Effect of exercise on glucose disposal: response to a maximal insulin stimulus

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Although the effect of exercise on insulin sensitivity seems well documented, the picture regarding the interactions between exercise and a maximal insulin stimulus is less clear. As reviewed above, in rat skeletal muscle there is initially an additive effect of exercise and a maximal insulin stimulus, which is followed by a transient increase in insulin responsiveness that is gone within 18 h and prevented by carbohydrate feeding (4). Whether similar phenomena occur at the whole-body level in humans is of considerable theoretical interest relative to the mechanisms that regulate and limit exercise and insulin-stimulated glucose disposal.

The primary purpose of the present study was to determine whether, as in rat muscle, exercise results in an increased rate of glucose disposal (GDR) in humans during and shortly after exercise even in the face of a maximal insulin stimulus. A secondary goal was to try to explain an apparent disagreement regarding whether a persistent increase in insulin responsiveness occurs in normal human subjects after exercise. Mikines et al. have reported that insulin responsiveness is increased 48 h after exercise in young untrained men (15) and 15 h after exercise in trained men (16). In contrast, Devlin and Horton (8) found no significant effect of a bout of exercise performed 16-18 h earlier on insulin responsiveness in young lean men. We also found no increase in insulin responsiveness in young athletes studied ~16 h after exercise compared with sedentary lean young men (12). Mikines et al. (15, 16), however, only raised plasma insulin concentration into the 350- to 450-μU/ml range and assumed that this was sufficient to give a maximal effect, whereas others (2, 8, 12) have generally raised plasma insulin to ~2,000 μU/ml. This raised the possibility that Mikines et al. (15, 16) might have used a submaximal insulin stimulus and observed the exercise-induced increase in insulin sensitivity rather than an increase in responsiveness. In this context, we determined whether a plasma insulin concentration similar to that studied by Mikines et al. produces a maximal GDR.

METHODS

Subjects and preliminary testing. Fourteen healthy physically active young men gave their informed consent before participating in this study, which was approved by the Human Studies Committee of Washington University. Means for age, height, and weight were 20 ± 2...
(SE) yr, 179 ± 7 cm, and 76.0 ± 2.9 kg, respectively. Percent body fat, estimated from body density (3, 21), averaged 15.5 ± 1.8%.

Peak $O_2$ uptake ($V_{O_2}$) was measured during a cycling exercise test on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands). After a 10-min warm-up period at ~175 W, the work rate was increased 50 W every 2 min until the subject was unable to continue. Inspiratory flow rate was measured continuously by a Parkinson Cowan CD 4 spirometer, and expired air was continuously sampled from a 4-liter mixing chamber and analyzed for $O_2$ content with a zirconium cell analyzer (Applied Electrochemistry, Sunnyvale, CA) and for $CO_2$ content with an infrared analyzer (Beckman Instruments, Fullerton, CA). The results were analyzed by an on-line computer. The leveling-off criterion for $V_{O_2}$ was used to establish that peak $V_{O_2}$ had been attained, i.e., no increase in $V_{O_2}$ with further increases in work rate. The mean peak $V_{O_2}$ was 52 ± 2 ml·kg$^{-1}$·min$^{-1}$ (range 45-64 ml·kg$^{-1}$·min$^{-1}$).

**General procedures.** Subjects were studied by use of the euglycemic hyperinsulinemic clamp procedure (7). For 4 days before each experiment, the men refrained from exercise and consumed at least 200 g carbohydrate/day. Subjects reported to the General Clinical Research Center at Washington University Medical Center at 0700 h after an overnight fast. A polyethylene catheter was placed in an antecubital vein for infusion of insulin, and a catheter was placed in the subject's hand to establish whether a plasma insulin concentration within the normal range by infusion of KC1 at 5-10 meq/l before the 2-h euglycemic clamp. During the euglycemic clamp with concurrent exercise (protocol III), biopsies were performed at rest before insulin infusion, after 2 h of insulin infusion, and immediately after exercise. One incision was used for all three biopsies in each experiment with care to redirect the needle for each biopsy. Muscle samples were frozen immediately by plunging the needle into liquid N$_2$. Samples were stored in liquid N$_2$ until analysis.

**Tissue analysis.** Plasma glucose concentration was determined by the glucose oxidase method (Beckman Instruments). Plasma insulin concentration was determined by radioimmunoassay (9).

Muscle samples were lyophilized for 7 days at ~40°C and <0.01 Torr. All visible blood and connective tissue experiments indicated that insulin infusion at 180 mU·m$^{-2}$·min$^{-1}$ produced less than a maximal increase in glucose disposal.

In four subjects, two-stage hyperinsulinemic clamps were extended for a period of at least 60 min during which the insulin infusion rate was increased from 400 to 600 mU·m$^{-2}$·min$^{-1}$ (protocol not illustrated); this resulted in no further increase in GDR. Infusion of insulin at 400 mU·m$^{-2}$·min$^{-1}$, which results in a plasma insulin concentration of ~2,000 $\mu$U/ml and causes a maximal (G1R) in young lean subjects, was therefore used in the experiments described below to assess the effects of prior or concurrent exercise on maximal insulin-stimulated glucose disposal.

The effect of prior exercise on maximal insulin-stimulated glucose disposal was determined in 11 subjects by having the men exercise on a cycle ergometer in the upright position for 1 h at ~80% of peak $V_{O_2}$ immediately before a 2-h euglycemic clamp (protocol II; Fig. 1). This work rate was chosen to involve a relatively large muscle mass and to cause glycogen depletion. Expired air during exercise was collected for 2 min every 20 min by use of a Daniel's breathing valve and meteorological balloons. The $O_2$ and $CO_2$ fractions were analyzed with a Perkin-Elmer MGA 1100 respiratory spectrometer, and ventilatory volumes were determined with a Tissot spirometer. Infusion of insulin was begun 30 min after the end of exercise. The results of this experiment were compared with those of another euglycemic clamp without prior exercise. At least 7 days elapsed between the two experiments, which were performed in a random order.

The effect of exercise during insulin infusion was studied in eight of these subjects by extending the euglycemic clamp without prior exercise by 2 h (protocol III; Fig. 1). During the 3rd h of insulin infusion, subjects performed supine cycle exercise for 10 min at a $V_{O_2}$ of ~15 ml·kg$^{-1}$·min$^{-1}$ and then for 50 min at a $V_{O_2}$ of ~30 ml·kg$^{-1}$·min$^{-1}$. This protocol was chosen because it used the highest work rate that the subjects could maintain for this period of time in the supine position. Expired air was again collected every 20 min for $O_2$ and $CO_2$ analysis. Infusion of glucose and insulin was continued for 1 h after exercise.

Vastus lateralis muscle specimens were also obtained in these eight subjects by needle biopsy (1) during both experimental protocols (Fig. 1). In the euglycemic clamp preceded by exercise (protocol II), biopsies were performed before exercise, ~15 min after exercise (i.e., ~15 min before insulin infusion), and at the end of the euglycemic clamp. During the euglycemic clamp with concurrent exercise (protocol III), biopsies were performed at rest before insulin infusion, after 2 h of insulin infusion, and immediately after exercise. One incision was used for all three biopsies in each experiment with care to redirect the needle for each biopsy. Muscle samples were frozen immediately by plunging the needle into liquid N$_2$. Samples were stored in liquid N$_2$ until analysis.

**Muscle biopsy procedures.** Four milliliters of the subject's blood were added to each of insulin, and the subject's hand was placed in a box heated to 70°C for sampling of arterialized blood (14). A second catheter was placed in an antecubital vein for infusion of insulin, 20% dextrose, and KC1. Human insulin (Squibb Novo, Princeton, NJ) was diluted in 0.9% saline. Four milliliters of the subject's blood were added to each 100 ml of saline to protect against insulin adherence to glassware and tubing. Arterialized plasma glucose concentration was measured every 5 min and maintained at 90 mg/dl by adjustment of the glucose infusion rate. Additional blood samples were drawn at 15-min intervals for subsequent determination of plasma insulin concentration. Plasma potassium concentration was maintained within the normal range by infusion of KC1 at 5-10 meq/h.

**Experimental design.** Preliminary experiments were conducted to establish whether a plasma insulin concentration of ~500 $\mu$U/ml, as used by Mikines and co-workers (15, 16), produces a maximal (G1R). Four subjects were studied during a two-stage euglycemic hyperinsulinemic clamp (protocol I; Fig. 1). Insulin was infused at a rate of 180 mU·m$^{-2}$·min$^{-1}$ for 2 h and then at 400 mU·m$^{-2}$·min$^{-1}$ for an additional 2 h. These preliminary experiments indicated that insulin infusion at 180 mU·m$^{-2}$·min$^{-1}$ produced less than a maximal increase in glucose disposal.
TABLE 1. Euglycemic clamp data: effect of insulin concentration

<table>
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<tr>
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<th>Moderate vs. High Concentration</th>
<th>Comparison at High Concentration</th>
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<tbody>
<tr>
<td>Insulin infusion rate, mU·min⁻¹·min⁻¹</td>
<td>180</td>
<td>400</td>
</tr>
<tr>
<td>Plasma glucose, mg/dl</td>
<td>90</td>
<td>90</td>
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<tr>
<td>Coefficient of variation in glucose, %</td>
<td>3.2</td>
<td>4.1</td>
</tr>
<tr>
<td>Plasma insulin, mU/l</td>
<td>750±60</td>
<td>2,700±100</td>
</tr>
<tr>
<td>GDR, mg·kg LBM⁻¹·min⁻¹</td>
<td>11.7±0.6</td>
<td>15.6±0.3*</td>
</tr>
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</table>

Values are means ± SE; n, no. of subjects. * P < 0.001 from low dose.

TABLE 2. Euglycemic clamp data: effect of exercise

<table>
<thead>
<tr>
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<th>Prior Exercise (n = 11)</th>
<th>Simultaneous Exercise (n = 8)</th>
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<tbody>
<tr>
<td></td>
<td>No exercise</td>
<td>Prior exercise</td>
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<tr>
<td></td>
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<tr>
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<td>During exercise</td>
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<tr>
<td></td>
<td></td>
<td>After exercise</td>
</tr>
<tr>
<td>Plasma glucose, mg/dl</td>
<td>89</td>
<td>90</td>
</tr>
<tr>
<td>Coefficient of variation in glucose, %</td>
<td>4.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Plasma insulin, mU/l</td>
<td>2,100±100</td>
<td>2,300±200</td>
</tr>
<tr>
<td>GDR, mg·kg LBM⁻¹·min⁻¹</td>
<td>15.4±0.9</td>
<td>15.7±0.7</td>
</tr>
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</table>

Values are means ± SE; n, no. of subjects. Insulin was infused at 400 mU·m⁻²·min⁻¹. * P < 0.01 from before exercise.

was then removed under a dissecting scope at 20°C. Fragments (1-2 mg) of each sample were weighed on an electronic balance (Sartorius model 4503, Gottingen, FRG), powdered, and extracted on ice for 10 min in 0.3 N perchloric acid containing 1 mM EDTA. Perchloric acid extracts were neutralized with KOH and imidazole, subjected to centrifugation at 4°C and 10,000 g for 30 s, and stored at −80°C until analyzed. Lactate, glucose, and glucose 6-phosphate (G-6-P) in the perchloric acid extract were measured by fluorometric techniques (13) with intra- and interassay coefficients of variation of 2.7 and 3.5% for lactate, 1.9 and 1.5% for glucose, and 6.9 and 11% for G-6-P, respectively. Total creatine was measured fluorometrically by the method of Hintz et al. (10) with intra- and interassay coefficients of variation of 4.5 and 4.4%, respectively. Glycogen was determined as glucose residues (13) after acid hydrolysis (2 h in 2 N HCl at 100°C) of the perchloric acid precipitate with intra- and interassay coefficients of variation of 5.0 and 6.1%, respectively. Metabolite concentrations were expressed as millimoles per kilogram dry weight. To correct for residual nonmuscle material, glycogen and G-6-P concentrations were adjusted to the highest total muscle creatine concentration observed in each individual (18). Muscle lactate and glucose concentrations were not corrected because of the high concentrations of these metabolites in the extracellular space.

Data analysis. GDR was calculated as the glucose infusion rate corrected for changes in the mass of the glucose in the estimated glucose space (7). Comparisons between means were made by analysis of variance for repeated measures. Missing muscle biopsy data (due to inadequate sampling in one subject after upright exercise) were estimated as described by Sokal and Rohlf (19). Significant differences identified by analysis of variance were isolated by the T method (Tukey's honestly significant difference method) (19).

RESULTS

Subjects were studied by use of three different protocols. The insulin infusion rates, mean plasma insulin, mean plasma glucose, and coefficient of variation in plasma glucose for each euglycemic clamp protocol are presented in Tables 1 and 2.

Effect of insulin infusion rate. Four subjects were studied during a two-stage hyperinsulinemic euglycemic clamp (protocol I; Fig. 1). When insulin was infused at 180 mU·m⁻²·min⁻¹, plasma insulin concentration reached a plateau of 750 ± 50 mU/ml by 15 min (Fig. 2). Mean GDR increased to 10.8 ± 0.6 mg·kg·LBM⁻¹·min⁻¹ during 30-60 min and then increased gradually to 11.7 mg·kg·LBM⁻¹·min⁻¹ for the final 30 min. When the insulin infusion rate was increased to 400 mU·m⁻²·min⁻¹, plasma insulin concentration increased three- to fourfold and GDR increased significantly (Fig. 2). GDR was constant during the last 30 min at each insulin infusion rate, GDR was 33% greater when insulin was infused at 400 vs. 180 mU·m⁻²·min⁻¹ (15.6 ± 0.3 vs. 11.7 ± 0.6 mgs-
kg LBM⁻¹·min⁻¹; P < 0.001; Table 1). That a plasma insulin concentration of ~2,000 μU/ml results in a maximal insulin-stimulated GDR in healthy young lean men was confirmed in four subjects in whom a further increase in plasma insulin concentration to ~4,500 μU/ml caused no further increase in GDR (Fig. 1).

Effect of exercise before insulin infusion. Eleven subjects exercised at 78 ± 3% of peak VO₂ for 60 min before the start of a 120 min euglycemic clamp procedure (protocol II, Fig. 1). These results were compared with the results of another clamp performed without prior exercise. Plasma glucose concentration before insulin infusion did not differ between clamps with and without prior exercise (90 ± 2 vs. 94 ± 3 mg/dl; NS). Although GDR tended to be higher during the first 60 min of the clamp with prior exercise (Fig. 3), these differences were not significant. GDR during the last 30 min did not differ between clamps with and without prior exercise (15.7 ± 0.9 vs. 16.4 ± 0.7 mg·kg⁻¹·LBM⁻¹·min⁻¹; NS). Muscle biopsies were performed in eight subjects for the measurement of total glucose, glycogen, G-6-P, lactate, and creatine (Table 3). Prior exercise resulted in an ~50% decrease in muscle glycogen (P < 0.01) and a twofold increase in muscle glucose concentration. After 2 h of insulin infusion, muscle glycogen concentration increased 60% only in previously exercised muscle.

Effect of exercise during insulin infusion. Eight subjects performed supine exercise at a mean VO₂ of 29 ± 1 ml·kg⁻¹·min⁻¹ during the 3rd h of a 4-h insulin infusion at 400 ml·m⁻²·min⁻¹ (protocol III; Fig. 1). GDR reached a plateau during the 2nd h of insulin infusion at rest, rose rapidly with the onset of exercise, and declined rapidly when exercise was stopped (Fig. 4). The mean GDR during the final 15 min of exercise (26.7 ± 1.3 mg·kg LBM⁻¹·min⁻¹) was 70% greater (P < 0.001) than the GDR before exercise (15.7 ± 1.0 mg·kg LBM⁻¹·min⁻¹). The mean GDR during the final 15 min of recovery (16.6 ± 0.7 mg·kg LBM⁻¹·min⁻¹) was not significantly different from the preexercise value. Vastus lateralis muscle biopsies were performed in eight subjects. Total muscle glucose was increased only in the biopsies obtained at the end of exercise (Table 3). Significant lactate accumulation was observed only in the muscle samples obtained immediately after supine exercise. Muscle G-6-P tended to be elevated immediately after exercise in those subjects with elevated muscle lactate (R = 0.92, P = 0.001). There was no increase in G-6-P concentration in resting muscle during the hyperinsulinemic euglycemic clamp.

**DISCUSSION**

Our results show that in lean healthy young men a strenuous bout of exercise does not result in an increase in GDR in response to a maximal insulin stimulus during a euglycemic clamp started 30 min after exercise. In contrast, when muscles from exercised and rested rats are treated with a maximal concentration of insulin in vitro (5) or in situ (22), the rate of glucose transport is significantly higher in the exercised than in the rested muscles. Shortly after exercise, this increased rate of glucose transport is due to an additive effect of exercise and insulin (5, 20); later, when the effect of exercise persists, an increased responsiveness to insulin becomes evident (4, 22).

There are a number of possible explanations for our finding that exercise followed by a maximal insulin stimulus did not result in a greater GDR than the maximal insulin stimulus alone. One explanation is that in humans, a maximal insulin stimulus by itself elicits the highest attainable rate of glucose uptake. This possibility is ruled out by our finding that superimposition of mild exercise on a maximally insulin-stimulated GDR resulted in a further large increase in glucose uptake. A second possibility is that in euglycemic humans, glucose metabolism limits glucose disposal in resting muscles exposed to a maximally effective insulin concentration and does not change with prior exercise. In this case, superimposed exercise might be expected to accelerate glucose removal by increasing the rate at which glucose is metabolized. Arguing against this explanation is our finding that G-6-P concentration did not increase in resting muscle in response to hyperinsulinemia, as it would if glucose uptake was limited by the rate of glucose metabolism (i.e., glycogen synthesis and glycolysis).

A third possibility is that glucose transport is rate limiting for glucose disposal in maximally insulin-stimulated resting muscles in humans and that exercise increases GDR by further stimulating glucose transport. If this were the case, the stimulating effect of muscle contractions on glucose transport would have to be lost extremely rapidly. We cannot rule out this possibility, but we think it unlikely that human skeletal muscle would behave so differently from rat skeletal muscle, in which the rate of transport is markedly higher in exercised compared with rested muscle exposed to a maximal
increased by insulin in vivo, glucose uptake is accelerated and the arteriovenous glucose concentration difference widens. The decrease in glucose concentration as blood flows from the arterial to the venous end of the muscle capillaries can be very large in insulinized muscles because insulin increases glucose uptake without increasing glucose delivery (i.e., blood flow). For example, DeFronzo et al. (6) found that a moderate increase in plasma insulin concentration (to ~75 μU/ml) resulted in widening of the arteriovenous glucose difference across the lower extremity with a drop in plasma glucose concentration from 82 (arterial) to 50 mg/dl (venous). The plasma insulin concentration used in the present study causes roughly twice as great an increase in GDR in young healthy nonexercised subjects as the insulin concentration used in the study by DeFronzo et al. (6). It is therefore probable that a large decrease in plasma glucose concentration occurred in the muscle capillaries during the hyperinsulinemic clamp in the present study.

In muscles that are extracting glucose from the blood, the glucose concentration in the fluid in the interstitial space must be lower than that in the plasma at the venous end of the capillaries. It is therefore reasonable to assume that 1) the glucose concentration in the interstitial fluid of resting muscles was far below that in arterial plasma during the hyperinsulinemic clamp in the present study and 2) if prior exercise resulted in a further stimulation of glucose transport activity, then the concentration of glucose in the interstitial fluid would have decreased even further and thus reduced or prevented an additional increase in the steady-state GDR in the exercised muscles.

In the study by DeFronzo et al. (6), when mild exercise was superimposed on hyperinsulinemia (~75 μU/ml), leg blood flow increased approximately ninefold and glucose uptake increased markedly, whereas, as a result of the increase in blood flow, the arteriovenous glucose concentration difference was smaller than that with hyperinsulinemia alone. DeFronzo and co-workers’ (6) interpretation of their findings was that the effect on glucose uptake of exercise superimposed on hyperinsulinemia was mediated by increased blood flow to and increased capillary surface area in the exercising muscles. This interpretation was supported by close correlations between the changes in blood flow and glucose uptake. It seems probable that this explanation also applies to our findings.

In the present study, exercise superimposed on hyperinsulinemia also resulted in a large increase in glucose uptake that reversed rapidly when exercise was stopped. In the above context, it seem probable that the glucose concentration in the interstitial fluid of muscles was markedly reduced by hyperinsulinemia and that the increase in glucose uptake caused by exercise was mediated primarily by a large increase in blood flow to the working muscles with a decrease in arteriovenous glucose concentration difference and a concomitant increase in the concentration of glucose in the interstitial fluid to which the muscle cells were exposed. Compatible with this

### Table 3. Muscle metabolites

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<tbody>
<tr>
<td>Glycogen, mmol/kg dry wt</td>
<td>262±44</td>
<td>187±45*</td>
<td>200±65</td>
<td>200±36</td>
<td>448±59</td>
</tr>
<tr>
<td>G-6-P, μmol/kg dry wt</td>
<td>410±100</td>
<td>330±54</td>
<td>350±65</td>
<td>270±41</td>
<td>244±34</td>
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<tr>
<td>Glucose, mmol/kg dry wt</td>
<td>2.3±0.3</td>
<td>5.1±0.6††</td>
<td>2.3±0.5</td>
<td>2.0±0.6</td>
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<td>Lactate, mmol/kg dry wt</td>
<td>8.6±1.1</td>
<td>17.5±3.1</td>
<td>13.2±2.4</td>
<td>7.1±1.3</td>
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<td>Total creatine, mmol/kg dry wt</td>
<td>125±6</td>
<td>120±9</td>
<td>113±10</td>
<td>123±4</td>
<td>124±8</td>
</tr>
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</table>

Values are means ± SE; n = 8 subjects. Compared with before-exercise value: * P < 0.01; † P < 0.05. ‡ P < 0.025 from after-clamp value. § P < 0.025 from before-clamp value.
interpretation is our finding of a large increase in glucose concentration in the muscle biopsies obtained after exercise.

Our finding that exercise followed 30 min later by a maximal insulin stimulus did not result in a greater GDR than a maximal insulin stimulus alone conflicts with the conclusion reached by Mikines et al. (15, 16), who used a maximum insulin concentration of only 470 μU/ml. In our subjects, a plasma insulin concentration of 750 μU/ml was submaximal in effect, providing evidence that the concentration used by Mikines et al. (15, 16) was insufficient to evaluate insulin responsiveness.

We conclude that prior exercise does not enhance maximally insulin-stimulated glucose disposal in young healthy men. Our results are compatible with (but do not prove) the hypotheses that glucose availability rather than glucose metabolism limits GDR in response to a maximal insulin stimulus in resting subjects and that the increase in glucose uptake in response to superimposed exercise is primarily due to an increase in glucose availability.

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