12). Glycogen is the substrate for the anaerobic glycolytic system and has been shown to be depleted to some extent during HIIE (11). Research using muscle biopsy has shown that PCr recovery was incomplete after 15 s of rest in a 15 s work, 15 s rest
intermittent exercise bout (12). These authors suggest that progressive depletion of PCr and glycogen, in conjunction with increased cytosolic citrate, act to inhibit glycolysis and lead to lowered lactate accumulation and enhanced fatty acid oxidation. Myoglobin O₂ usage during the work bouts has been estimated to contribute to about 44% of the oxygen deficit and has been shown 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 IMTG stores provide some substrate (32, 34). Other possible sources of FFA are plasma FFAs, TGs, LDL, VLDL, 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 pre-exercise 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 lactates, to have higher catecholamine concentrations (7, 27). In the Christmass et al. (6) study, fat oxidation decreased over time in the LS exercise which may be a function of the higher lactate concentrations and lower epinephrine concentrations as 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” where there are repeated shifts from anaerobic to aerobic energy sources. As discussed previously, there is evidence that ATP, creatine phosphate, glycogen, and myoglobin O₂ 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 O₂, and a recycling of lactate (12). Glycogen does not appear to be resynthesised 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 which inhibits PFK 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 current data that showed 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 be likely restored postexercise along with glycogen. So, restoration of glycogen and IMTGs combined with this “shuttle” effect
may have metabolic consequences in the recovery from exercise and be partially responsible for an elevated postexercise oxygen consumption. 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 (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 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 to 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**


5. **Børsheim E, Lonnroth P, Knardahl S, Jansson P-A.** No difference in the lipolytic response to β-adrenoceptor stimulation in situ but a delayed increase in adipose tissue blood flow in moderately obese compared with lean men in the postexercise period.


Table 1. Subject characteristics

<table>
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<tr>
<th></th>
<th>Age (years)</th>
<th>Mass (kg)</th>
<th>Height (cm)</th>
<th>VO\textsubscript{2peak} (l.min\textsuperscript{-1})</th>
<th>BMI</th>
<th>Σ 9 skin folds</th>
<th>%Body fat</th>
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<td>2.8</td>
<td>22.0</td>
<td>115.5</td>
<td>22.6</td>
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<td>± 2.2</td>
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<td>± 1.1</td>
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<td>163 - 174.5</td>
<td>2.1 - 3.3</td>
<td>18.5 - 26.7</td>
<td>80.7 - 164</td>
<td>18.8 - 28.3</td>
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<tr>
<td><strong>Trained</strong> (n = 8)</td>
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<td>68.2</td>
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Figure captions

**Figure 1.** Heart rate response of trained (T) and untrained (U) subjects during short (SS) and long (LS) bouts of high intensity intermittent exercise.

**Figure 2.** Oxygen uptake response of trained (T) and untrained (U) subjects during short (SS) and long (LS) bouts of high intensity intermittent exercise.

**Figure 3.** Glycerol response of T and U subjects during SS and LS bouts of exercise.

**Figure 4.** Lactate response of trained and untrained subjects during short and long bouts of exercise.

**Figure 5.** Epinephrine response of trained and untrained subjects pre- and postexercise.
Figure 6. Norepinephrine response of trained and untrained subjects pre- and postexercise.

***denotes a significant difference between pre- and postexercise for both exercise protocols and both groups, $P < 0.001$. 
Figure 1
Figure 2
Figure 3
Figure 4.
Figure 5.
Figure 6