Acute Effects of Exercise Intensity on Insulin Sensitivity under Energy Balance

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Abstract:

Exercise is known to improve insulin sensitivity (SI), however studies to date have been confounded by negative energy deficits following exercise. **Purpose:** The primary objective of this study was to assess the effect of 8-16 weeks of aerobic exercise training on the SI of untrained women under rigorously controlled energy balanced conditions. The secondary objective was to determine if one acute bout of moderate (MIC)- or high (HII)-intensity exercise further affected SI. **Methods:** SI was assessed in 28 untrained women at baseline, following 8-16-weeks of training with no-exercise prior to assessment (NE), 22hrs after MIC (50% VO$_2$peak), and 22hrs after HII (84% VO$_2$peak) using a hyperinsulinemic-euglycemic clamp. Participants were in a whole-room indirect calorimeter during each condition and food intake was adjusted to ensure energy balance across 23 hours prior to each clamp. **Results:** There were no significant differences in acute energy balance between each condition. Results indicated a significant main effect of time, such that SI was higher during the HII condition compared to both baseline and NE (P < 0.05). No significant differences in SI were observed following NE or MIC. **Conclusion:** Widely reported improvements in SI in response to chronic exercise training may be mediated in part by shifts in energy balance. However, an acute bout of HII exercise may increase SI even in the context of energy balance.

**Keywords:** aerobic exercise, exercise intensity, insulin sensitivity, energy balance
INTRODUCTION

It is well known that a single exercise session and exercise training can improve insulin sensitivity (1, 2). However, the duration in which exercise-induced improvements in insulin sensitivity persists varies significantly among studies, ranging from 0- to 72- hours following the last bout of exercise (2-6). One factor that may explain the variability in the duration of improved insulin sensitivity among studies is the magnitude of energy deficit following exercise. While it is often assumed that chronic skeletal muscle adaptations following exercise are primarily responsible for the improvements in insulin sensitivity (7), data from caloric restriction studies demonstrate that acute changes in energy balance with no changes in body composition can lead to acute improvements in insulin sensitivity during negative energy balance and acute reductions in insulin sensitivity during positive energy balance (8, 9). Further evidence was demonstrated by Toledo et al. (2007) in which the amount of energy expended during exercise was the strongest predictor of improvements in insulin sensitivity, and the mean daily energy expenditure accounted for 75% of the variance in improved insulin sensitivity (3). Additionally, Black et al. (2005) found that six days of exercise training without energy replacement led to significant improvements in insulin action, whereas there were no changes when participants were refed the energy expended from exercise (10). Last, Segal et al. (1991) demonstrated that refeeding the amount of energy expended during 12 weeks of training led to no improvement in insulin action or insulin resistance in obese and diabetic men (11). Therefore, it is important to control for energy balance when trying to compare the acute vs chronic effects of exercise on insulin sensitivity.

It has also become increasingly clear that exercise intensity can influence improvements in insulin sensitivity (6, 12, 13). A recent meta-analysis comparing high intensity interval...
training (HII) to continuous moderate exercise intensity training (MIC) demonstrated greater improvements in insulin resistance in HII as compared to MIC (14, 15). However, while there is convincing evidence that HII exercise may provide greater improvements for insulin sensitivity there have been several studies that have shown no differences on insulin sensitivity between low intensity and high intensity exercise protocols (16, 17). One of the challenges when trying to elucidate the mechanisms through which higher intensity exercise influences insulin sensitivity is controlling for greater amounts of energy expended per unit time as compared to lower intensity exercise. Ross et al. (2015) attempted to address this by comparing the effects of habitual exercise training of differing intensities on glucose tolerance while controlling for energy balance using dietary recalls (13). The primary finding from this study was higher intensity exercise was required in order to improve 2-hour glucose levels. While this study did attempt to control for energy balance using a dietary recall approach it is well known that these approaches are limited and are highly variable in regards to accuracy (18, 19). Thus, it is critical to conduct well-controlled studies that utilize state of the art equipment to control for energy balance when assessing the independent effects of exercise intensity on changes in insulin sensitivity.

While the benefits of exercise training per se for improving glucose tolerance and insulin sensitivity has been widely reported, there is also convincing evidence that a single exercise session performed at high or moderate exercise intensities can improve insulin sensitivity for up to 24-48 hours (20-22). Additionally, Nelson et al. (2014) found that a single session of exercise in sedentary overweight adults could improve insulin sensitivity to similar levels of individuals that were physically active (23). Thus, although routine exercise training is associated with long term health benefits and reduces risk for onset of chronic diseases it remains unclear if these benefits are due to the most recent exercise session or if they are a result of long term
physiological adaptations that occur as a result of routine exercise training. Furthermore, given recent evidence that exercise intensity may impact the magnitude of exercise induced improvements in insulin sensitivity studies are needed to compare the acute effects of exercise intensity for improving insulin sensitivity. We are aware of no studies that have assessed the effects of an acute bout of high intensity interval exercise versus moderate intensity continuous exercise for improving insulin sensitivity when controlling for energy balance.

Thus, the overall purpose of this study was to compare the chronic effects of 8-16-weeks of aerobic exercise training and the acute effects of a single bout of moderate-intensity continuous aerobic exercise (MIC) or high-intensity interval exercise (HII) for improving insulin sensitivity under rigorously controlled energy balanced conditions using whole room indirect calorimetry.

METHODS

Study Participants

Twenty-eight women between 20-40 years of age participated in this study. Participants reported normal menstrual cycles and were not taking oral contraceptives or any medications known to influence glucose and/or lipid metabolism. Additional inclusion criteria were: i) normotensive; ii) non-smoker; iii) sedentary as defined by participating in any exercise-related activities < 1x per week; and iv) normoglycemic as evaluated by postprandial glucose response to a 75-g oral glucose tolerance test. All participants provided written informed consent. Study procedures were approved by the Institutional Review Board at the University of Alabama at Birmingham and conformed to the guidelines set forth by the Declaration of Helsinki.
Procedures

After initial screening and fitness assessments, all participants were evaluated four times during the follicular phase of their menstrual cycle. Participants stayed in a room calorimeter for the 23 hours prior to testing. Food was provided for two days prior room calorimeter visits, and food intake was adjusted during the stay in the room calorimeter to maintain energy balance. The first evaluation was considered baseline. Post training evaluations took place after 8, 12, and 16 weeks of exercise training (Figure 1). The three post training evaluations were performed in a randomized order and consisted of the following: 1) Following 60 hours of no exercise (NE), designed to evaluate the effects of chronic exercise training); 2) ≈22 hours after one hour of continuous stationary cycle ergometry at 50% peak VO$_2$ (MIC); and 3) ≈22 hours after one hour of interval stationary cycle ergometry at 84% peak VO$_2$ (HII). Details regarding exercise sessions in the room calorimeter are outlined in the room calorimeter exercise methods section below. Post training assessments (NE, MIC and HII exercise) were randomized to reduce the risk of bias by ordered effects, and were 1 month apart to permit testing during the follicular phase of the menstrual cycle. Forty-eight hours prior to testing, participants were required to abstain from any exercise or vigorous physical activity. Food was provided to participants during the two days preceding testing. Caloric intake during these two days was estimated from doubly labeled water estimates of free-living energy expenditure of 330 sedentary premenopausal women collected from our laboratory. The following equation was used: Equation 1 = 750 kcal + [(31.47 FFM) – (.31 x FM) – (155 x race); race coded 1 for African American and 2 for European American](24). Diets were prepared by the Clinical Research Unit and consisted of ≈60% energy as carbohydrates, ≈25% energy as fat, and ≈15% energy as protein. Dietary sodium and the ratio
between polyunsaturated: saturated fats were held constant to reduce the potential confounding effects of thermic processes.

**Energy Balance**

Our goal for providing food was to achieve energy balance (energy intake to match energy expenditure) especially during the room calorimeter visit. Breakfast was at 9:30 AM (35% of planned energy intake), lunch was at 1:00 PM (25% of planned energy intake), and supper was at 6:00 PM (40% of planned energy intake). We used multiple regression to develop three equations (equations 2-3) to estimate energy expenditure while the subjects were in the room calorimeter as follows. An equation for estimating the room calorimeter energy intake was developed from room calorimeter visits of >200 pre-menopausal women: Equation 2 = 465 Kcal + [(27.8 FFM)- (2.4 x FM) – (188 x race); race coded 1 for African American and 2 for European American]. The estimated energy cost of the exercise (based on ACSM metabolic equations) during the MIC and HII visits was added to the equation 2 result: Equation 3 = (equation 2 estimated energy expenditure + energy cost of MIC or HII exercise). We recognized that the estimates may result in overfeeding or underfeeding individual subjects. Thus, we developed a correction equations for the room calorimeter visit that was based on energy expenditure during the room calorimeter stay up to 5:30 pm. This equation was as follows: Equation 4 = 9(390 kcal + average energy expenditure in kcal/min between 8:00 am and 5:30 pm) x 925 kcal) – equation 3 estimated of energy expenditure). Food intake was adjusted during the evening meal to match the results of equation 4.
**Exercise Training**

After the baseline assessment, all participants trained three times/week on a bicycle ergometer for the duration of the study. Participants trained initially for 20 minutes at 65% of maximum heart rate and progressively increased their training until they were training continuously for 40 minutes at 80% of maximum heart rate by week four. Heart rate was monitored throughout each session by a Polar Vantage XL heart rate monitor. All sessions were under the supervision of an exercise physiologist in a training facility dedicated to research. Participants were instructed to refrain from all other exercise training for the duration of the study.

**Peak Aerobic Capacity**

A peak aerobic capacity test was conducted two to four days prior to each room calorimeter visit. After an initial warm-up, participants completed a cycle ergometer graded exercise test to measure peak oxygen uptake (VO$_{2peak}$) as determined by the highest level reached in the final stage of exercise. Power output began at 25 W and increased by 25 W every 2 minutes until participants reached volitional exhaustion. Oxygen uptake, ventilation, and respiratory exchange ratio were determined by indirect calorimetry using a MAX-II metabolic cart (Physio-Dyne Instrument Company, Quogue, NY). Heart rate was continuously monitored by Polar® Vantage XL heart rate monitors (Polar Beat, Port Washington, NY). Criteria for achieving VO$_{2peak}$ were heart rate within 10 bpm of estimated maximum, RER of at least 1.10, and plateauing of VO$_2$. All subjects achieved at least one criteria and all but three subjects achieved at least two criteria during each of the four tests.
Room Calorimeter

Participants spent 23 h in a whole-room respiration chamber (3.38-m long, 2.11-m wide, and 2.58-m high) for measurement of total energy expenditure (TEE) and resting energy expenditure (REE). The design characteristics and calibration of the calorimeter were described previously [14]. Oxygen consumption and carbon dioxide production were continuously measured with the use of a magnetopneumatic differential oxygen analyzer (Magnos206; ABB, Frankfurt, Germany) and a nondispersive infrared industrial photometer differential carbon dioxide analyzer (Uras26, ABB, Frankfurt, Germany). The calorimeter was calibrated before each participant entered the chamber. Zero calibration was carried out simultaneously for both analyzers. The full scale was set for 0–1% for the carbon dioxide analyzer and 0–2% for the oxygen analyzer. Each participant entered the calorimeter at 0800 h. Metabolic data were collected throughout the 23h stay. Each participant was awakened at 0630 the next morning. REE was then measured for 30 min before the subject left the calorimeter at 0700 h. Hyperinsulinemic euglycemic clamp measures were conducted immediately after participants exited the room calorimeter.

Exercise Sessions in Room Calorimeter

Workload for the MIC exercise was calculated from the VO_{2peak} that corresponded to each follow-up assessment using the appropriate metabolic equation for cycle ergometry \([\text{VO}_2 (\text{mL/kg/min}) + 2 \times (\text{mass (kg)} \times \text{length (m)})/(\text{time (min)}) + (3.5 \times \text{body mass})]\) in accordance with the American College of Sports Medicine . During the MIC exercise, participants cycled continuously for 60 minutes at an intensity of 50% VO_{2peak}. Fifteen work intervals were performed for 2 min 24 s with rest intervals of 1 min 43 s for the HII exercise. Work was calculated by multiplying 1.66 to the MIC exercise workload, which equated to 84% VO_{2peak}. 
Total work was identical among the two exercise bouts. Work load was controlled outside the room calorimeter using a Collins electronically braked cycle ergometer (Warren E. Collins, Braintree, MA).

**Body Composition**

Total and regional body composition (i.e., % fat, fat mass and lean mass) were determined by dual-energy X-ray absorptiometry (DEXA; iDXA, GE-Lunar, Madison, WI). Participants wore light clothing and remained supine in compliance with normal testing procedures. Scans were analyzed with enCORE 2011 software (GE Healthcare Lunar, Madison, WI).

**Hyperinsulinemic-Euglycemic Clamp**

A hyperinsulinemic-euglycemic clamp was administered providing a continuous infusion of regular insulin (Humulin, Eli Likely & Co., Indianapolis, IN) at 40 mU/m² body surface area/min through the brachial vein. Body surface area was calculated using the method of Du Bois (25). Blood glucose was monitored at bedside at 5 min intervals using a YSI 2300 STAT Plus (YSI Life Sciences, Yellow Springs, OH) and a 20% dextrose solution was infused at a variable rate to maintain euglycemia (targeting 90 mg/dL). Serum samples were collected from the antecubital vein every 10 minutes for laboratory analysis of insulin and glucose. The procedure lasted approximately 2 hr. The protocol for the steady state period was a period of 30 min or longer (at least 1 hr after beginning the insulin infusion) during which the coefficients of variation for serum glucose, and the recorded dextrose infusion rate were less than 5% . Mean parameter values during the steady state period for each individual clamp were used to calculate the SI\textsubscript{Clamp} [defined as \( M/(ssG \times \Delta I) \)], where \( M \) is the steady state glucose infusion rate (mg/kg/min), \( ssG \) is the steady state blood glucose concentrations (milligrams per dL), and \( \Delta I \) is the difference between basal and steady state serum insulin concentrations (µU/ml)] (26).
Statistics

All statistical analyses were conducted using SPSS Statistics for Macintosh Version 22.0 (IBM Corp, Armonk, NY). Descriptive statistics and primary outcome variables (SI<sub>clamp</sub>) are reported as mean ± standard deviation. A one way repeated measures ANOVA was used to compare differences across conditions (baseline, NE, MIC, and HII) for each dependent variable. Condition sequence was assessed as a covariate to determine if there was an order effect. When significance was observed, Bonferroni pairwise comparisons were used to compare differences between each condition. An alpha level of 0.05 was used to determine statistical significance.

RESULTS

Descriptive statistics at baseline and the effects of each condition are shown in Table 1. There were no significant differences between conditions for exercise adherence (NE = 84%, MIC = 89%, and HII = 81%). Additionally, there was no significant effect of condition sequence. A significant time effect was observed for VO<sub>2</sub> peak, such that a 6.5% improvement was observed for VO<sub>2</sub> peak following 8-16 weeks of exercise training (P < 0.05). No significant differences were observed for %fat, fasting glucose, or fasting insulin.

There were no significant differences in energy balance (Kcals consumed – Kcals expended) measured in the room calorimeter between each condition (Figure 2), demonstrating effective control of energy balance among all four conditions. A significant time effect was observed for 24- hour respiratory exchange ratio following the initial 12-weeks of exercise training (P < 0.05), (Figure 3). A significant main effect of time was observed for SI<sub>clamp</sub> (P < 0.05). Bonferroni Pairwise comparisons between time points showed a significant improvement
in $\text{SI}_{\text{clamp}}$ following HII compared to baseline and NE (P < 0.05). No significant improvements in $\text{SI}_{\text{clamp}}$ were observed between baseline, NE or MIC time points (Figure 4).

**DISCUSSION**

The purpose of this study in healthy premenopausal women was to determine the effects of aerobic exercise training and the acute effects of a moderate- or high intensity- exercise bout for improving whole body insulin sensitivity when rigorously controlling energy balance using a room calorimeter. The primary finding was that HII exercise significantly improved SI 22 hours following exercise when we controlled energy balance. We did not see any significant improvements following the 8-12 weeks of aerobic exercise training (NE) or following an acute bout of moderate intensity exercise (MIC). These observations are in agreement with Ross et al. 2015, who found that 2-hour glucose tolerance only improved in individuals performing high intensity exercise (13). However, given the fact that we controlled for energy balance our findings suggest that the benefits of performing higher intensity exercise for improving insulin sensitivity may be due some other factor other than energy deficit following the higher intensity exercise.

Our study was unique in that we utilized a longer term training period, 8-16 weeks of training, to assess the chronic effects of exercise training. We did not see any significant improvements in SI following chronic exercise training. Importantly, energy balance was tightly controlled during the ensuing 22-hrs prior to the hyperinsulinemic euglycemic clamp assessment and there was no significant weight loss or change in body composition throughout the study. The lack of a training induced significant increase in SI in the NE group is in agreement with
several previous studies that suggest that energy deficit during chronic exercise training is the primary factor responsible for improving SI. Indeed, we (27) and others (28, 29) have shown that energy deficit using caloric restriction alone, exercise alone, or combined led to similar improvements in SI. Furthermore, previous investigations in both men (30) and women (31) that adjusted dietary intake to account for increased energy expenditure during exercise training found no significant improvements in SI following 12- and 14-weeks of training, which suggests that training adaptations are driven at least in part by negative energy balance elicited during exercise training.

In addition to chronic exercise training it is also well known that an acute bout of exercise (1, 2, 32) and short term exercise training (2, 33) can improve SI. However, an important potential confounder in the majority of these studies is the likelihood that subjects remained in energy deficit prior to assessment of SI. We attempted to control energy balance while assessing the impact of two different exercise intensities on SI. Several (6, 12-14) but not all (34, 35) previous studies have demonstrated greater improvements in SI following high intensity exercise training, however these studies were unable to determine if improvements were due to exercise mediated effects or an energy surplus or deficit. When accounting for energy balance, we found a significant improvement in SI following the HII condition but not the MIC condition. These data support the notion that an acute bout of high intensity exercise may provide superior benefits for improving SI compared to lower intensity exercise. Furthermore, these data also suggest that improvements following HII are at least in part independent of energy balance.

While we attempted to control energy balance prior to assessment of SI, we could not control for carbohydrate balance. Carbohydrate deficit rather than energy deficit is thought to be the primary driver of exercise-induced improvements in insulin-stimulated glucose uptake.
Indeed, a number of previous investigations have shown that improvements in SI with exercise are associated with reductions in muscle glycogen (36, 37). Additionally, work from Fox et al. (38) has found that sustaining a carbohydrate deficit rather than an energy deficit is the key contributor for improving SI. Furthermore, when CHO intake following exercise was controlled and excess lipid was infused to create an 1100 kcal surplus there was no difference in SI (39). Because exercise at higher intensities selectively depletes glycogen (40), it is possible that glycogen deficit explained the increase in SI following high intensity exercise in this study. While these data suggest that carbohydrate balance may be critical in determining the effects of exercise induced SI, there is evidence from a one-legged cycling study that this may not be the case. Stephens et al (41) conducted a study in humans in which they performed 90 minutes of a one-legged cycling exercise protocol at 60% VO\textsubscript{2}max followed by a hyperinsulinemic euglycemic clamp 22 hours post-exercise. Skeletal muscle glycogen content measured prior to the clamp was similar between the exercise and non-exercise leg, however there was 17% greater glycogen stored in the exercise- vs non-exercise leg following the clamp, demonstrating an improvement in insulin action in the exercise leg independent of glycogen content (41). It is possible that the high intensity exercise served to increase glycogen storage in the exercised leg, thus increasing glucose removal. In our study urine nitrogen was collected on a subset of participants (n=13), in our exploratory analyses we found that carbohydrate balance was not associated with insulin sensitivity. Therefore, it remains to be determined if muscle glycogen content is critical for exercise-induced improvement in SI. There is compelling evidence that sustaining a glycogen or carbohydrate deficit during the post-exercise period may lead to greater- or prolonged- improvements in SI following exercise. Further work is needed to better
understand the interactions between exercise volume, intensity, and post-exercise macronutrient intake for improving SI.

Strengths of our study included use of the whole room indirect calorimeter to control for energy balance prior to assessment of SI and use of the hyperinsulinemic euglycemic clamp for assessment of SI. All assessments were conducted during the follicular phase of the menstrual cycle, enabling tight control of the hormonal effects on metabolism. Additionally, exercise training was performed under supervision by trained exercise physiologists throughout the study and acute sessions were rigorously monitored while participants were within the room calorimeter. A limitation in this study was the absence of an energy deficit group that did not perform exercise. Additionally, the conclusions from this study are limited to healthy premenopausal women. Furthermore, without direct measures of muscle glycogen and only collecting urine nitrogen on half of the cohort we are only able to speculate as to the potential contributions of energy balance versus carbohydrate balance per se. Finally, although the changes in Si were relatively small and not significant between the NE and baseline as well as the NE and MIC the power was relatively low (below 0.75 for both contrasts) so it is impossible to determine whether chronic exercise training and an acute bout of exercise influenced insulin sensitivity. However, these results are consistent with the concept that a bout of high intensity exercise induces a larger increase in insulin sensitivity for at least 24 hours compared to either chronic exercise training or an acute bout of moderate intensity exercise.

In conclusion, the overall implication from the present study was that 12-weeks of aerobic exercise training did not improve SI in a cohort of premenopausal women when energy balance was controlled; thus, widely reported improvements in SI in response to chronic exercise training may be at least partly mediated by shifts in energy balance. However, an acute bout of
HII exercise may increase SI even in the context of energy balance. Thus, high intensity exercise may be more effective for treatment and prevention of metabolic diseases related to insulin resistance.
Acknowledgements: The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by ACSM. This work was supported by the NIH grants R01DK049779, P30 DK56336, P60 DK079626, UL 1RR025777. The authors would like to thank David Bryan, Bob Petri, and Paul Zuckerman for help in data acquisition.
REFERENCES


Figures:

**Figure 1:** Study schematic. Following baseline evaluations, all participants completed at least 8 weeks of supervised, aerobic exercise training before further assessments were made at 12 and 16 weeks. At least one 1 month separated follow-up assessments to ensure measures corresponded to the follicular phase of the menstrual cycle. Participants continued to exercise train until completing 16 weeks. All follow-up assessments were randomized. Before evaluations were made in the “trained state,” participants abstained from any exercise (No Exercise) 48 h prior to testing for the purpose of attenuating the acute effects of exercise. Measures of moderate-intensity continuous exercise (MIC) and high-intensity interval exercise (HII) were completed ≈22 h an overnight stay in a room calorimeter. Procedures were conducted under standardized conditions at the same time of day.

**Figure 2:** Energy consumed vs. energy expended at baseline, NE, and at MIC and HII. No significant differences between conditions.

**Figure 3:** 24-Hour- RER at baseline, NE, and at MIC and HII. All follow-up measures were significantly lower than baseline (* = p < 0.05)

**Figure 4:** Insulin sensitivity measured at baseline, NE, and MIC ad HII. SI$_{clamp}$ was significantly increased in HII compared to baseline and NE (* = p < 0.05)
Figure 1
Figure 2a
Figure 3
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Table 1: Descriptive statistics at baseline and for each condition.