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


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 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).
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 diabetes or glycogen synthase activity.

Criteria. All nondiabetic subjects 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 diabetes. Three of the seven diabetic subjects were taking glyburide, than having diabetes, diabetic subjects were in good health. None of the subjects had participated in a regular exercise program for ≥1 yr before entering the study. Peak aerobic capacity [i.e., peak O2 uptake (V\textsubscript{O2 peak})] was determined using an incremental cycle ergometer protocol. Criteria for test completion were a respiratory exchange ratio >1.1 and no further increase in O2 uptake (V\textsubscript{O2}) and/or heart rate. The heart rate corresponding to 70% V\textsubscript{O2 peak} was used for the insulin clamp + exercise protocol.

Insulin clamp without simultaneous exercise. At least 1 wk after the V\textsubscript{O2 peak} test, the subjects reported to the General Clinical Research Center (GCRC) at 8 AM after consuming nothing but water since the prior evening (Fig. 1A). An antecubital vein was cannulated for infusion of [3-3H]glucose, 20% dextrose, and insulin (Humulin, Eli Lilly, Indianapolis, IN). A hand vein was cannulated in a retrograde fashion, and the hand was placed in a heated box (60°C) for sampling of arterialized blood. To ensure isotopic equilibrium, a primed (25 \mu Ci × fasting plasma glucose/90), continuous (0.25 \mu Ci/min) infusion of [3-3H]glucose was started 2 h (nondiabetic subjects) or 3 h (diabetic subjects) before the start of insulin infusion. At 60 min before the start of insulin infusion, a percutaneous biopsy of the vastus lateralis muscle was obtained with a Bergstrom cannula under local anesthesia (2). Muscle biopsy specimens were immediately blotted free of blood, frozen, and stored in liquid nitrogen until used. Arterialized blood was sampled for measuring plasma glucose, insulin, and [3-3H]glucose specific activity. Blood samples were obtained at baseline and every 10 min during the last 30 min of the isotopic equilibration period. Continuous indirect calorimetry was performed with a ventilated hood system (DeltaTrac, Sensor Medics, Anaheim, CA) during the last 30 min of the tracer equilibration (basal) and insulin clamp periods for measurement of carbohydrate and lipid oxidation rates. Leg blood flow was measured using strain-gauge plethysmography basally and after 55 and 120 min of insulin infusion. After completion of the tracer equilibration period, a primed, continuous infusion of insulin was started at 40 mU·m\textsuperscript{-2}·min\textsuperscript{-1} for 120 min. Plasma glucose was measured every 5 min throughout the study with a glucose oxidase analyzer (Beckman Instruments, Fullerton, CA) and maintained at euglycemia (90–100 mg/dl) using a variable infusion of 20% dextrose. After 30 min of insulin infusion, a second biopsy was obtained from the opposite vastus lateralis muscle.

Insulin clamp with simultaneous exercise. On another day ≥2 wk after the first euglycemic, hyperinsulinemic clamp, subjects returned to the GCRC at 8 AM after consuming nothing but water since the prior evening. On this occasion, the previous protocol was followed with the following modifications. At the initiation of the insulin infusion, subjects began exercising on a recumbent cycle ergometer for 30 min at a heart rate corresponding to 70% V\textsubscript{O2 peak} (Fig. 1B). Heart rate was monitored by electrocardiography, and exercise intensity was adjusted as necessary to maintain the target heart rate. After 30 min of exercise, a second biopsy of the vastus lateralis muscle was performed as soon as possible.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Nondiabetic (n = 16)</th>
<th>Diabetic (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>35 ± 2</td>
<td>45 ± 4*</td>
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<tr>
<td>BMI, kg/m\textsuperscript{2}</td>
<td>28.3 ± 0.4</td>
<td>30.8 ± 1.2*</td>
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<tr>
<td>HbA\textsubscript{1c}</td>
<td>4.9 ± 0.1</td>
<td>7.8 ± 0.9†</td>
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<tr>
<td>Fasting plasma glucose, mg/dl</td>
<td>94 ± 2</td>
<td>149 ± 16‡</td>
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<tr>
<td>Fasting plasma insulin, uU/ml</td>
<td>9 ± 3</td>
<td>12 ± 3</td>
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<tr>
<td>Cholesterol, mmol/l</td>
<td>186 ± 11</td>
<td>192 ± 4</td>
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<tr>
<td>Triglycerides, mmol/l</td>
<td>106 ± 12</td>
<td>319 ± 140*</td>
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<tr>
<td>Ethnicity</td>
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<td>2C/5H</td>
</tr>
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</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 (Ser\(^{473}\)) 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 (Ser\(^{473}\)) 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 [\(\gamma\)\(^{32}\)P]ATP was obtained from NEN (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 \(\beta\)-glycerophosphate, 10 mM NaF, 2 mM Na\(_3\)VO\(_4\), 2 mM EDTA (pH 8.0), 1% Nonidet P-40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 1 mM MgCl\(_2\), 1 mM CaCl\(_2\), 10 \(\mu\)g/ml leupeptin, and 10 \(\mu\)g/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 \(\times\) 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). Supernatant was stored at –80°C until used.

SDS-PAGE and immunoblotting. For phospho-Akt (Ser\(^{473}\)), 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 (Ser\(^{473}\)) immunoblot was stripped using a buffer containing 0.7% \(\beta\)-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 \(\mu\)g) was immunoprecipitated with anti-IRS-1 antibody, and PI3-kinase activity was measured by determining incorporation of [\(\gamma\)\(^{32}\)P]ATP into [\(\gamma\)\(^{32}\)P]phosphatidylinositol phosphate, as previously described (8).

Glycogen synthase activity. Glycogen synthase (GS) activities were assayed using 0.1 mM (GS\(_{0.1}\)) and 10 mM (GS\(_{10}\)) glucose 6-phosphate, as previously described (32). Glycogen synthase fractional velocity (GSF\(_{V}\)) was calculated as the ratio of GS\(_{0.1}\) to GS\(_{10}\). Changes in GSF\(_{V}\) 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 \(\dot{V}_O_2\) and \(\dot{V}_CO_2\) 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-
During the exercise, by design, was relative to an individual trained at 70% \( V_{\text{O}_2}\) peak concomitant exercise (Fig. 1). When exercise was performed on separate occasions, once without and once with insulin infusion, are given in Table 2. Diabetic subjects had reduced maximum aerobic capacity (\( V_{\text{O}_2}\) peak) compared with control subjects (\( P < 0.001\)). In conjunction with the decreased aerobic capacity of the diabetic subjects, less work was performed during a \( V_{\text{O}_2}\) peak test (\( P < 0.01\)). Maximum heart rate achieved during the \( V_{\text{O}_2}\) peak test, as well as the heart rate at which subjects exercised during the insulin infusion, was not significantly different between the groups.

**Euglycemic clamps.** Subjects underwent two euglycemic, hyperinsulinemic clamps (40 mU·m\(^{-2}·\text{min}^{-1}\)) on separate occasions, once without and once with concomitant exercise (Fig. 1). When exercise was performed together with insulin infusion, subjects exercised at 70% \( V_{\text{O}_2}\) 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 \( V_{\text{O}_2}\) peak, the diabetic subjects on average exercised at a lower \( V_{\text{O}_2}\) (\( P < 0.001\)) 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 \( \mu \text{U/ml} \) and fell to 61 ± 3 \( \mu \text{U/ml} \) during the first 30 min of insulin alone, whereas concentrations peaked at 90 ± 5 \( \mu \text{U/ml} \) and fell to 72 ± 2 \( \mu \text{U/ml} \) 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)\(^{-1}·\text{min}^{-1}\) at 30 min and then further to an average of 6.6 ± 0.7 mg·kg FFM\(^{-1}·\text{min}^{-1}\) 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\(^{-1}·\text{min}^{-1}\) after 30 min and then decreased gradually after cessation of exercise to an average of 8.1 ± 0.7 mg·kg FFM\(^{-1}·\text{min}^{-1}\) 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 disposal was significantly greater than that of the control subjects (Fig. 4B). In contrast, exercise did not increase the rate of glucose disposal in diabetic subjects (Fig. 4C).
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 Ser473 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 Ser473 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 GS0.1 and GS10 to determine active and total forms of the enzyme. Under basal conditions, neither GS0.1 nor GSFV differed with the insulin-alone and insulin + exercise for control or diabetic subjects (Table 4, Fig. 7). Insulin alone stimulated GS0.1 and GSFV 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 GS0.1 and GSFV 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-
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 FFM⁻¹·min⁻¹, an increase of 4.9 ± 0.6 mg·kg FFM⁻¹·min⁻¹ over that achieved 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 FFM⁻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 FFM⁻¹·min⁻¹, an increase of 3.7 ± 0.8 mg·kg FFM⁻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 FFM⁻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

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**Table 3. Glucose metabolism during euglycemic, hyperinsulinemic clamps**

<table>
<thead>
<tr>
<th></th>
<th>Nondiabetic</th>
<th></th>
<th>Diabetic</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>−Exercise</td>
<td>+Exercise</td>
<td>−Exercise</td>
<td>+Exercise</td>
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<td>Basal</td>
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<tr>
<td>Oxidation</td>
<td>2.3 ± 0.2</td>
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<td>1.4 ± 0.5</td>
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<tr>
<td>Storage</td>
<td>0.5 ± 0.2</td>
<td>2.9 ± 0.9</td>
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<tr>
<td>Disposal</td>
<td>2.8 ± 0.1</td>
<td>6.9 ± 1.3</td>
<td>2.9 ± 0.2</td>
<td>4.2 ± 0.6</td>
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</table>

Values are means ± SE in mg·kg fat-free mass⁻¹·min⁻¹; n = 7. Subjects underwent 2 euglycemic, hyperinsulinemic clamps (40 mU/m·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.

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**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.

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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 VO2 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

<table>
<thead>
<tr>
<th></th>
<th>− Exercise Basal</th>
<th>− Exercise Insulin</th>
<th>+ Exercise Basal</th>
<th>+ Exercise Insulin</th>
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<td>Nondiabetic (n = 8)</td>
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<tr>
<td>GS0.1</td>
<td>1.3±0.3</td>
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<td>1.5±0.4</td>
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<td>GS10</td>
<td>13.2±1.9</td>
<td>14.3±1.9</td>
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<td>11.7±0.9</td>
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<tr>
<td>Diabetic (n = 6)</td>
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<tr>
<td>GS0.1</td>
<td>2.2±0.5</td>
<td>3.6±0.7</td>
<td>2.3±0.6</td>
<td>4.1±0.9†</td>
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<tr>
<td>GS10</td>
<td>15.2±2</td>
<td>15.6±1.3</td>
<td>13.5±2.2</td>
<td>13.0±1.8</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 (GS0.1) and 10 mM (GS10) glucose 6-phosphate. *P < 0.05; †P < 0.01 vs. basal. §P < 0.05 vs. −exercise.
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.

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
effect (unpublished observation). Nevertheless, blood flow to working muscle was doubtlessly increased (10).

In summary, exercise performed in conjunction with insulin administration synergestically increases glucose disposal compared with the effect of insulin alone in nondiabetic and diabetic subjects. The exercise-induced increase in glucose uptake is then selectively routed toward glycogen synthesis. This event is likely mediated by increased glycolgen synthase activity. At least in the nondiabetic group, increased PI3-kinase signaling may contribute to the exercise-induced synergestic enhancement of glucose disposal. Therefore, although increased blood flow to working muscle undoubtedly increases delivery of insulin and glucose, qualitative changes within the muscle contribute to the increase in glucose uptake and routing of this additional glucose to glycogen stores.

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DISCLOSURES

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