Effects of treadmill exercise to exhaustion on the insulin response to hyperglycemia in untrained men

J. P. KIRWAN, R. E. BOUREY, W. M. KOHRT, M. A. STATEN, AND J. O. HOLLOSZY
Section of Applied Physiology, Department of Internal Medicine, Washington University
School of Medicine, St. Louis, Missouri 63110

KIRWAN, J. P., R. E. BOUREY, W. M. KOHRT, M. A. STATEN, AND J. O. HOLLOSZY. Effects of treadmill exercise to exhaustion on the insulin response to hyperglycemia in untrained men. J. Appl. Physiol. 70(1): 246-250, 1991.—The effects of a single bout of exercise to exhaustion on pancreatic insulin secretion were determined in seven untrained men by use of a 3-h hyperglycemic clamp with plasma glucose maintained at 180 mg/100 ml. Clamps were performed either 12 h after an intermittent treadmill run at ~77% maximum O₂ consumption or without prior exercise. Arterialized blood samples for glucose, insulin, and C-peptide determination were obtained from a heated hand vein. The peak insulin response during the early phase (0–10 min) of the postexercise clamp was higher (81 ± 8 vs. 59 ± 9 μU/ml; P < 0.05) than in the nonexercise clamp. Incremental areas under the insulin (376 ± 33 vs. 246 ± 51 μU·min⁻¹·min) and C-peptide (17 ± 2 vs. 12 ± 1 ng·min⁻¹·min) curves were greater (P < 0.05) during the early phase of the postexercise clamp. No differences were observed in either insulin concentrations or whole body glucose disposal during the late phase (15-180 min). Area under the C-peptide curve was greater during the late phase of the postexercise clamp (560 ± 53 vs. 536 ± 75 ng·min⁻¹·min, P < 0.05). The exercise bout induced muscle soreness and caused an elevation in plasma creatine kinase activity (142 ± 32 vs. 206 ± 31 IU/l; P < 0.05) before the postexercise clamp. We conclude that in untrained men a bout of running to exhaustion increased pancreatic β-cell insulin secretion during the early phase of the hyperglycemic clamp. Increased insulin secretion during the late phase of the clamp appeared to be compensated by increased insulin clearance.

It has been suggested that the last bout of exercise may be responsible for much of the improvement in insulin action that has previously been attributed to training (9, 13). Heath et al. (9) found that in trained subjects, the reduced insulin response to an oral glucose load was lost within 10 days of inactivity but was restored after a single bout of exercise. However, Miikens et al. (23) recently reported that a single bout of moderate exercise (60 min at 150 W) did not affect the insulin response of untrained subjects during a hyperglycemic clamp. The reduced insulin response of trained individuals may possibly be related to the intensity and duration of the last exercise bout. Therefore the purpose of this study was to measure insulin secretion in untrained subjects by use of the hyperglycemic clamp procedure performed either without prior exercise or 12 h after a single prolonged bout of intense exercise.

METHODS

Subjects. Seven untrained men volunteered as subjects. Selected physical characteristics are shown in Table 1. This study was approved by the Human Studies Committee of Washington University. All subjects signed an informed consent in accordance with the University guidelines for the protection of human subjects. None of the subjects was on medication, and all had a normal response to a 75-g oral glucose tolerance test. Exercise. Maximal oxygen consumption (VO₂max) was determined before the hyperglycemic clamp studies by use of an incremental treadmill protocol. Gas volumes were measured by a dry gas meter (PARKINSON-COWAN). O₂ and CO₂ concentrations were measured on an electrochemical O₂ analyzer (Applied Electrochemistry S-3A) and infrared CO₂ analyzer (Beckman LB-2), respectively. Heart rate was monitored by use of a modified V5 tracing. Skinfold measurements were obtained at the triceps, subscapular, pectoral, umbilical, suprailliac, front thigh, and midaxillary sites for estimation of percent body fat according to the equation of Jackson and Pollock (11).

On the evening before the postexercise hyperglycemic clamp, the subjects reported to the laboratory, where they performed a treadmill run to exhaustion. The run was performed at ~77% VO₂max by use of an intermittent protocol consisting of 30-, 25-, 20-, 15-, 10-, and 5-min duration bouts with a 5-min rest between bouts. Subjects who completed all six bouts continued to per-
form 5-min intermittent bouts until they could no longer complete the full 5 min. Heart rate and \( V_O_2 \) consumption measurements were obtained during each bout. After the run, the subjects were admitted to the Clinical Research Center of the Washington University Medical Center. The evening before the nonexercise clamp, subjects reported directly to the Clinical Research Center. On both occasions, subjects ate a similar meal (~730 kcal, 102 g carbohydrate) ~12 h before the clamp.

**Hyperglycemic clamp.** The hyperglycemic clamps, performed according to the procedure described by De Fronzo et al. (5), were randomized and conducted ~10 days apart. After an overnight fast, the subjects voided, were weighed, and then remained supine throughout the procedure. A polyethylene catheter was inserted into an antecubital vein for glucose infusion (20% dextrose). A second catheter was inserted retrograde into a dorsal hand vein that was warmed in a heated box (75 °C) for sampling of arterialized venous blood (22).

Three baseline blood samples were drawn at 5-min intervals for the determination of plasma glucose, insulin, and C-peptide concentrations. One sample was also assayed for creatine kinase activity according to Szasz et al. (28) by the use of a Sigma test kit (Sigma Chemical, St. Louis, MO). Plasma glucose concentration was then raised to 180 mg/100 ml within 15 min by use of a primed infusion and was maintained at that level for a further 165 min by a variable-speed infusion pump (Harvard Apparatus, Millis, MA). Plasma glucose concentration was measured at 5-min intervals by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Blood samples for determination of plasma insulin and C-peptide concentrations were drawn at 2-min intervals for the first 10 min and at 15-min intervals for the remainder of the clamp and were dispensed into chilled tubes containing Trasylol (aprotinin; FBA Pharmaceuticals, New York) and EDTA. The samples were centrifuged and stored at -20 °C for subsequent analysis. Samples were analyzed in duplicate by a double antibody radioimmunoassay for both insulin (25) and C-peptide (16).

At the end of 180 min, the glucose infusion was stopped, and the subjects remained supine for a further 20 min. A urine sample was then obtained for the determination of C-peptide and glucose concentration.

**Calculations and statistics.** Areas under the early-phase (0–10 min) and late-phase (15–180 min) insulin and C-peptide curves were determined from the trapezoidal model used previously by Seals et al. (27). Glucose disposal rate was calculated for each 30-min interval as described by King et al. (13). Differences between the dependent variables were examined by analyses of variance with and without repeated measures where appropriate. Because we were interested in determining whether the exercise bout increased or decreased the dependent variables, two-tailed testing was used. Specific mean differences were identified by a Newman-Keuls post hoc test. All values are expressed as means ± SE. The acceptable level for statistical significance was set at 0.05.

**RESULTS**

**Exercise.** The treadmill run to exhaustion was performed at ~77% \( V_O_2_{\text{max}} \), which elicited a mean heart rate of 174 ± 4 beats/min. The mean treadmill running time was 80.7 ± 6.0 min. At the end of the exercise bout, the subjects complained of leg fatigue and were unable to complete a full 5-min bout at the required intensity. The morning after exercise, the subjects complained of soreness in both the calf and thigh muscles. Plasma creatine kinase levels were significantly elevated (P < 0.05) before the postexercise clamp (305 ± 31 vs. 142 ± 32 IU/l). No differences were observed in hemoglobin (14.8 ± 0.5 and 14.9 ± 0.6 g/dl) or hematocrit (44.3 ± 1.4 and 44.4 ± 1.2%) for the clamps without exercise and postexercise, respectively.

**Plasma glucose.** Plasma glucose levels during the hyperglycemic clamps were not different between the postexercise and nonexercise clamps (Table 2). During the last 165 min of hyperglycemia, plasma glucose concentration averaged 178 ± 1 and 179 ± 1 mg/100 ml for the postexercise and nonexercise clamps, respectively. Coefficients of variation were 3.8 ± 0.4 postexercise and 3.4 ± 0.4% nonexercise. Plasma glucose levels at 0, 2, and 4 min of the postexercise clamp (90 ± 1, 146 ± 3, and 164 ± 5 mg/100 ml, respectively) were similar to those of the nonexercise clamp (96 ± 2, 144 ± 4, and 164 ± 2 mg/100 ml, respectively). Whole body glucose disposal, calculated at 30-min intervals, was not significantly different between clamps (Table 2).

**Early-phase insulin and C-peptide.** During the early phase (0–10 min) of the clamps, insulin levels (Fig. 1) peaked at 4 min during both clamps and were higher

<table>
<thead>
<tr>
<th>Value</th>
<th>23±4.2</th>
<th>172±6±1.7</th>
<th>72±2.3</th>
<th>24±1±8.3</th>
<th>14±1±8.9</th>
<th>49±1±6.5</th>
</tr>
</thead>
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<tr>
<td><strong>TABLE 1. Subject characteristics</strong></td>
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Values are means ± SE; n = 7 men.

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**TABLE 2. Plasma glucose concentrations and glucose disposal rates during hyperglycemic clamps performed either 12 h after exercise or without prior exercise**

<table>
<thead>
<tr>
<th>0-30</th>
<th>30-60</th>
<th>60-90</th>
<th>90-120</th>
<th>120-150</th>
<th>150-180</th>
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<tr>
<td><strong>Plasma glucose, mg/100 ml</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>PEx</td>
<td>15±5</td>
<td>18±1</td>
<td>176±2</td>
<td>176±2</td>
<td>17±1</td>
</tr>
<tr>
<td>NEx</td>
<td>15±5</td>
<td>18±1</td>
<td>176±2</td>
<td>180±2</td>
<td>176±2</td>
</tr>
<tr>
<td><strong>Glucose disposal, mg kg⁻¹ min⁻¹</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEx</td>
<td>4.4±0.5</td>
<td>4.6±0.7</td>
<td>5.8±0.9</td>
<td>7.9±0.9</td>
<td>10.4±1.2</td>
</tr>
<tr>
<td>NEx</td>
<td>5.1±0.6</td>
<td>5.3±0.7</td>
<td>6.3±0.9</td>
<td>7.9±1.0</td>
<td>9.5±1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 men. PEx, 12 h postexercise; NEx, no prior exercise.
during the postexercise clamp (81 ± 8 vs. 59 ± 9 μU/ml; P < 0.05). The incremental area under the insulin curve was also significantly greater during the postexercise clamp (376 ± 33 vs. 245 ± 51 μU·mL⁻¹·min⁻¹).

C-peptide levels during the early phase were highest at 4 min of the nonexercise clamp (3.2 ± 0.4 ng/ml) and at 6 min of the postexercise clamp (3.6 ± 0.4 ng/ml) but were not significantly different (Fig. 2). However, the incremental area under the C-peptide curve (Table 3) was greater (P < 0.05) during the postexercise clamp (17 ± 2 vs. 12 ± 1 ng·mL⁻¹·min⁻¹).

Late-phase insulin and C-peptide. Insulin levels (Fig. 1) increased continuously throughout the late phase (15-180 min) of both clamps. There were no differences in insulin concentration or incremental area under the insulin curve between the two clamps. Plasma C-peptide levels also increased continuously throughout the late phase of both clamps (Fig. 2). Incremental area under the C-peptide curve (Table 3) was higher (P < 0.05) during the postexercise clamp (650 ± 53 and 536 ± 76 ng·mL⁻¹·min postexercise and nonexercise, respectively). The insulin-to-C-peptide area ratio was reduced during the postexercise clamp; however, the difference

<table>
<thead>
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<th>Early Phase</th>
<th>Late Phase</th>
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<tbody>
<tr>
<td>NEx</td>
<td>PEx</td>
</tr>
<tr>
<td>Insulin area, μU·mL⁻¹·min⁻¹</td>
<td>245±51</td>
</tr>
<tr>
<td>C-peptide area, ng·mL⁻¹·min⁻¹</td>
<td>12±1</td>
</tr>
<tr>
<td>Insulin-to-C-peptide molar ratio</td>
<td>0.37±0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 men. Early phase, 0-10 min; late phase, 15-180 min; NEx, no prior exercise; PEx, 12 h postexercise. *P < 0.05 between clamps.
was not statistically significant (Table 3). Urinary C-peptide clearance rate was not significantly different between clamps (23.6 ± 2.6 and 14.5 ± 3.4 ml/min for nonexercise and postexercise, respectively; P = 0.09).

**DISCUSSION**

Using the hyperglycemic clamp procedure, we found that an acute bout of exercise to exhaustion increased pancreatic insulin secretion during the early phase (0-10 min) of the hyperglycemic stimulus and appeared to increase insulin secretion and clearance during the late phase (15-180 min). This finding was unexpected in light of previous studies using the hyperglycemic clamp that showed no change in insulin secretion in untrained subjects after moderate exercise (23) and decreased insulin secretion in trained compared with untrained subjects (14). Furthermore, Bogardus et al. (3) have shown enhanced insulin action in active subjects after a single bout of glycogen-depleting exercise by use of the hyperinsulinemic-euglycemic clamp.

Compared with oral and intravenous glucose tolerance tests, the hyperglycemic clamp offers the advantage of control over the plasma glucose levels and consequently the glycemic stimulus presented to the β-cell. When glucose concentration is rapidly increased and maintained at hyperglycemic levels, insulin secretion from the β-cell is biphasic (4). Secretion during the early phase (0-10 min) is believed to arise from a pool of insulin secretory granules located at the periphery of the β-cell. The increasing insulin concentration through the late phase (15-180 min) is thought to partially represent de novo synthesis of insulin by the pancreas (4). In the present study, insulin secretion was greater during the early phase of the postexercise clamp compared with the nonexercise clamp. This may represent an expansion of the insulin pool located at the periphery of the cell. Mikines et al. (23) recently examined the effects of an acute bout of cycling exercise (60 min, 150 W) on insulin secretion in untrained subjects by use of a four-stage sequential hyperglycemic clamp (7, 11, 20, and 35 mM). In contrast to the findings of the present study, they did not observe any effect of acute exercise on the insulin response to the glucose stimulus. However, the marked difference in exercise intensity, total work, mode of exercise, and control of food intake between the two studies may help explain the different findings.

Insulin and C-peptide are secreted into the portal circulation by the pancreas in equimolar amounts (10). Unlike insulin, C-peptide is not extracted by the liver and has a constant metabolic clearance rate within the range of plasma levels that prevailed in this study (20). Consequently, plasma C-peptide levels provide a good index of pancreatic insulin secretion. Thus the augmented C-peptide secretion during both the early and late phase of the postexercise clamp may be used as evidence that insulin secretion was increased as a result of the exercise bout.

The increase in plasma C-peptide levels without a concomitant increase in insulin during the late phase suggests either an increased insulin clearance or a decreased C-peptide clearance. Despite an ~38% difference in clearance between the two clamps, urinary C-peptide clearance was not significantly impaired during the postexercise clamp. Although this finding is supported by the data of Blix et al. (2), who reported that urinary C-peptide clearance was not affected 9-12 h after exercise (90 min treadmill running at 75% of maximal heart rate), the magnitude of the difference appears too large to totally disregard its possible physiological significance.

Enhanced insulin clearance has been reported in endurance-trained athletes after intravenous glucose administration (30) and in physically trained rats after insulin infusion (31). However, neither King et al. (13) nor Mikines et al. (24) observed any change in insulin clearance during euglycemic-hyperinsulinemic clamps in trained subjects after 14 days of inactivity (13) or in untrained subjects after a single bout of exercise (24). Insulin clearance cannot be determined directly from the present study. However, the increased C-peptide levels in conjunction with unchanged insulin levels tend to support the possibility that the acute bout of exercise may have enhanced insulin clearance.

The present study differs from previous investigations of the insulin response after exercise in that running to exhaustion was the mode of exercise rather than cycling (23). The eccentric component of running is considerably greater than that of cycling and is therefore more likely to induce muscle soreness. On the morning of the postexercise clamp, the subjects complained of muscle soreness and stiffness, and plasma creatine kinase levels were significantly elevated. Elevated creatine kinase levels are associated with muscle damage and increased membrane permeability (15, 26). It has been noted that exercise-induced muscle damage may lead to impaired insulin action (17). Our observations suggest that insulin resistance may have developed after the exercise bout because glucose disposal rates were similar for the two clamps despite a greater insulin secretion during the postexercise clamp. Although we did not directly measure changes in insulin sensitivity, recent data suggest that increases in the acute insulin response to glucose (which is similar to our early-phase insulin response) are an early marker of insulin resistance (8, 12).

The mechanism by which insulin secretion was increased after running to exhaustion is not readily apparent from these data. Calcium-induced stimulation of the arachidonic cascade via the lipoxygenase-regulated pathway has been reported to be involved in sarcosomal damage (6). Turk et al. (29) have found that 12-lipoxygenase products also stimulate insulin secretion in isolated human pancreatic islets. Some neural or hormonal changes possibly occur after extreme exertion, which stimulates arachidonate metabolism, leading to increased sarcolemmal permeability in skeletal muscle and increased insulin secretion from the pancreatic islets.

In conclusion, in untrained subjects, exercise to exhaustion increased insulin secretion from the pancreatic β-cell during the early phase of the hyperglycemic clamp and appeared to increase insulin secretion and clearance during the late phase. The exercise bout also
induced muscle damage as evidenced by elevated plasma creatine kinase levels and complaints of muscle soreness. Further investigation is required to determine whether there is any relationship between the increased insulin secretion and muscle damage.

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REFERENCES