Gender differences in insulin action after a single bout of exercise

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Perreault, Leigh, Jennifer M. Lavely, Bryan C. Bergman, and Tracy J. Horton. Gender differences in insulin action after a single bout of exercise. J Appl Physiol 97: 1013–1021, 2004. First published May 14, 2004; 10.1152/japplphysiol.00186.2004.—Effects of a single exercise bout on insulin action were compared in men (n = 10) and women (n = 10). On an exercise day, subjects cycled for 90 min at 85% lactate threshold, whereas on a rest (control) day, they remained semirecumbent. The period of exercise, or rest, was followed by a 3-h hyperinsulinemic-euglycemic clamp (30 mU·m−2·min−1) and indirect calorimetry. Glucose kinetics were measured isotopically by using an infusion of [6,6-2H2]glucose. Glucose infusion rate (GIR) during the clamp on the rest day was not different between the genders. However, GIR on the exercise day was significantly lower in men compared with women (P = 0.01). This was mainly due to a significantly lower glucose rate of disappearance in men compared with women (P = 0.05), whereas no differences were observed in the endogenous glucose rate of appearance. Nonprotein respiratory quotient (NPRQ) increased significantly during the clamp from presclamp measurements in men and women on the rest day (P < 0.01). Exercise abolished the increase in NPRQ seen during the clamp on the rest day and tended to decrease NPRQ in men. Our results indicate the following: 1) exercise abolishes the usual increase in NPRQ observed during a hyperinsulinemic-euglycemic clamp in both genders, 2) men exhibit relatively lower whole body insulin action in the 3–4 h after exercise compared with women, and 3) gender differences in insulin action may be explained by a lower glucose rate of disappearance in the men after acute exercise. Together, these data imply gender differences in insulin action postexercise exist in peripheral tissues and not in liver.

isotopes; glucose; insulin sensitivity; clamp; activity

THERE ARE CLEAR DIFFERENCES in the prevalence and prognoses of metabolic diseases between men and women (1). Gender differences are also observed in response to lifestyle interventions commonly used to modify disease risk. For example, substantial data suggest that, for a given submaximal exercise intensity, women oxidize a greater proportion of lipid relative to carbohydrate than men (7, 18), although not all agree (25, 30). In addition, the relative utilization and type of muscle fuel stores during exercise also appear to be different between genders (33, 36). Whether gender-specific patterns of exercise fuel utilization affect postexercise metabolism is not known. Differences in intramuscular fuel utilization during exercise, such as muscle glycogen stores, could most certainly affect postexercise glucose utilization differently in men and women. Better understanding gender differences in postexercise fuel metabolism has implications with respect to glucose homeostasis, fuel oxidation, and postprandial nutrient partitioning. Such differences may underlie gender differences in disease prevalence and prognoses, especially in insulin-resistant states.

Improved insulin action is an important metabolic consequence of both acute and chronic exercise. In aerobically trained individuals, increased peripheral insulin sensitivity and ability of insulin to suppress endogenous glucose production have been observed at rest (13). Increased peripheral insulin sensitivity, as a result of chronic training, has largely been attributed to anatomic changes in skeletal muscle such as increased capillarization (2), which enhances blood flow (11) and can increase glucose uptake. In contrast, the effect of acute exercise on muscle insulin action appears to be due to biochemical rather than anatomic changes. Translocation of GLUT4 vesicles to cell surface membranes and T tubules dictates glucose transport via insulin-dependent (8) and contraction-mediated (insulin-independent) mechanisms (17). The relative contribution of each mechanism to net glucose uptake during and immediately after exercise is controversial; however, they are clearly synergistic (15). It is unknown whether acute exercise enhances insulin action similarly in men and women. There is reason to believe there could be differences between the genders in postexercise insulin action.

Greater insulin sensitivity has been observed in women compared with men at rest (39), during exercise (4), and after a meal (29). It is not known whether gender differences in substrate use during exercise confer gender differences in insulin action immediately postexercise. In addition, if women were more insulin sensitive than men before exercise, there may be less capacity (or need) for them to increase insulin action after acute exercise. Few studies addressing the effects of exercise on insulin action have included both men and women, and even fewer have attempted to directly compare the response between genders. Historically, studies that have included both men and women lacked the statistical power to discern gender differences in the primary end points (3, 26). Furthermore, matching of subjects for habitual activity and fitness level, as well as controlling pretest diet, exercise, and menstrual cycle phase (in women), have been frequently overlooked, making data difficult to interpret. Lastly, timing of measurements is critical because the effect of acute exercise on insulin action is not uniform over time. Previous authors have appreciated a decrement in insulin action immediately after exercise with improvements seen farther out (21). Taken together, there is reason to believe that women may be more insulin sensitive at baseline and/or during exercise. Furthermore, an intervention aimed at an improvement in insulin action may result in a lower absolute change in women than that of men. Therefore, it was hypothesized that exercise...
combined with hyperinsulinemia would cause a greater change in insulin action postexercise in men than women and that this would be manifest by greater nonoxidative glucose disposal (NOGD) in men during the 3–4 h after exercise. Statistics were powered to discern gender differences. Careful prestudy diet, exercise, and menstrual cycle phase controls were employed.

METHODS

Subjects

Lean, healthy men and women (21–44 yr old) were recruited for the study. Female subjects were eumenorrheic with regular menstrual cycles (>5 cycles over the past 6 mo). Women were studied in the follicular phase of their menstrual cycle. All subjects were habitually active (>90 min of aerobic activity per week). Men and women were pair matched on the basis of habitual physical activity and maximal oxygen uptake ($V\text{O}_2\text{max}$) per kilogram of fat-free mass (FFM) (Table 1). Medical exclusions included past or present history of cardiovascular disease, high blood pressure, diabetes, any hormonal imbalance or metabolic abnormality, or use of pharmacological contraception. A total of 20 subjects completed the study. The study was approved by the University of Colorado Institutional Review Board for the Protection of Human Subjects. All subjects read and signed an informed consent form before admission into the study.

Preliminary Assessments

Preliminary assessments included a medical history and physical examination, blood tests (for determination of thyroid-stimulating hormone, electrolytes, hepatic and renal function, insulin, glucose, and lipid profile), and an assessment of food preferences. Body composition, resting metabolic rate (RMR), and $V\text{O}_2\text{max}$ were measured (described below).

Body composition. Body composition was determined by dual-energy X-ray absorptiometry. Fat percent $>25\%$ in men and $>30\%$ in women excluded individuals from participating.

Table 1. Subject Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Men (n = 10)</th>
<th>Women (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>34 ± 2</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.80 ± 0.02</td>
<td>1.67 ± 0.03</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>79.5 ± 5.1</td>
<td>62.1 ± 2.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.2 ± 0.7</td>
<td>22.4 ± 0.5</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>16.4 ± 1.4</td>
<td>24.5 ± 1.6</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>66.4 ± 2.6</td>
<td>46.7 ± 2.0</td>
</tr>
<tr>
<td>$V\text{O}_2\text{max}$, ml/min</td>
<td>3.939 ± 162</td>
<td>2.797 ± 131</td>
</tr>
<tr>
<td>$V\text{O}_2\text{max}$, ml/kg-FFM$^{-1}$-min$^{-1}$</td>
<td>59.4 ± 1.3</td>
<td>60.2 ± 2.0</td>
</tr>
<tr>
<td>Lactate threshold, % $V\text{O}_2\text{max}$</td>
<td>61.4 ± 2.7</td>
<td>61.2 ± 2.1</td>
</tr>
<tr>
<td>Habitual exercise, min/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild (&lt;3.9 MET)</td>
<td>15 ± 4</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>Moderate (4–8.9 MET)</td>
<td>28 ± 4</td>
<td>29 ± 4</td>
</tr>
<tr>
<td>Intense (&gt;9 MET)</td>
<td>16 ± 4</td>
<td>17 ± 5</td>
</tr>
<tr>
<td>Habitual exercise, % type</td>
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<td></td>
</tr>
<tr>
<td>Aerobic</td>
<td>82 ± 7</td>
<td>87 ± 5</td>
</tr>
<tr>
<td>Interval training</td>
<td>2 ± 2</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>Resistance</td>
<td>16 ± 7</td>
<td>12 ± 6</td>
</tr>
<tr>
<td>Estradiol, pg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest day</td>
<td>26 ± 3</td>
<td>67 ± 14</td>
</tr>
<tr>
<td>Exercise day</td>
<td>25 ± 3</td>
<td>86 ± 19</td>
</tr>
<tr>
<td>Progesterone, pg/ml</td>
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<td></td>
</tr>
<tr>
<td>Rest day</td>
<td>1.1 ± 0.3</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Exercise day</td>
<td>0.9 ± 0.1</td>
<td>0.8 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. BMI, body mass index; $V\text{O}_2\text{max}$, maximal oxygen uptake; FFM, fat-free mass; MET, metabolic equivalents. *Significant difference between men and women, $P < 0.05$. †Significant difference between men and women, $P < 0.005$.

$RMR$. $RMR$ was measured by using indirect calorimetry via a metabolic cart system (model 2900, Sensormedics, Yorba Linda, CA). Subjects were tested in the morning after a 12-h fast. After 30 min of rest, a 15- to 20-min measurement of metabolic rate was made by using a ventilated canopy. Gas concentrations were measured in the air exiting the hood. Oxygen consumption and carbon dioxide production were used to calculate metabolic rate, as well as the oxidation of carbohydrate and fat after correction for protein oxidation. Protein oxidation was estimated by urinary nitrogen excretion corrected for changes in blood urea nitrogen concentrations. $RMR$ was used to determine caloric requirements of subjects during prestudy diet control to maintain energy balance.

$V\text{O}_2\text{max}$ and determination of lactate threshold. $V\text{O}_2\text{max}$ was determined by using a graded exercise test on a cycle ergometer (Lode Medical Technology, Groningen, The Netherlands). Subjects cycled at a constant cadence (70–90 rpm) while resistance was gradually increased until volitional exhaustion. To ensure that maximum effort had been achieved, two of the following criteria had to be fulfilled: whole body respiratory quotient $\geq 1.1$, maximum heart rate within 5% of the age-predicted maximum, and/or an increase in oxygen consumption in response to the final workload of $<2.0$ ml/kg body weight $^{-1}$-min$^{-1}$. For determination of lactate threshold, a retrograde dorsal hand intravenous catheter was placed and the heated hand technique used to arterialize the blood. At the end of each workload stage (every 2 min), 0.5 ml of blood was drawn, placed in 8% perchloric acid (1.5 ml), vortexed, and placed on ice. Lactate concentrations were measured and adjusted for dilution. The log of the lactate concentration was plotted against the log of the oxygen consumption at the time of sampling. A two-line regression model was used to describe the two phases of the lactate accumulation. The point of intersection of these two lines was taken as the lactate threshold (38).

Menstrual cycle monitoring and scheduling. Confirmation of menstrual cycle phase was based on estrogen and progesterone levels measured on the study day. At least 4 wk separated the 2 study days for all subjects, and the condition of the study day (exercise vs. rest) was randomly assigned. All subjects completed an exercise and a rest study day.

Prestudy diet and exercise control. Subjects were fed a controlled diet for 3 days before each study day. All food was prepared by the General Clinical Research Center (GCRC) diet kitchen at the University of Colorado Health Science Center, and subjects were required to consume breakfast in the GCRC with other food prepared to take away. No other food was permitted, and subjects were required to consume all the food given. The only optional part of the diet were two food modules ($840$ kJ each), one or both of which the subjects could eat if they were hungry. The diet composition was $25\%$ fat, $15\%$ protein, and $60\%$ carbohydrate and the initial energy intake was calculated at $1.6 – 1.75 \times RMR$ on the basis of the subject’s habitual activity level. Body weight was measured daily before the subjects ate breakfast, and the diet adjusted as needed to maintain weight stability. Table 2 shows dietary intake information for the subjects on each study day. Subjects followed their usual exercise routine for the first 2 days of the diet, and on the last day they refrained from exercise. No subject exercised within 36 h of the onset of the study protocol.

Study Protocol

Subjects spent the evening before each study day in the GCRC. The study began the next day between 0700 and 0730 (≈9 h fasted). An intravenous catheter was placed in an antecubital vein for infusion of isotopes, dextrose, and insulin. A sampling catheter was placed retrograde in a dorsal hand or distal wrist vein of the contralateral arm. For all blood samples, the heated hand technique was used to arterialize the blood. Background samples were drawn 30 min after sampling catheters were placed.
Resting day. Subjects remained semirecumbent throughout the study day. A primed (19 μmol/kg) and constant (0.22 μmol·kg⁻¹·min⁻¹) infusion of [6,6-H₂]glucose was started at 0800 for the determination of glucose kinetics. Resting measurements were made after 90 min of constant infusion to allow for equilibration of the tracer in the glucose pool. Indirect calorimetry was performed, and blood samples taken for resting substrate kinetics, hormones, and substrates during the final 30 min of the 120-min rest period.

Exercise day. After baseline blood draws and 30 min of rest, subjects were moved to the stationary bicycle (Lode Medical Technology). A primed (19 μmol/kg) and continuous (0.44 μmol·kg⁻¹·min⁻¹) infusion of [6,6-H₂]glucose commenced with the onset of exercise. Subjects cycled for 90 min at 85% of lactate threshold. Indirect calorimetry was performed throughout exercise, and workload adjusted to maintain the relative intensity. Four blood samples for substrate levels were taken during the final 30 min of the 90-min exercise. At the cessation of exercise, the isoence infusate rate was decreased to 0.22 μmol·kg⁻¹·min⁻¹ for the remainder of the study day.

Hyperinsulinemic-euglycemic clamp. One hour after blood sampling on the rest and exercise days, a one-stage 3-h hyperinsulinemic–euglycemic clamp commenced and continued throughout the remainder of the study day. A primed (35.2 μmol/kg) and continuous (0.44 μmol·kg⁻¹·min⁻¹) infusion of [6,6-H₂]glucose commenced with the onset of exercise. Subjects cycled for 90 min at 85% of lactate threshold. Indirect calorimetry was performed throughout exercise, and workload adjusted to maintain the relative intensity. Four blood samples for substrate levels were taken during the final 30 min of the 90-min exercise. At the cessation of exercise, the isoence infusate rate was decreased to 0.22 μmol·kg⁻¹·min⁻¹ for the remainder of the study day.

Values are means ± SE; n, no. of subjects. For each individual, the average of their 3-day dietary intake was used to calculate the group means. Weight change was estimated from the fasting body weight measured the morning of the first day of diet control – fasting body weight measured on the morning of the study day. *Significant difference between men and women, P < 0.05.

<table>
<thead>
<tr>
<th>Energy intake, MJ/day</th>
<th>Men (n = 10)</th>
<th>Women (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest study day</td>
<td>14.7 ± 0.8</td>
<td>11.4 ± 0.7</td>
</tr>
<tr>
<td>Exercise study day</td>
<td>14.8 ± 0.7</td>
<td>10.6 ± 0.3</td>
</tr>
<tr>
<td>Weight change, kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest study day</td>
<td>0.4 ± 0.2</td>
<td>0.0 ± 0.2</td>
</tr>
<tr>
<td>Exercise study day</td>
<td>0.2 ± 0.2</td>
<td>0.1 ± 0.2</td>
</tr>
</tbody>
</table>

Whole Body Substrate Oxidation

Whole body substrate oxidation during exercise and rest, as well as during the clamp on both study days, was measured by indirect calorimetry. Oxygen consumption and carbon dioxide production were used to calculate metabolic rate, as well as the oxidation of carbohydrate and fat after correction for protein oxidation (19). Protein oxidation was estimated by urinary nitrogen excretion corrected for changes in BUN concentrations.

Isotopic Enrichment

Glucose isotope enrichment was measured via gas chromatography-mass spectroscopy (GC-MS; GC model 5992 and MS model 5985B, Hewlett-Packard, Palo Alto, CA) by using the pentacetate derivative of glucose, with [U-13 C]glucose added as an internal standard. Plasma samples were deproteinized with 1 ml of iced ethanol, and the supernatant was lyophilized. Samples were derivatized with 200 μl of acetic anhydride-pyridine (1:1 vol/vol) and heated for 10 min at 60°C. Injector temperature of the GC-MS was set at 250°C, and initial oven temperature was set at 190°C. Oven temperature was increased 10°C/min until a final temperature of 300°C was achieved. Helium was used as the carrier gas, with electron impact ionization and selective ion monitoring of fragments with mass-to-charge ratio of 242-247.

Calculations

Rates of glucose appearance (Rₐ) and disappearance (Rₕ) and metabolic clearance rate (MCR) were calculated by using a modified Steele equation as described by Finegood et al. (12). The following equations were used for measurements made during rest or exercise (preclamp):

\[
R_a = \frac{F - V[(C_i + C_t)/2][E_2 - E_1](t_2 - t_1)}{(E_1 + E_2)/2} - F
\]

\[
R_d = R_a - V(C_i + C_t)(t_2 - t_1)
\]

\[
MCR = R_a(C_i + C_t)/2
\]

To account for the tracer in the “spiked” dextrose solution, the following equations were used for measurements made during the clamp:
Data Analysis

All data are presented as means ± SE. A repeated-measures ANOVA was used to compare gender differences during exercise vs. rest and pre- vs. during clamp. The three-way interaction (gender × day × clamp) was first examined. If the three-way interaction was nonsignificant, then two-way interactions with gender were considered (gender × day, gender × clamp). Finally, if these two-way interactions were nonsignificant, then the overall gender difference and within-gender effects (exercise vs. rest day, pre- vs. during clamp, and the day × clamp interaction) were examined. Data were analyzed in the same way after being adjusted for FFM. This was done to remove the confounding effect of FFM, which is known to be a major determinant of fuel metabolism and insulin action in and of itself. Significant differences were established where P < 0.05.

RESULTS

Demographics

Subject demographics are shown in Table 1. There were no differences in age between groups. However, men were significantly taller (P < 0.05), heavier, with greater body mass index, FFM, and absolute VO2 max (P < 0.005). Women had a higher percentage of body fat (P < 0.005). When VO2 max was adjusted for FFM, no differences were observed (59.4 ± 1.3 vs. 60 ± 2.0 ml·kg FFM⁻¹·min⁻¹, men vs. women, respectively). Furthermore, men and women were very closely matched in terms of habitual activity. There were no significant changes in body weight changes over the 3 days before each study (Table 2). Energy and nutrient intakes were similar for the 3 days preceding the control or exercise day.

Circulating Substrate and Hormone Concentrations

There was no significant change in catecholamine or lactate concentrations during the clamp on the rest day (Table 3), as well as no difference in catecholamine and lactate concentrations during the clamp between study days. As expected, preclamp epinephrine, norepinephrine, and lactate concentrations were all significantly higher on the exercise compared with rest day, and the clamp values on the exercise day. No gender differences were observed in these hormone and substrate levels during rest, exercise, or the clamp. Glucagon concentrations decreased significantly during the clamp on both study days. Exercise resulted in significantly higher glucagon concentrations in men compared with women, as well as in men compared with preclamp values on the rest day. No gender differences in glucagon concentration were noted during the clamp on either day.

Energy Expenditure and Substrate Oxidation

Energy expenditure (EE) increased in women during the clamp on either study day (P < 0.001). Exercise EE during exercise was greater than during rest or on either study day (P < 0.01). Absolute EE, above RMR, during the 90-min exercise session was 25.2 ± 3 kcal in women and 83.7 ± 38 kcal in men. The decline in EE postexercise was similar between the genders.

The relative contribution of carbohydrate and fat to total EE on the 2 study days is represented by the nonprotein respiratory...
Nonprotein respiratory quotient (NPRQ), as depicted in Fig. 1. On the rest day, NPRQ during the hyperinsulinemic-euglycemic clamp significantly increased in both genders, indicating an increase in carbohydrate and decrease in lipid utilization. In contrast, NPRQ did not change during the clamp from preclamp values on the exercise day and tended to decrease in men (P = 0.08).

Divergent trends between study days resulted in an overall tendency for the NPRQ to be lower on the exercise vs. rest day (P = 0.15 men and P = 0.06 women). This implies that exercise attenuates insulin-stimulated carbohydrate oxidation.

Although absolute rates of carbohydrate, fat, and protein oxidation were greater in men than women at all times, values were not different when adjusted for differences in FFM.

Hyperinsulinemic-Euglycemic Clamp

Insulin concentrations during the clamp were similar in men and women and were not different between study days (Table 3). On the rest day, men were clamped at a higher glucose level than the women (93 ± 2 vs. 85 ± 1 mg/dl, men vs. women; P < 0.01). Otherwise, no differences were seen in glucose concentrations between the genders across the study days. No difference between genders was seen in glucose infusion rate (GIR) during the final 30 min of the clamp on the rest day (1.33 ± 0.004 vs. 1.23 ± 0.006 mg glucose·kg FFM\(^{-1}\)·min\(^{-1}\), women vs. men). On the exercise day, women had a significantly higher GIR than the men (1.35 ± 0.008 vs. 1.06 ± 0.005 mg glucose·kg FFM\(^{-1}\)·min\(^{-1}\), women vs. men, P = 0.01) because of a nonsignificant increase in the GIR in women and a relative decrease in the GIR in men (P = 0.04) compared with their rest day (Fig. 2).

Glucose Kinetics

Glucose enrichment was stable over the period of measurement (mean change <4.4%, P = not significant) during rest, exercise, and clamp periods. The hyperinsulinemic-euglycemic clamp, as well as exercise, significantly and independently affected parameters of glucose metabolism (Figs. 3 and 4). On rest and exercise days, the clamp significantly increased peripheral glucose R\(_d\), glucose MCR, and NOGD, with a corresponding significant decrease in glucose R\(_a\) (P < 0.01). As would be expected, preclamp measures of glucose R\(_a\), glucose R\(_d\), and MCR were significantly increased during exercise.

Fig. 1. Nonprotein respiratory quotient (NPRQ) before and at the end of the hyperinsulinemic-euglycemic clamp in women (A) and men (B). Preclamp values on the exercise day were measured during the final 30 min of the 90-min cycling bout at 85% of lactate threshold. During clamp, measurements of energy expenditure were made during the final 60 min of the 180-min clamp.*Difference from preclamp to clamp NPRQ (P < 0.01). NPRQ tended to decrease in men from preclamp to clamp on the exercise day (P = 0.08).

Fig. 2. Glucose infusion rates (GIR) in men and women on rest (A) and exercise (B) days during the hyperinsulinemic-euglycemic clamp. Blood glucose concentration was measured every 5 min during the clamp, and the GIR was adjusted to maintain euglycemia (blood glucose ~90 mg/dl). No difference between genders was seen in GIR on the rest day. On the exercise day, women had a significantly higher GIR than the men [1.35 ± 0.008 vs. 1.06 ± 0.005 mg glucose·kg FFM\(^{-1}\)·min\(^{-1}\), women vs. men; P = 0.01] because of a nonsignificant increase in the GIR in women and a relative decrease in the GIR in men (P = 0.04) compared with their rest day. FFM, fat-free mass.
exercise vs. rest day (P < 0.01) suggesting more of the glucose was directed toward glycogen storage.

Less expected, men and women demonstrated divergent responses to exercise and/or insulin with respect to glucose $R_d$ and NOGD. Glucose $R_d$ was higher during the clamp on the exercise day in the women compared with the men ($P = 0.05$; Fig. 3). Glucose $R_d$ also tended to be higher in women across the entire exercise day ($P = 0.06$), not just during the clamp. NOGD and MCR, in part, a function of $R_d$, were also higher (or tended to be higher) in the women vs. the men on the exercise

compared with rest ($P < 0.01$). Conversely, NOGD during exercise was negative compared with rest ($P < 0.01$) because of glycogen utilization. Interestingly, glucose $R_a$ during the clamp on the exercise day was higher in both genders compared with the rest day ($P < 0.01$), suggesting a lesser suppression of endogenous glucose production by insulin post-exercise. However, we did not observe any significant overall effect of prior exercise on glucose $R_d$ or MCR during the clamp. Nevertheless, during-clamp NOGD was greater on the

Fig. 3. Glucose rates of appearance ($R_a$) on the rest (A) and exercise (B) days as well as the rates of disappearance ($R_d$) on rest (C) and exercise (D) days are depicted in men and women. On rest and exercise days, the clamp significantly increased peripheral glucose $R_d$ with a corresponding significant decrease in glucose $R_a$ (*$P < 0.01$). As would be expected, preclamp measures of glucose $R_a$ and $R_d$ were significantly increased during exercise compared with rest ($P < 0.01$). Glucose $R_d$ was higher during the clamp on the exercise day in the women compared with the men (***$P = 0.05$).

Fig. 4. Metabolic clearance rate (MCR) on the rest (A) and exercise (B) days, as well as nonoxidative glucose disposal (NOGD) rate on rest (C) and exercise (D) days are depicted in men and women. On rest and exercise days, the clamp significantly increased MCR and NOGD to a similar degree in men and women (*$P < 0.01$). On the rest day, preclamp glucose $R_a$ and $R_d$ were signifi
cantly increased during exercise compared with rest ($P < 0.01$). Glucose $R_d$ was higher during the clamp on the exercise day in the women compared with the men (***$P = 0.05$).
day ($P = 0.03$ for gender difference in NOGD, $P = 0.07$ for gender difference in MCR). There was no gender difference in glucose $Ra$ at any time point. No gender difference in any parameter of glucose metabolism was observed during the clamp on the rest day. On the rest day, there was a tendency, however, for preclamp glucose $R_{G}$ to be higher in women vs. men ($P = 0.06$), and MCR was significantly greater ($P = 0.04$).

**DISCUSSION**

This study investigated gender differences in insulin action and glucose metabolism after an acute bout of exercise. The major finding of this investigation was that acute exercise resulted in significantly lower whole body insulin action in men vs. women when measured 3–4 h postexercise. This was due to a relative decrease in the stimulation of peripheral glucose uptake by insulin in men vs. women, whereas there was no gender difference in suppression of endogenous glucose production. Taken together, these findings suggest gender differences in postexercise insulin action exist in peripheral tissues, most likely in skeletal muscle.

Data from the present investigation confirm previous observations that men and women oxidize a similar proportion of CHO and fat at rest (18). Furthermore, these data also suggest similar proportions of macronutrients were oxidized by men and women during exercise. The latter finding is in disagreement with some (7, 35), but not all (30). Nevertheless, perhaps the most striking observation was the effect of exercise on whole body substrate oxidation during the clamp. The data demonstrate a similar increase in NPRQ during the clamp in men and women over the course of the rest day. Interestingly, antecedent exercise significantly blunted the increase in NPRQ observed during the clamp on the rest day in both genders. Depletion of muscle glycogen during exercise, and the requirement for repletion postexercise, could be one factor explaining the blunting of NPRQ seen during the clamp after exercise (16). To our knowledge, these data are novel, suggesting that the effect of exercise supersedes that of hyperinsulinemia on substrate use during the first 3–4 h of recovery.

The predominant paradigm is simply that exercise improves insulin action. DeFronzo et al. (9) were among the first to describe the synergistic effect of exercise and insulin on peripheral glucose uptake. Since then, exercise has repeatedly been shown to improve insulin sensitivity in the 4–48 h after a single bout (27, 28, 37). In the present study, however, we observed no overall increase in insulin action measured in the 3–4 h postexercise despite the ability of moderate-dose insulin to suppress endogenous glucose production. In review of the literature, whole body insulin action during the 0–4 h after an exercise bout has been shown to increase (37), not change (5), or decrease (21, 31). Many of the conflicting findings can be attributed to differences in study design (37) or research methodology (5, 21, 31). Most recently, Rose et al. (31) demonstrated impairment in glucose tolerance in men in the immediate postexercise period using a double-tracer technique. Taken together, this suggests that the effect of exercise on insulin action may be a continuum with a decrement, or no change, immediately postexercise and enhanced action farther from the bout. It also raises the question of whether the specific mechanisms explaining changes in insulin action postexercise differ across the postexercise time course.

Our data suggest gender may contribute to differences in insulin action immediately postexercise. Others have reported greater insulin sensitivity in women compared with men at rest (39), during exercise (4), and after a meal (29). In addition, antecedent exercise may have divergent effects between men and women in terms of the counterregulatory response to hypoglycemia (14). Cumulatively, these data lend credence to increased postexercise insulin action in women vs. men observed in the present investigation, as well as possible subtle baseline differences on the rest day. Our study specifically identifies the peripheral tissues (most likely skeletal muscle) as the primary site(s) for the observed gender differences.

The significantly lower peripheral insulin action observed in men vs. women postexercise in the present study is in contrast to what was originally hypothesized. It had been hypothesized that men would have a greater improvement in insulin action postexercise and that this would be due to a greater increase in NOGD due to a higher exercise depletion of muscle glycogen. Although a more negative NOGD during exercise was observed in men vs. women, suggesting greater glycogen utilization, a less positive NOGD in men was observed during the postexercise clamp, suggesting lower glycogen resynthesis. The data of the present study, therefore, imply that the level of glycogen depletion is not a primary determinant of the immediate postexercise glucose $R_{G}$ and NOGD under hyperinsulinemic conditions. To more directly address this issue, however, measurement of muscle glycogen is required. It could also be speculated that NOGD in women was directed toward glycogen resynthesis, as well as other fats, during the postexercise clamp. Other forms of NOGD include increased glycolysis and lactate release and increased utilization of glucose for glycerol synthesis and triglyceride synthesis in adipose tissue and muscle. Interestingly, we did observe a tendency for a higher lactate concentration in the exercise day clamp in women vs. men. Nonetheless, these possibilities require further investigation.

Factors that could be contributing to the lower peripheral insulin action during the postexercise clamp in men vs. women include differences in circulating substrates, and/or hormones, known to affect insulin action. However, we did not observe any significant gender differences in clamp concentrations of lactate or catecholamines. On each study day, clamp FFA and glycerol levels were significantly suppressed in both genders, but these levels were significantly higher in men vs. women on the exercise day (25). Nevertheless, it is unlikely that such a modest elevation in FFA concentration impaired peripheral insulin action in men because the absolute concentrations were extremely low ($73 \pm 3$ vs $60 \pm 2$ μequiv/l in men and women, respectively). Additionally, it could be speculated that a greater propensity to oxidize lipid during the postexercise clamp in men, as evidenced by their tendency for a lower NPRQ vs. women, could contribute to a decrease in glucose $R_{G}$. However, key enzymes regulating glucose uptake and storage in muscle, including glycolgen synthase or hexokinase, have not been shown to differ between men and women postexercise (30, 36) nor have gender differences in GLUT4 translocation been observed after acute or chronic exercise (20, 22). None of these factors, however, have been measured under hyperinsulinemic conditions postexercise, the more relevant situation for com-
comparison to the present study. In summary, the exact mechanism(s) responsible for the differences in peripheral insulin action observed in the present investigation were not identified and require further investigation.

Skeletal muscle metabolism during exercise is known to be influenced by the sex steroids, with estrogen and progesterone having opposing effects on muscle glycogen utilization (10). Pharmacological estrogen supplementation decreases glucose utilization during exercise (6, 32). Normal fluctuations in the sex steroid over the course of the menstrual cycle appear to have only subtle effects on exercise substrate use. Such effects are typically dwarfed by nutritional state as well as the intensity and duration of the exercise bout (34). Therefore, in our investigation, prestudy diet and exercise were rigorously controlled, and subjects were studied in the fasting state. Furthermore, women were studied in the follicular phase of the menstrual cycle when circulating estrogen and progesterone are low, compared with the luteal phase. Thus effects of sex steroids on metabolism were minimized, while potential confounders of prestudy diet and exercise were removed to allow determination of underlying gender differences in insulin action.

Certain limitations exist with respect to the present investigation that warrant further discussion. For example, a small sample size led to borderline $P$ values for three-way interactions for $R_d$, MCR, and NOGD. The observed trends are provocative, but should be interpreted with caution. Statistical analyses in the present investigation were adjusted for multiple comparisons, and erroneous results are unlikely as the isotopic, clamp, and calorimetry data all agree. It is relevant to point out that the insulin concentrations were lower than those generally used in studies performing hyperinsulinemic-euglycemic clamps. This was, in part, by design because a 30 mU·m⁻²·min⁻¹ insulin infusion was used (compared with the standard 40 mU·m⁻²·min⁻¹ dose) to mimic physiological hyperinsulinemia. The insulin infusion setup, in part, precluded us from reaching expected insulin concentrations in the blood (23) and may have skewed the results by allowing residual endogenous insulin production. Nonetheless, the total insulin exposure over the 3-h clamp would more resemble the insulin incremental area under the curve during the postprandial period than the standard insulin infusion dose. Therefore, our findings may be more physiologically relevant to postprandial metabolism in men and women. Lastly, the men were inadvertently clamped at a higher glucose concentration than the women on the rest day. This may have obscured subtle gender differences in insulin action on the rest day, such as those observed by others (24, 39). Nevertheless, men and women were clamped at comparable glucose concentrations on the exercise day, thus not confounding the differences observed postexercise.

In conclusion, this study investigated gender differences in the effect of an acute bout of exercise on insulin action and glucose metabolism. Interestingly, we observed that exercise abolishes the usual increase in NPQR observed during a hyperinsulinemic-euglycemic clamp and also decreased insulin’s ability to suppress endogenous glucose production in both genders. The major finding of this investigation was that acute exercise resulted in significantly lower whole body insulin action in men vs. women when measured 3–4 h after the exercise bout. Decreased insulin action was due to a decrease in the stimulation of peripheral glucose uptake by insulin in men vs. women, whereas there was no gender difference in the suppression of endogenous glucose production. Together, these data imply gender differences in insulin action postexercise exist in peripheral tissues, but not liver. Exact mechanisms underlying the observed differences were not identified. An examination of the time course of changes in postexercise insulin action, and whether these gender differences persist, is warranted. In addition, elucidating the complex chronology of the mechanisms underlying time course changes in insulin action would be important area of future study.

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GENDER DIFFERENCES IN INSULIN ACTION