Increased insulin receptor signaling and glycogen synthase activity contribute to the synergistic effect of exercise on insulin action

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Christ-Roberts, Christine Y., Thongchai Pratipanawatr, Wilailak Pratipanawatr, Rachele Berria, Renata Belfort, and Lawrence J. Mandarino. Increased insulin receptor signaling and glycogen synthase activity contribute to the synergistic effect of exercise on insulin action. J Appl Physiol 95: 2519–2529, 2003. First published August 8, 2003; 10.1152/japplphysiol.00605.2003.—The purpose of this study was to determine the factors contributing to the ability of exercise to enhance insulin-stimulated glucose disposal. Sixteen insulin-resistant nondiabetic and seven Type 2 diabetic subjects underwent two hyperinsulinemic (40 mU·m−2·min−1) clamps, once without and once with concomitant exercise at 70% peak O2 consumption. Exercise was begun at the start of insulin infusion and was performed for 30 min. Biopsies of the vastus lateralis were performed before and after 30 min of insulin infusion (immediately after cessation of exercise). Exercise synergistically increased insulin-stimulated glucose disposal in nondiabetic (from 4.6 ± 0.4 to 9.5 ± 0.8 mg·kg−1·min−1) and diabetic subjects (from 4.3 ± 1.0 to 7.9 ± 0.7 mg·kg−1·min−1) subjects. The rate of glucose disposal also was significantly greater in each group after cessation of exercise. Exercise enhanced insulin-stimulated increases in glycogen synthase fractional velocity in control (from 0.07 ± 0.02 to 0.22 ± 0.05, P < 0.05) and diabetic (from 0.08 ± 0.03 to 0.15 ± 0.03, P < 0.01) subjects. Exercise also enhanced insulin-stimulated glycogen storage (glycogen synthesis) in nondiabetic (2.9 ± 0.9 vs. 4.9 ± 1.1 mg·kg−1·min−1) and diabetic (1.7 ± 0.5 vs. 4.2 ± 0.8 mg·kg−1·min−1) subjects. Increased glucose storage accounted for the increase in whole body glucose disposal when exercise was performed during insulin stimulation in both groups; effects of exercise were correlated with enhancement of glucose disposal and glucose storage (r = 0.93, P < 0.001). Exercise synergistically enhanced insulin-stimulated insulin receptor substrate 1-associated phosphatidylinositol 3-kinase activity (P < 0.05) and Akt Ser473 phosphorylation (P < 0.05) in nondiabetic subjects but had little effect in diabetic subjects. The data indicate that exercise, performed in conjunction with insulin infusion, synergistically increases insulin-stimulated glucose disposal compared with insulin alone. In nondiabetic and diabetic subjects, increased glycogen synthase activation is likely to be involved, in part, in this effect. In nondiabetic, but not diabetic, subjects, exercise-induced enhancement of insulin stimulation of the phosphatidylinositol 3-kinase pathway is also likely to be involved in the exercise-induced synergistic enhancement of glucose disposal.

phosphatidylinositol 3-kinase; Akt

IN VIVO AND IN VITRO STUDIES demonstrate that muscle contraction enhances insulin stimulation of glucose disposal when contracting muscle is stimulated with insulin (10, 11). This enhanced glucose disposal persists for several hours after the cessation of exercise (33, 36). However, the mechanism for this enhancement remains unknown. Although insulin and muscle contraction independently stimulate GLUT-4 translocation and glucose uptake, the mechanisms by which they bring about these effects are distinct. Insulin acts by binding to its receptor, resulting in tyrosine phosphorylation of the receptor and insulin receptor substrates (primarily IRS-1), which serve as docking proteins for proteins containing Src homology (SH2) domains. Association of the SH2 domain of the regulatory subunit of phosphatidylinositol 3-kinase (PI3-kinase) with IRS-1 activates the catalytic subunit of PI3-kinase. Numerous studies have shown that insulin-stimulated glucose uptake is dependent on the activation of PI3-kinase (6, 20). A potential downstream effector of PI3-kinase is Akt, a serine/threonine kinase also known as PKB (3, 23, 34). Unlike insulin, contraction stimulates glucose uptake independent of PI3-kinase (3, 27). Although the signaling mechanisms are unclear, increased 5′-adenosine monophosphate-activated protein kinase activity has been linked to contraction-induced glucose uptake (17).

Because of the distinct upstream signaling mechanisms utilized by insulin and contraction, it is not surprising that the combination of the two stimuli increases glucose uptake to a degree greater than either stimulus alone. A previous study in human subjects showed that exercise, when performed simultaneously with an insulin infusion, synergistically increased whole body glucose disposal (10). This result was contributed to enhanced blood flow to the working muscles.
muscle, resulting in a greater presentation of insulin and glucose to muscle (10). It is not clear, however, whether the synergistic increase in glucose disposal is due solely to a greater mass of muscle being perfused or whether there are qualitative effects on the muscle as well. One logical mechanism to increase insulin-stimulated glucose disposal might be to increase skeletal muscle insulin receptor signaling.

Data from studies in rodents (15) and humans (27) indicate strongly that exercise or muscle contraction alone does not enhance insulin signaling in the absence of an increase in insulin concentration. However, a single bout of exercise can increase the subsequent response of insulin signaling to an insulin infusion. For instance, 24 h after a single bout of exercise, insulin receptor and IRS-1 tyrosine phosphorylation are increased in response to insulin in insulin-resistant subjects compared with unexercised controls. These increases were not accompanied by an increase in PI3-kinase activity or glucose disposal, however (9). Because there were effects 24 h after exercise, the results raise the possibility that exercise during an insulin infusion might further increase insulin receptor signaling at more distal steps, thereby mediating an increase in glucose disposal. Glycogen synthase activity was also increased (9), suggesting that downstream effectors are also involved. The present study was undertaken, therefore, to determine whether, during a simultaneous insulin infusion and exercise bout, enhanced glucose disposal can be attributed to increased insulin receptor signaling or glycogen synthase activity.

METHODS

Subjects. Sixteen nondiabetic and seven Type 2 diabetic subjects participated in the study (Table 1). A complete history was obtained from each subject, and each subject underwent a physical examination, including a 75-g oral glucose tolerance test to determine the presence or absence of diabetes using established American Diabetes Association criteria. All nondiabetic subjects denied a family history of Type 2 diabetes and had normal glucose tolerance. Other than having diabetes, diabetic subjects were in good health. Three of the seven diabetic subjects were taking glyburide, which was withdrawn 3 days before clinical studies. The remaining four diabetic subjects were treated with diet alone. None of the subjects had a history of smoking.

Because there were effects 24 h after exercise, the peak aerobic capacity of an increase in insulin concentration. However, a single bout of exercise can increase the subsequent response of insulin signaling to an insulin infusion. For instance, 24 h after a single bout of exercise, insulin receptor and IRS-1 tyrosine phosphorylation are increased in response to insulin in insulin-resistant subjects compared with unexercised controls. These increases were not accompanied by an increase in PI3-kinase activity or glucose disposal, however (9). Because there were effects 24 h after exercise, the results raise the possibility that exercise during an insulin infusion might further increase insulin receptor signaling at more distal steps, thereby mediating an increase in glucose disposal. Glycogen synthase activity was also increased (9), suggesting that downstream effectors are also involved. The present study was undertaken, therefore, to determine whether, during a simultaneous insulin infusion and exercise bout, enhanced glucose disposal can be attributed to increased insulin receptor signaling or glycogen synthase activity.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Nondiabetic (n = 16)</th>
<th>Diabetic (n = 7)</th>
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</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>35 ± 2</td>
<td>45 ± 4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.3 ± 0.4</td>
<td>30.8 ± 1.2</td>
</tr>
<tr>
<td>HbA₁c</td>
<td>4.9 ± 0.1</td>
<td>7.8 ± 0.9</td>
</tr>
<tr>
<td>Fasting plasma glucose, mg/dl</td>
<td>94 ± 2</td>
<td>149 ± 16</td>
</tr>
<tr>
<td>Fasting plasma insulin, μU/ml</td>
<td>9 ± 3</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
<td>186 ± 11</td>
<td>192 ± 4</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>106 ± 12</td>
<td>319 ± 140</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>7/CH/1A/1A</td>
<td>2/5/5</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; H, Hispanic; C, Caucasian; A, Asian; AA, African-American. *P < 0.05; †P < 0.01; ‡P < 0.001 vs. nondiabetic.
The clamp then continued as described in Insulin clamp without simultaneous exercise.

Materials. Polyclonal anti COOH-terminal IRS-1 and polyclonal antiphospho-Akt (Ser473) antibodies were purchased from Upstate Biotechnology (Lake Placid, NY). A polyclonal anti-Akt antibody was purchased from Cell Signaling (Beverly, MA). Platelet-derived growth factor-stimulated NIH 3T3 L1 cell lysate (Upstate Biotechnology) served as a positive control for phospho-Akt (Ser473) immunoblotting. Rat liver homogenate served as a standard control for the PI3-kinase assay. Goat anti-rabbit and rabbit anti-sheep antibodies coupled to horseradish peroxidase (Amersham, Piscataway, NJ) were used as secondary antibodies. Protein A and phosphatidylinositol were purchased from Sigma Chemical (St. Louis, MO), and [γ-32P]ATP was obtained from NEN Life Science Products (Boston, MA).

Muscle processing. Muscle samples were weighed while still frozen and homogenized in ice-cold lysis buffer (1:10, wt/vol) containing 50 mM HEPES (pH 7.6), 150 mM NaCl, 20 mM sodium pyrophosphate, 20 mM β-glycerophosphate, 10 mM NaF, 2 mM Na3VO4, 2 mM EDTA (pH 8.0), 1% Nonidet P-40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 1 mM MgCl2, 1 mM CaCl2, 10 μg/ml leupeptin, and 10 μg/ml aprotinin. A Polytron homogenizer (Brinkman Instruments, Westbury, NY) set on maximum speed for 30 s was used for homogenization. Homogenates were incubated on ice for 20 min and then centrifuged at 15,000 g for 20 min at 4°C. Muscle debris was removed, and protein concentrations of crude extracts were estimated by the method of Lowry et al. (28).

SDS-PAGE and immunoblotting. For phospho-Akt (Ser473), equal amounts of protein were resolved on 7.5% (Akt) SDS-polyacrylamide gel and transferred to nitrocellulose membranes. After they were blocked, the membranes were incubated with antibodies, and protein bands were visualized using an enhanced chemiluminescence detection system according to the manufacturer’s protocol (Amersham). Images were digitized by scanning, and band intensity was quantified using Image Tool Software (University of Texas Health Science Center at San Antonio). For determining Akt expression, the phospho-Akt (Ser473) immunoblot was stripped using a buffer containing 0.7% β-mercaptoethanol, 7 mM SDS, and 6 mM Tris·HCl (pH 6.7) for 20 min, washed with Tris-buffered saline three times for 10 min each, blocked with Tris-buffered saline + Tween 20 containing 5% milk, and reprobed with anti-Akt antibody overnight. The detection procedures were the same as those described above.

PI3-kinase assay. Muscle protein (250 μg) was immunoprecipitated with anti-IRS-1 antibody, and PI3-kinase activity was measured by determining incorporation of [32P]ATP into [32P]phosphatidylinositol phosphate, as previously described (8).

Glycogen synthase activity. Glycogen synthase (GS) activities were assayed using 0.1 mM (GS0.1) and 10 mM (GS10) glucose 6-phosphate, as previously described (32). Glycogen synthase fractional velocity (GSSFV) was calculated as the ratio of GS0.1 to GS10. Changes in GSSFV are indicative of insulin’s effects.

Laboratory analyses. Plasma insulin concentration was determined by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Plasma tritiated glucose specific activity was determined on barium hydroxide-zinc sulfate-precipitated plasma samples.

Calculations. Glucose disposal rates were calculated using steady-state equations or, where appropriate for non-steady-state conditions, Steele’s equation (14). Glucose and fat oxidation rates were calculated from V\(^{\text{O}_2}\) and V\(^{\text{CO}_2}\) output data by the equations of Frayn (13). Insulin clearance was calculated as previously described by DeFronzo et al. (10).

Statistical analysis. Values are means ± SE. A t-test was used to test for differences between study groups with regard to subject clinical characteristics. Statistical differences among groups were determined by two-way repeated-measures analysis of variance and Fisher’s post hoc tests using StatView 4.0 software. Correlation analysis was performed by the Pearson product-moment method. For all analyses, P < 0.05 was considered to be statistically significant.

RESULTS

Subjects. The characteristics of the subjects are given in Table 1. As expected, diabetic subjects had elevated HbA1c and fasting plasma glucose levels compared with control subjects. There was no difference in fasting insulin levels between the groups. Diabetic subjects were somewhat older (P < 0.05) and had higher triglyceride levels than control subjects. Although both groups were moderately obese, diabetic subjects had a slightly higher body mass index. Maxi-
Table 2. Exercise characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Nondiabetic</th>
<th>Diabetic</th>
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<tr>
<td></td>
<td>Peak</td>
<td>Exercise during clamp</td>
</tr>
<tr>
<td>$\dot{V}O_2$, ml O$_2$·kg FFM$^{-1}$·min$^{-1}$</td>
<td>35.4 ± 1.7 †</td>
<td>24.9 ± 1.3</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>166 ± 4 † †</td>
<td>138 ± 4</td>
</tr>
<tr>
<td>Work, W</td>
<td>169 ± 12 †</td>
<td>116 ± 10</td>
</tr>
</tbody>
</table>

Values are means ± SE. Peak measurements constitute maximum values obtained during a peak $O_2$ consumption ($V_{O_2 peak}$) test. Exercise measurements correspond to 70% of each individual’s $V_{O_2 peak}$ and are characteristic of intensity at which subjects exercised during euglycemic, hyperinsulinemic clamp. $V_{O_2}$, $O_2$ uptake; FFM, fat-free mass. *$P < 0.01$; †$P < 0.001$ vs. exercise; ‡$P < 0.001$ vs. nondiabetic.

Euglycemic clamps. Subjects underwent two euglycemic, hyperinsulinemic clamps (40 mU·m$^{-2}$·min$^{-1}$) on separate occasions, once without and once with concomitant exercise (Fig. 1). When exercise was performed together with insulin infusion, subjects exercised at 70% $V_{O_2 peak}$ for 30 min beginning at the start of the insulin infusion (Table 2). Because the exercise intensity, by design, was relative to an individual’s $V_{O_2 peak}$, the diabetic subjects on average exercised at a lower $V_{O_2}$ ($P < 0.001$) and performed less work ($P < 0.01$) than the control subjects during the clamp (Table 2).

Plasma glucose levels were maintained at euglycemia by a variable glucose infusion throughout the clamps for control subjects (Fig. 2). Because of the increased fasting glucose levels of the diabetic subjects, their glucose concentrations were significantly greater during the initial part of the clamps than those of the control subjects (Fig. 2). During insulin alone, plasma glucose concentrations were significantly greater in the diabetic than in the control subjects during the first 40 min of insulin infusion ($P < 0.05$), whereas insulin + exercise was associated with a more rapid fall in glucose concentrations in the diabetic subjects so that, by 30 min, glucose concentrations were not significantly different from those of control subjects.

Plasma insulin concentrations were determined during the initial 30 min and final 30 min of the clamps. In the control subjects, plasma insulin concentrations were elevated during insulin + exercise compared with insulin alone (Fig. 3A). Plasma insulin concentrations peaked at $76 ± 4$ μU/ml and fell to $61 ± 3$ μU/ml during the first 30 min of insulin alone, whereas concentrations peaked at $90 ± 5$ μU/ml and fell to $72 ± 2$ μU/ml during the same period of insulin + exercise. Because the insulin infusion rate was the same on both occasions, we calculated the insulin clearance rates. Whole body insulin clearance was decreased when exercise was performed along with insulin infusion (Fig. 3B). After completion of the exercise, plasma insulin concentrations fell and insulin clearance increased to match values reached during insulin alone. In contrast, exercise did not affect insulin concentrations or insulin clearance in the diabetic subjects (Fig. 3, C and D).

Glucose disposal. In control subjects, insulin alone gradually increased the rate of glucose disposal calculated using tritiated glucose to $4.6 ± 0.4$ mg·kg fat-free mass (FFM)$^{-1}$·min$^{-1}$ at 30 min and then further to an average of $6.6 ± 0.7$ mg·kg FFM$^{-1}$·min$^{-1}$ over the last 30 min of insulin infusion (90–120 min). When exercise was performed during the first 30 min of insulin infusion in control subjects, the rate of glucose disposal increased steeply to $9.5 ± 0.8$ mg·kg FFM$^{-1}$·min$^{-1}$ after 30 min and then decreased gradually after cessation of exercise to an average of $8.1 ± 0.7$ mg·kg FFM$^{-1}$·min$^{-1}$ over the last 30 min of insulin infusion. At each time point, the rate of glucose disposal was greater when exercise was performed during the first 30 min of insulin infusion (Fig. 4A). In insulin-infused nonexercised diabetic subjects, the rate of glucose dis-
posal increased gradually to 4.3 ± 1.0 mg·kg FFM⁻¹·min⁻¹ at 30 min and then remained constant throughout the insulin infusion, with an average of 4.2 ± 0.6 mg·kg FFM⁻¹·min⁻¹ over the last 30 min of insulin infusion (90–120 min). When exercise was performed during the first 30 min of insulin infusion in diabetic subjects, the rate of glucose disposal increased steeply to 7.9 ± 0.7 mg·kg FFM⁻¹·min⁻¹ after 30 min and then rapidly decreased to 6.0 ± 0.8 mg·kg FFM⁻¹·min⁻¹ and remained constant throughout the duration of the insulin infusion (Fig. 4B). Throughout the studies, the rate of glucose disposal was significantly decreased in the diabetic subjects compared with the control subjects during insulin alone and insulin + exercise.

The rate of whole body glucose oxidation was determined basally (−30 to 0 min) and during the last 30 min of insulin infusion (90–120 min) using VO₂ and CO₂ output measured by systemic indirect calorimetry. Glucose storage was calculated as the difference between the rate of glucose disposal and oxidation. Results (Table 3, Fig. 4C) indicate that, as described above, the rate of insulin-stimulated glucose disposal was increased in both groups when exercise was performed during the first 30 min of insulin infusion. However, the rate of systemic glucose oxidation was not increased (60–90 min after exercise had ceased) in either group. Basal and insulin-stimulated rates of glucose oxidation remained significantly decreased in diabetic subjects compared with control subjects, whether or not exercise was performed. Therefore, in control and diabetic subjects, exercise significantly enhanced the rate of insulin-stimulated glucose storage (glycogen synthesis). This was especially true for the diabetic subjects, in whom insulin alone failed to increase glucose storage above basal values, but exercise increased the rate of insulin-stimulated glucose storage to a value not significantly different from that of control subjects studied during insulin infusion alone. The effect of exercise was strongly correlated with enhancement of glucose disposal and glucose storage (r = 0.93, P < 0.001).

Insulin signaling. To evaluate the effect of exercise on insulin’s ability to stimulate the PI3-kinase signaling pathway, muscle biopsies were performed in the basal period and during insulin stimulation with and
without simultaneous exercise. IRS-1-associated PI3-kinase activity and Akt serine phosphorylation and protein expression were measured in lysates from these muscle biopsies. Muscle samples from insulin and insulin + exercise clamps for each subject were analyzed on the same immunoblot to reduce variability.

The effect of exercise on insulin stimulation of IRS-1-associated PI3-kinase activity is shown in Fig. 5 (normalized to 100% for insulin alone for each group). Consistent with their general level of insulin resistance, insulin alone modestly but significantly increased IRS-1-associated PI3-kinase activity in both groups. Exercise significantly increased insulin-stimulated IRS-1-associated PI3-kinase activity compared with insulin alone in the control subjects (P < 0.05); however, this was not the case in the diabetic subjects.

Insulin infusion alone increased Akt Ser\(^{473}\) phosphorylation 10-fold in control and diabetic subjects (Fig. 6). To determine whether Akt protein expression was different between the two groups, muscle samples obtained during the basal period of the insulin-alone clamp were analyzed on the same immunoblot to reduce variability. Akt protein expression was decreased 23% in the diabetic subjects compared with the control subjects (P = 0.05). In control subjects, exercise performed during the insulin infusion significantly increased Akt Ser\(^{473}\) phosphorylation (P < 0.05), but this effect of exercise did not occur in the diabetic subjects.

Glycogen synthase activity. Glycogen synthase activity was assayed using GS\(_{0.1}\) and GS\(_{10}\) to determine active and total forms of the enzyme. Under basal conditions, neither GS\(_{0.1}\) nor GS\(_{PV}\) differed with the insulin-alone and insulin + exercise for control or diabetic subjects (Table 4, Fig. 7). Insulin alone stimulated GS\(_{0.1}\) and GS\(_{PV}\) to a similar extent in the obese control and diabetic subjects consistent with their degree of insulin resistance. Exercise performed during the first 30 min of insulin infusion resulted in a significant increase in GS\(_{0.1}\) and GS\(_{PV}\) compared with the value achieved during insulin alone (P < 0.05) for the obese control and diabetic patients.

**DISCUSSION**

Exercise performed simultaneously with an insulin infusion synergistically increases glucose disposal compared with insulin infusion alone (10). Acute exercise can enhance subsequent (24 h later) insulin stimulation of proximal insulin receptor signaling and glycogen synthase activity (9). We sought to determine whether the synergistic effect of exercise on insulin-

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**Fig. 4.** Effect of exercise on glucose disposal rates for control (A) and diabetic subjects (B). Subjects underwent 2 euglycemic, hyperinsulinemic clamps (40 mU·m\(^{-2}\)·min\(^{-1}\)), once without (Ex) and once with (+Ex) concomitant exercise, during which tritiated glucose was infused for determination of glucose disposal. See METHODS for calculation. Control, insulin alone; control, insulin + exercise; diabetic, insulin alone; diabetic, insulin + exercise. *P < 0.001; §P < 0.01; %P < 0.05 vs. insulin alone. C: effect of exercise on insulin-stimulated whole body glucose metabolism. Whole body glucose disposal (height of bar) was determined using tritiated glucose, and indirect calorimetry was used to determine whole body glucose oxidation (open bars). Nonoxidative glucose storage (solid bars) represents difference between total glucose disposal and glucose oxidation. Basal measurements were subtracted from stimulated measurements for each subject to determine effect of insulin alone and insulin + exercise. FFM, fat-free mass. Values are means ± SE for 7 control and 7 diabetic subjects. *P < 0.05; †P < 0.01 vs. insulin alone; ‡P < 0.05 vs. control.
stimulated glucose disposal can be attributed to increased insulin signaling or glycogen synthase activity. We chose to study insulin-resistant subjects to gain insight into the mechanism of the ability of exercise to overcome insulin resistance.

Results of the present study confirm that exercise performed simultaneously with insulin administration increases glucose disposal to a degree greater than that predicted on the basis of their separate individual contributions (10). For example, glucose disposal at the end of insulin + exercise was 9.5 ± 0.8 mg·kg⁻¹·FMM⁻¹·min⁻¹, an increase of 4.9 ± 0.6 mg·kg⁻¹·FMM⁻¹·min⁻¹ over that with insulin alone. Previous studies in our laboratory using similar exercise intensity (27) show that if the effect of exercise and insulin were additive, one would expect an increase of only 1.5 ± 0.4 mg·kg⁻¹·FMM⁻¹·min⁻¹. Therefore, this study design results in exercise synergistically increasing insulin-stimulated glucose disposal in nondiabetic subjects. Similarly, in the diabetic subjects, glucose disposal at the end of insulin + exercise was 7.9 ± 0.7 mg·kg⁻¹·FMM⁻¹·min⁻¹, an increase of 3.7 ± 0.8 mg·kg⁻¹·FMM⁻¹·min⁻¹ over that achieved by insulin alone. Minuk et al. (30) demonstrated that exercise-induced glucose disposal in diabetic subjects is similar to that measured in nondiabetic subjects. Therefore, if we assume that exercise alone stimulates glucose disposal to the same extent as in obese nondiabetic subjects (27), the predicted additive effect of insulin and exercise would be 5.8 ± 1.1 mg·kg⁻¹·FMM⁻¹·min⁻¹, suggesting that exercise synergistically increases insulin-stimulated glucose disposal in diabetic subjects as well.

With regard to insulin receptor signaling, we chose to assess distal components of the PI3-kinase signaling pathway, that is, IRS-1-associated PI3-kinase activity and Akt Ser⁴⁷³ phosphorylation. Our laboratory previously showed that the effect of exercise can be dissociated from changes in insulin receptor and IRS-1 tyrosine phosphorylation (9), so the present study was designed to assess the involvement of the distal portion of the PI3-kinase pathway. We and other investigators showed that voluntary (27) exercise alone and electrically stimulated muscle contraction (15) do not increase IRS-1-associated PI3-kinase in the absence of insulin or in the presence of low insulin concentrations. However, whether exercise performed simultaneously with insulin infusion, leading to synergistic increases in glucose disposal, is associated with increased IRS-1-associated PI3-kinase activity is not known. In the present study, exercise performed in conjunction with insulin infusion increased IRS-1-associated PI3-kinase activity over that obtained with insulin alone in nondiabetic subjects. Because exercise alone does not increase IRS-1-associated PI3-kinase activity (15, 27), the increase in response to insulin + exercise is synergistic by definition. Therefore, in nondiabetic subjects, a synergistic increase in IRS-1-associated PI3-kinase activity is consistent with the synergistic increase in glucose disposal. In contrast, even though simultaneous exercise enhanced insulin-stimulated glucose disposal in the diabetic group, there was no increase of IRS-1-associated PI3-kinase activity. Therefore, in contrast to the nondiabetic subjects, it is unlikely that increased IRS-1-associated PI3-kinase activity is involved in the synergistic effect of exercise on insulin-stimulated glucose uptake in patients with Type 2 diabetes.

Contrary to IRS-1-associated PI3-kinase activity, insulin alone significantly increased Akt Ser⁴⁷³ phosphorylation in both groups. These results are comparable to those of Kim et al. (22). Similar to PI3-kinase activity, exercise performed in conjunction with insulin

Table 3. Glucose metabolism during euglycemic, hyperinsulinemic clamps

<table>
<thead>
<tr>
<th></th>
<th>Nondiabetic</th>
<th>Diabetic</th>
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<tbody>
<tr>
<td></td>
<td>+Exercise</td>
<td>+Exercise</td>
</tr>
<tr>
<td>Oxidation (mg·kg⁻¹·FMM⁻¹·min⁻¹)</td>
<td>2.3 ± 0.2</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>Storage (mg·kg⁻¹·FMM⁻¹)</td>
<td>0.5 ± 0.2</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Disposal (mg·kg⁻¹·FMM⁻¹)</td>
<td>2.8 ± 0.1</td>
<td>2.9 ± 0.2</td>
</tr>
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</table>

**Control**

**Diabetic**

Values are means ± SE in mg·kg⁻¹·fat-free mass⁻¹·min⁻¹; n = 7. Subjects underwent 2 euglycemic, hyperinsulinemic clamps (40 mU/m²·min), once without (−Exercise) and once with concomitant exercise (+Exercise). Tritiated glucose was used to assess glucose disposal, and indirect calorimetry was used to calculate glucose oxidation. Difference between glucose disposal and glucose oxidation represents glucose storage. *P < 0.05; †P < 0.001 vs. basal. ‡P < 0.05; ††P < 0.01 vs. insulin. *P < 0.05 vs. nondiabetic.

**Fig. 5. Effect of exercise on insulin stimulation of insulin receptor substrate 1 (IRS-1)-associated phosphatidylinositol (PI) 3-kinase.** Data are expressed relative to average value for insulin alone for each group. Open bars, basal values; solid bars, insulin-stimulated values. *P < 0.05 vs. basal; †P < 0.05 vs. insulin (Ins) alone.
infusion significantly increased Akt serine phosphorylation only in the nondiabetic group. Because exercise alone does not stimulate Akt serine phosphorylation (3, 35), similar to the results for PI3-kinase, exercise synergistically increased insulin-stimulated Akt Ser473 phosphorylation in the nondiabetic subjects (27) but had no effect in the diabetic subjects. Taken together, the results of the present study indicate that exercise synergistically enhances signaling through PI3-kinase and Akt in nondiabetic, but not Type 2 diabetic, subjects.

Recent studies suggest that decreased insulin stimulation of Akt isoforms 2 and 3 is involved in the development of insulin resistance and diabetes (4, 7). In the present study, we were unable to identify individual Akt isoforms, and therefore we are unable to determine whether there is a difference between the two study groups with regard to the effects of insulin or exercise on individual isoforms of Akt.

There are several possible explanations for the difference between the obese control and Type 2 diabetic subjects. 1) Because of their lower V\textsubscript{O2} peak, work was performed at a lower absolute rate by the diabetic subjects, and perhaps this rate was insufficient to induce the insulin signaling increase observed in the control subjects. 2) Insulin signaling abnormalities are more profound in patients with Type 2 diabetes than in obese control subjects (9). These defects may be too profound to be overcome by a single bout of exercise. 3) The diabetic subjects were, on average, older than the nondiabetic control subjects; thus age may have contributed to the discrepancies between the two groups. However, when we investigated this matter, we found no correlation between age and the effects of exercise on insulin action and signaling. Nevertheless, glucose disposal was increased in both groups of subjects when

### Table 4. Glycogen synthase activity for nondiabetic and diabetic subjects

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<thead>
<tr>
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<th>Exercise</th>
<th>+ Exercise</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Insulin</td>
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<tr>
<td><strong>Nondiabetic</strong></td>
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<td></td>
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<tr>
<td>GS\textsubscript{0.1}</td>
<td>1.3±0.3</td>
<td>2.4±0.5*</td>
</tr>
<tr>
<td>GS\textsubscript{10}</td>
<td>13.2±1.9</td>
<td>14.3±1.9</td>
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<tr>
<td><strong>Diabetic</strong></td>
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<td></td>
</tr>
<tr>
<td>GS\textsubscript{0.1}</td>
<td>2.2±0.5</td>
<td>3.6±0.7*</td>
</tr>
<tr>
<td>GS\textsubscript{10}</td>
<td>15.2±2</td>
<td>15.6±1.3</td>
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</table>

Values are means ± SE. Glycogen synthase activity was assayed in muscle biopsies obtained basally and during insulin infusion. Activity was measured in the presence of 0.1 mM (GS\textsubscript{0.1}) and 10 mM (GS\textsubscript{10}) glucose 6-phosphate. *P < 0.05; †P < 0.01 vs. basal. ‡P < 0.05 vs. no exercise.

Fig. 6. Effect of exercise on insulin-stimulated Akt serine phosphorylation for control (A) and diabetic (B) subjects. Akt Ser\textsuperscript{473} phosphorylation was determined relative to Akt protein expression for each subject from muscle samples obtained during the basal period and during insulin infusion without (−) or with (+) concomitant exercise. Values (means ± SE) are expressed as percentage of insulin-stimulated muscle sample, which is set to 100% for each group. Open bars, basal; solid bars, insulin. *P < 0.05; †P < 0.001 vs. basal. ‡P < 0.01; §P < 0.05 vs. no exercise.
exercise was performed together with an insulin infusion. This indicates that if effects on insulin signaling are involved, they only partially explain the phenomenon.

Downstream mediators of glucose metabolism, such as glycogen synthase (24, 27, 29) and GLUT-4 (16), are also influenced by exercise. Cusi et al. (9) demonstrated that 24 h after an acute bout of exercise insulin-stimulated glycogen synthase activity was enhanced, even though insulin-stimulated IRS-1-associated PI3-kinase activity was unaffected. In the present study, insulin alone modestly increased glycogen synthase activity to the same extent in nondiabetic and diabetic subjects. However, insulin-stimulated glycogen synthase activity was significantly increased when exercise was performed during the first 30 min of insulin infusion compared with insulin stimulation alone in both groups. Because exercise performed in conjunction with insulin stimulation enhanced glycogen synthase activity to the same extent in nondiabetic and diabetic subjects, exercise is likely to mediate its effects on glycogen synthase independent of its effects on insulin stimulation of IRS-1-associated PI3-kinase and Akt under these conditions. Compared with the effect of insulin or exercise alone (27) to independently increase glycogen synthase activity, exercise performed during the first 30 min of insulin infusion approximately additively increased glycogen synthase activity in nondiabetic subjects. These results are consistent with reports that exercise and insulin stimulate glycogen synthase by distinct mechanisms in skeletal muscle (1, 37).

In the present study, enhanced glucose disposal when exercise was performed in conjunction with insulin infusion was associated with increased rates of glucose storage (glycogen synthesis). This is especially true for the diabetic subjects, in whom insulin alone only minimally stimulated glucose storage. Glucose oxidation rates for diabetic subjects were significantly reduced compared with those for nondiabetic subjects basally and during insulin stimulation with or without exercise. This finding is consistent with previous studies and may be contributed to decreased pyruvate dehydrogenase activity basally and during insulin stimulation (21). Nevertheless, exercise did not increase subsequent insulin stimulation of glucose oxidation in either group. Therefore, the exercise-induced increase in insulin-stimulated glucose uptake was selectively shunted toward glycogen synthesis in nondiabetic and diabetic subjects. It is likely that increased routing of intracellular glucose to glycogen synthesis was due to the increase in glycogen synthase activity. However, it is less clear that the increase in glycogen synthase activity led to increased glucose disposal. Rather, because insulin and muscle contraction induce translocation of GLUT-4 transporters to the plasma membrane of muscle cells (16, 19), it is likely that the increase in glucose disposal was due to increased GLUT-4 translocation, even though this was not measured in the present study. It has been proposed that exercise and insulin recruit GLUT-4 from distinct pools of transporters (12). If this is the case, it might be predicted that exercise performed in conjunction with insulin infusion would result in an additive increase in glucose uptake. Because the effect of exercise on insulin-stimulated glucose disposal was synergistic in the present study, mechanisms in addition to GLUT-4 translocation are likely to be involved. It is also possible that an exercise-induced increase in hexokinase activity contributed to the increase in glucose uptake (18, 26).

There were clear indications of changes in blood flow during exercise. For instance, insulin clearance was decreased in control subjects, suggesting that an increase in blood flow to working muscle may have resulted in decreased splanchnic blood flow. This did not occur in the diabetic subjects, possibly because of the lower absolute work rate. The net result was slightly higher insulin concentration with exercise + insulin in the obese nondiabetic control subjects. This may have contributed to the increase in insulin signaling in this group. However, results from higher insulin infusion rates indicate that the magnitude of increase in insulin concentrations was insufficient to have much of an

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**Fig. 7.** Effect of insulin and insulin + exercise on glycogen synthase activity for control (A) and diabetic (B) subjects. Basal (open bars) and insulin-stimulated (solid bars) glycogen synthase activity in muscle samples without and with concomitant exercise was assayed in the presence of 0.1 and 10 mM glucose 6-phosphate (GS0.1 and GS10). Glycogen synthase fractional velocity was then calculated as the ratio of GS0.1 to GS10 for 8 control and 6 diabetic subjects. *P < 0.01; $P < 0.05$ vs. basal. †$P < 0.01$; ‡$P < 0.05$ vs. no exercise.
SYNERGISTIC EFFECT OF EXERCISE ON INSULIN ACTION


