Insulin Signaling and Insulin Sensitivity After Exercise in Human Skeletal Muscle

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Muscle glucose uptake, glycogen synthase activity, and insulin signaling were investigated in response to a physiological hyperinsulinemic (600 pmol/l)-euglycemic clamp in young healthy subjects. Four hours before the clamp, the subjects performed one-legged exercise for 1 h. In the exercised leg, insulin more rapidly activated glucose uptake (half activation time [$t_{1/2}$] = 11 vs. 34 min) and glycogen synthase activity ($t_{1/2}$ = 8 vs. 17 min), and the magnitude of increase was two- to four-fold higher compared with the rested leg. However, prior exercise did not result in a greater or more rapid increase in insulin-induced receptor tyrosine kinase (IRTK) activity ($t_{1/2}$ = 50 min), serine phosphorylation of Akt ($t_{1/2}$ = 1–2 min), or serine phosphorylation of glycogen synthase kinase-3 (GSK-3) ($t_{1/2}$ = 1–2 min) or in a larger or more rapid decrease in GSK-3 activity ($t_{1/2}$ = 3–8 min). Thirty minutes after cessation of insulin infusion, glucose uptake, glycogen synthase activity, and signaling events were partially reversed in both the rested and the exercised leg. We conclude the following: *1*) physiological hyperinsulinemia induces sustained activation of insulin-signaling molecules in human skeletal muscle; *2*) the more distal insulin-signaling components (Akt, GSK-3) are activated much more rapidly than the proximal signaling molecules (IRTK as well as insulin receptor substrate 1 and phosphatidylinositol 3-kinase [Wojtaszewski et al., *Diabetes* 46:1775–1781, 1997]); and *3*) prior exercise increases insulin stimulation of both glucose uptake and glycogen synthase activity in the absence of an upregulation of signaling events in human skeletal muscle. *Diabetes* 49:325–331, 2000

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%FV, fractional velocity; %I-form, percent of glucose-6-phosphate–independent glycogen synthase; AUC, area under the curve; G-6-P, glucose-6-phosphate; GSK-3, glycogen synthase kinase 3; IRS-1, insulin receptor substrate 1; IRTK, insulin receptor tyrosine kinase; PI, phosphatidylinositol; $t_{1/2}$, half activation time; TBST buffer, 10 mmol/l Tris-base (pH 7.8) buffer containing 50 mmol/l NaCl and 0.05% Tween-20.

Muscle glucose transport and glycogen synthase activity are increased immediately after a single bout of exercise (1). In human skeletal muscle, the effects of exercise per se on muscle glucose transport are relatively short-lived (2–4 h), whereas the enhanced sensitivity for glucose transport activation by insulin has been observed >48 h after an exercise bout in human subjects (3–5). In rat skeletal muscle, it has been demonstrated that there is a marked increase in insulin sensitivity for both glucose transport and glycogen synthase activation after exercise (2,6). These changes facilitate glycogen resynthesis, and they may be the mechanism by which muscle glycogen storage is increased above pre-exercise values, known as "supercompensation" (7,8). Whether prior exercise also increases the sensitivity for glycogen synthase activation by insulin in human skeletal muscle is unknown.

We have previously hypothesized that an upregulation of insulin signaling is involved in the increased insulin sensitivity after exercise (9). However, if humans are subjected to physiological hyperinsulinemia or if rat muscles are incubated in the presence of insulin 3–4 h after exercise, insulin receptor tyrosine kinase (IRTK) activity, insulin receptor substrate 1 (IRS-1) tyrosine phosphorylation, and phosphatidylinositol (PI) 3-kinase activity are not enhanced in skeletal muscle (9,10). This suggests that exercise may modulate insulin signaling further downstream or affect processes directly involved in glucose transporter translocation and activation.

Signaling involving D-3 phosphorylated inositol lipids, generated by the action of PI 3-kinases, has been suggested to lead to the metabolic effects of insulin, including the activation of glucose transport and glycogen synthase (11,12). PI-dependent kinase-1 (PDK-1) and Akt are signaling intermediaries downstream of PI 3-kinase that are activated with insulin treatment (13,14). Akt has been linked to glucose transport activation based on the findings that overexpression of constitutively active Akt constructs leads to enhanced glucose transport in 3T3-L1 adipocytes and L6 muscle cells (15–17). However, this link is still controversial: studies have reported a mismatch between Akt and glucose transport activation by insulin in skeletal muscle (18–21), and expression of a dominant negative Akt construct did not affect insulin-stimulated glucose transport in Chinese hamster ovary cells (22).

Recent studies suggest that insulin activates muscle glycogen synthase in part by decreasing the activity of an upstream kinase, glycogen synthase kinase 3 (GSK-3) (23,24). In vitro, several kinases (JNK, $p90^{RSK}$, $p70^{S6K}$, Akt)

have been shown to phosphorylate and inactivate GSK-3 (25–28). In skeletal muscle, Akt has been identified as the major GSK-3 kinase, and the sites of GSK-3 phosphorylation by Akt are reported to be the same as those phosphorylated in response to insulin in vivo (26). Thus, Akt and GSK-3 may be involved in insulin's regulation of glucose transport and glycogen synthase activity.

We have investigated the time course of Akt activation and GSK-3 deactivation in response to physiological hyperinsulinemia in human skeletal muscle. We have determined the temporal relationship between activation of these molecules and the increases in glucose uptake and glycogen synthase activity. In addition, we investigated whether an upregulation of these insulin-signaling intermediaries may account for increased insulin sensitivity for glucose uptake and glycogen synthase activity after exercise.

RESEARCH DESIGN AND METHODS

Subjects. Seven healthy men (age 22 ± 1 years) gave their informed consent to participate in the study, which was approved by the Copenhagen Ethics Committee. Body weight, height, and BMI were 76 ± 4 kg, 184 ± 3 cm, and 22 ± 1 kg/m^2, respectively. One to two weeks before the experiment, maximal pulmonary oxygen consumption was determined during an incremental bicycle ergometer test (53 ± 2 ml · kg^{-1} · min^{-1}), and the subjects were accustomed to the one-leg dynamic knee-extensor ergometer with one leg (29) before an incremental knee-extensor test was performed to determine the peak work capacity of the knee-extensor (52 ± 5 W) as previously described (9,30).

Experimental protocol. Subjects were instructed to eat a mixed diet and to abstain from strenuous physical activity for 30 h before the experiment. The subjects ate a light breakfast (~1,500 kJ) 2 h before arrival at the laboratory. Subjects performed 60 min of repeated dynamic one-leg knee-extensor exercise (1 kick/s), using a slight modification of our previously described protocols (9,30). The workload was varied every 5 min and alternately set at 75 and 95% of each subject's maximum one-legged knee-extensor work capacity. Four subjects were selected at random to exercise the dominant leg, and the three other subjects exercised the nondominant leg. After the exercise bout, the subjects rested in the supine position, and Teflon catheters were inserted below the inguinal ligament in one femoral artery and in both femoral veins, as described (9,30). Through each venous catheter, a thermistor (Edslab probe 94-030-2.5F; Baxter, Allerod, Denmark) was inserted and advanced 6–8 cm proximal to the catheter tip. Additional catheters were placed in an antecubital and a forearm vein for glucose and insulin infusion, respectively.

Four hours after the exercise bout, blood samples were drawn simultaneously from the arterial catheter and both venous catheters, and thigh blood flow was measured in both legs by the constant-infusion thermodilution method as previously described (9,30). Needle biopsies from both quadriceps femoris muscles were obtained under local anesthesia and quickly frozen in the needles within ~5 s. Four subjects had the biopsies taken in the rested leg before the exercised leg. At each time point, the two biopsies were taken within 30 s. A one-step euglycemic-hyperinsulinemic clamp was initiated by an intravenous bolus injection of insulin given over 1 min (9 mU/kg), followed by constant infusion of insulin for 120 min (1.5 mU · min^{-1} · kg^{-1}) (Actrapid; Novo Nordisk, Bagsvaerd, Denmark). After termination of the insulin infusion, the experiment was continued for an additional 30 min. Blood samples were drawn before (0 min), during (7, 15, 30, 45, 60, 90, and 120 min), and after (125, 130, 140, and 150 min) insulin infusion. Blood flow in both thighs was measured immediately after each blood sampling. Needle biopsies were taken from both legs at 7, 15, 60, 120, and 150 min. Three incisions spaced 4–5 cm apart were made in each thigh, and two biopsies were taken through each incision, with the needle pointed distally during the first biopsy and proximally during the second biopsy. Our control experiments have shown that the enzymes studied (insulin receptor kinase, Akt, GSK-3, and glycogen synthase) are not activated by this sampling technique. To prevent a decrease in plasma potassium concentration during the insulin clamp, 30 mmol KCl (Kaleorid, Leo, Denmark) was administered orally.

Analytical procedures. Glucose concentrations in blood and plasma, plasma insulin concentrations, blood hemoglobin content, blood oxygen saturation, and pulmonary oxygen uptake were all measured as previously described (9). For determination of glycogen content, muscle biopsies were freeze-dried and dissected free of blood, fat, and connective tissue before analysis. Glycogen was measured as glycosyl units after acid hydrolysis (31). Muscle glycogen synthase activity was determined by a modification of the method of Thomas et al. (32) as described by Richter et al. (30). Glycogen synthase activity was determined in the presence of 0, 0.17, and 8 mmol/l glucose-6-phosphate (G-6-P) and given either as the percent G-6-P–independent glycogen synthase (%I-form) (100 × activity in the absence of G-6-P divided by the activity at 8 mmol/l G-6-P [saturated]) or as the fractional velocity (%FV) (100 × activity in the presence of 0.17 mmol/l G-6-P divided by the activity at 8 mmol/l G-6-P).

For measurements of IRTK activity, GSK-3α activity, and immunoblotting, the frozen biopsies were dissected free of visual blood, fat, and connective tissue and processed as described (9). Solubilized protein concentrations were determined using a BCA Protein Assay Reagent kit and a microtiter plate protocol at 37°C for 30 min (Pierce, Rockford, IL). Unless stated otherwise, the chemicals used were all of analytical grade from Sigma Chemical (St. Louis, MO).

IRTK activity and insulin binding were measured as described previously (9). A filter paper (P-81) assay using a phospho-GS2 peptide (Upstate Biotechnology, Lake Placid, NY) as substrate was used to measure GSK-3 activity, as described previously (33). GSK-3 was immunoprecipitated from 100 µg of muscle protein using an anti–GSK-3α antibody (Upstate Biotechnology) bound to protein G Sepharose.

For immunoblotting, equal amounts of solubilized proteins (80 µg) were resolved by SDS-PAGE and transferred to nitrocellulose membranes. The membrane was blocked with 5% skim milk in a 10 mmol/l Tris-base (pH 7.8) buffer containing 50 mmol/l NaCl and 0.05% Tween-20 (TBST buffer) and incubated with primary antibody—α-phospho-specific serine^{473} Akt (New England Biolabs, Beverly, MA), α-phospho-specific GSK-3αSer^{21} (Upstate Biotechnology), α-COOH-terminal insulin receptor (β-subunit) (provided by C.R. Kahn, Joslin Diabetes Center [34]), α-IRS-1 (polyclonal antiserum made against a GST fusion peptide of the 511–589 amino acid residues of the mouse IRS-1; provided by C.R. Kahn), α-p85 (Upstate Biotechnology), α-GLUT4 (provided by R.J. Smith, Joslin Diabetes Center [35]), and α-Akt (Upstate Biotechnology)—in TBST buffer containing 3% bovine serum albumin or 3% skim milk. Immune complexes were visualized by either chemiluminescence (Amersham Life Science, Little Chalfont, U.K.) or [^{125}I]protein A (New England Nuclear, Boston, MA) detection. For enhanced chemiluminescence detection, specific bands were quantified using densitometric scanning. For experiments using ^{125}I detection, a phosphoimager was used for quantification.

Calculations and statistics. Exchange of glucose, oxygen, and insulin was calculated by multiplying arteriovenous differences with blood or plasma flow as appropriate. Exchange of substrate was calculated and expressed per kilogram thigh muscle (7.7 ± 0.4 kg, n = 14). Control samples were added to all immunoblots and activity assays, and assay-to-assay variation was accounted for by expressing data relative to these samples. Assuming that the different cellular processes have a mono-exponential time course, the half activation time ($t_{1/2}$) was calculated from the velocity constant (k) obtained by fitting data (SigmaPlot, Jandel Scientific Software) using the least-squares method to the equation: $y = \alpha[1 - \exp(-xk)] + \beta$.

Data are expressed as means ± SE. Statistical evaluation was done by paired Student's t test and one- or two-way analysis of variance with repeated measurements, as appropriate. When analysis of variance revealed significant differences, a post hoc test was used to correct for multiple comparisons (Student-Newman-Keuls test). Differences between groups were considered statistically significant if P was <0.05.

RESULTS

One-legged exercise. One-legged exercise was performed in 5-min intervals with an alternating work intensity of 75% (39 ± 3 W) and 95% (50 ± 4 W) of the maximal aerobic work capacity of the knee extensor. During these intervals, pulmonary oxygen uptake was on average 18% (0.98 ± 0.05 liters O_2/min) and 23% (1.2 ± 0.1 liters O_2/min) of the maximal whole-body oxygen uptake.

Hyperinsulinemic-euglycemic clamp. A hyperinsulinemic-euglycemic clamp was initiated 4 h after the one-legged exercise bout. The arterial plasma insulin concentrations increased immediately after the bolus injection of insulin and reached a plateau of ~600 pmol/l within 15 min (Fig. 1*A*). The plasma insulin concentrations rapidly decreased to a level not different from preclamp values 30 min after insulin infusion was terminated. Insulin clearance was the same in the two legs throughout the time course and, interestingly, became negative in both legs in the period following the insulin infusion (data not shown). Euglycemia was maintained with a coefficient of variation <7% (Fig. 1*B*) by

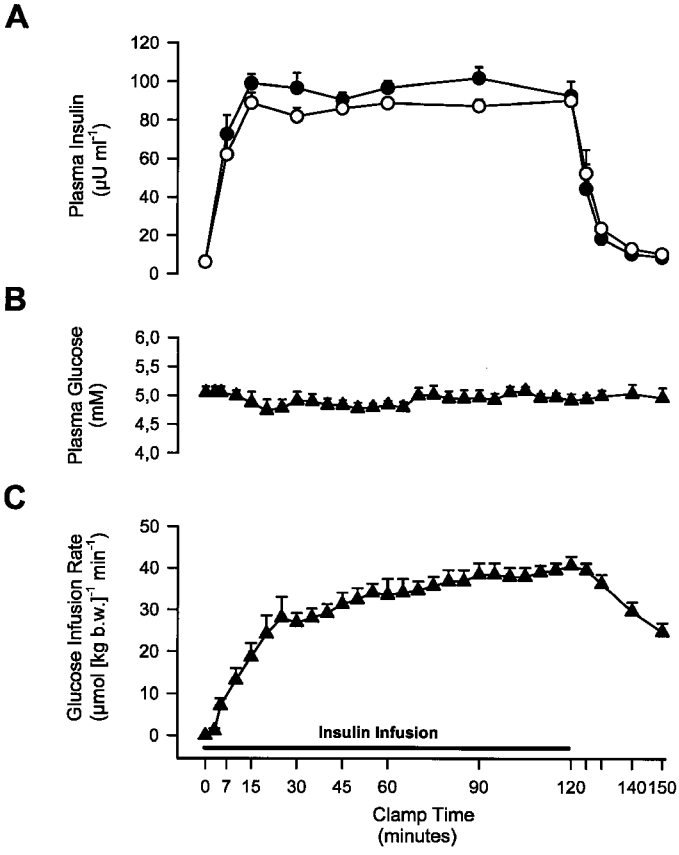

FIG. 1. Hyperinsulinemic-euglycemic clamp. Femoral venous (○) and arterial (●) plasma insulin concentrations (*A*), arterial plasma glucose concentrations (*B*), and glucose infusion rates (*C*) before, during, and after the insulin infusion. The femoral venous concentrations of insulin did not differ between rested and exercised legs and thus for clarity are presented as the average values for the two legs. Plasma insulin concentrations and glucose infusion rates were significantly increased during and significantly decreased after insulin infusion (both, $P < 0.001$). 1 μU/ml = 6 pmol/l insulin. Data are means ± SE, $n = 7$.

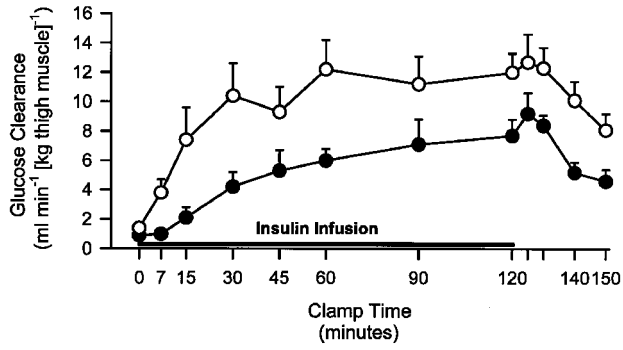

FIG. 2. Leg glucose clearance. Glucose clearance in the rested (●) and exercised (○) leg before, during, and after the clamp. Glucose clearance was calculated from the blood glucose concentration and blood flow in accordance to the equation $\{([G]_a - [G]_v)F\}[G]_a^{-1}$ and expressed to total thigh muscle mass (average 7.7 ± 0.4 kg, $n = 14$). Glucose clearance was similar before insulin infusion, but increased faster and to a greater extent in the exercised than in the rested leg during the clamp ($P < 0.005$). A partial but significant decrease in glucose clearance was observed in both legs following insulin infusion ($P < 0.05$). Data are means ± SE, $n = 7$.

increasing the glucose infusion rate to a steady-state level of ~40 μmol · kg^{-1} · min^{-1} (Fig. 1*C*). The time to reach half of the steady-state glucose infusion rate ($t_{1/2}$) was ~17 min. The glucose infusion rate was significantly decreased in the period after insulin infusion, but it was still markedly elevated compared with zero at the latest time point (150 min).

Leg glucose clearance. Four hours after exercise, leg blood flow (43 ± 4 vs. 47 ± 4 ml · min^{-1} · kg^{-1} muscle) and the arteriovenous glucose concentration difference (0.12 ± 0.03 vs. 0.16 ± 0.04 mmol/l) were not different between the rested and the exercised leg, respectively, resulting in an equal glucose clearance in the two legs (Fig. 2). In response to insulin infusion, glucose clearance increased faster ($t_{1/2}$ = 11 vs. 34 min) and to a greater extent in the previously exercised leg than in the rested leg. Over the 2 h of insulin infusion, total leg glucose clearance (calculated as the area under the curve $[AUC]_{0-120}$) was approximately twofold higher in the exercised leg compared with the rested leg (AUC_{0-120} 1,110 ± 147 vs. 590 ± 110 ml/kg muscle, $P < 0.005$). A minor part of this enhanced clearance was due to a slightly higher blood flow during insulin infusion in the previously exercised leg compared with the rested leg (average$_{7-120}$ 50 ± 2 vs. 42 ± 3 ml · min^{-1} · kg^{-1} muscle, $P < 0.05$), whereas the majority was due to a higher glucose extraction indicated by a significantly greater arteriovenous difference across the exercised leg (average$_{7-120}$ 0.82 ± 0.06 vs. 0.50 ± 0.05 mmol/l, $P < 0.001$). These results indicate that increased glucose uptake in the exercised leg reflects cell-related processes (glucose transport activation) rather than changes in hemodynamics. Thirty minutes after cessation of insulin infusion, glucose clearance was still significantly higher than basal values. Lactate release was similar for the two legs throughout the experiment (data not shown) and increased on average from 3 μmol · min^{-1} · kg^{-1} muscle at basal to 5 μmol · min^{-1} · kg^{-1} muscle after 2 h of insulin infusion.

Muscle glycogen concentration. Four hours after exercise, glycogen concentrations were 40% lower in the exercised than in the rested thigh muscle (Table 1). Insulin infusion resulted in a small, but significant, increase in glycogen content in both rested and exercised muscle (~6%). Based on total glucose uptake and lactate release during the clamp, and assuming that the glucose taken up into the muscles was not oxidized, it can be predicted that there would only be a modest increase in muscle glycogen content. Thus, 2 h of physiological euglycemic hyperinsulinemia only slightly

TABLE 1
Skeletal muscle glycogen concentration

	Clamp time (min)		
	0	60	120
Rested	495 ± 18	468 ± 28	524 ± 12
Exercised	289 ± 26	291 ± 27	307 ± 23

Data are means ± SE, $n = 7$. Glycogen content (mmol/kg muscle) in vastus lateralis muscle before (0 min) and during (60 and 120 min) the clamp. Glycogen content was lower in exercised muscle compared with rested muscle at all time points ($P < 0.003$). Insulin induced a small but significant increase in glycogen content after 120 min of infusion in both rested and exercised muscle ($P < 0.05$).

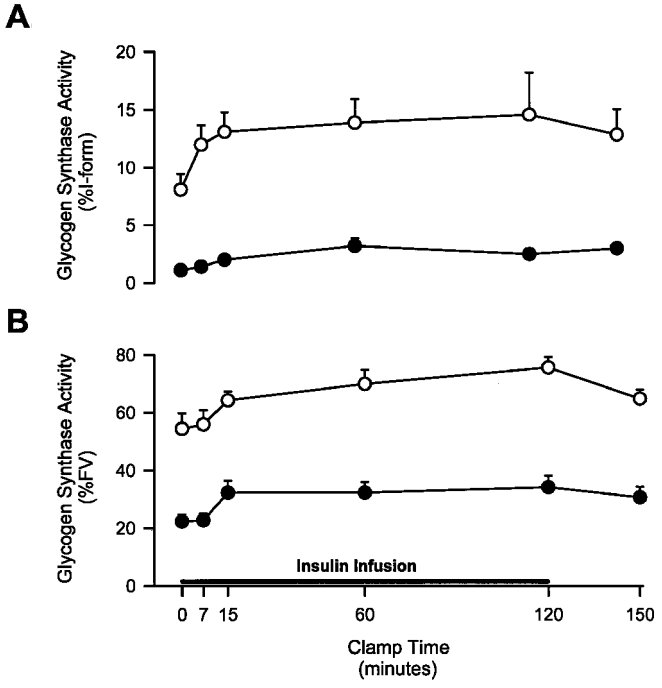

FIG. 3. Skeletal muscle glycogen synthase activity. Glycogen synthase activity, given as both %I-form (*A*) and %FV (*B*), was higher at all time points in the exercised muscle (○) compared with the rested muscle (●) ($P < 0.003$ and $P < 0.001$, respectively). Glycogen synthase activity (both forms) was activated by insulin in both rested and exercised muscle ($P < 0.002$), and the synthase activation (both forms) was greater in the exercised thigh muscle compared with rested muscle ($P < 0.02$ and $P < 0.06$ for %I-form and %FV, respectively). Total glycogen synthase activity was not different between rested and exercise muscles at any time point, nor was total synthase activity changed by insulin infusion. The average total glycogen synthase activity for rested and exercised muscle was 4.4 ± 0.2 and 4.8 ± 0.2nmol· min^{-1} · (mg wet $wt)^{-1}$, respectively ($n = 42$). Data shown are means ± SE, $n = 7$.

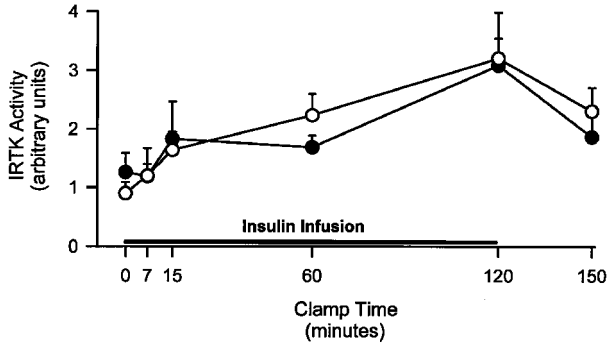

FIG. 4. Skeletal muscle IRTK activity. IRTK activity in rested (●) and exercised thigh muscle (○) was increased ($P < 0.001$) by insulin treatment and significantly decreased ($P < 0.05$) 30 min after termination of insulin infusion. IRTK activity was not different at any time point between the two legs. IRTK activity was measured as phosphorylation of poly-Glu-Tyr and expressed per amount of insulin receptors present in each sample as determined subsequently by [^{125}I]insulin binding. Data are means ± SE, $n = 7$.

increases glycogen content in human muscle, even in the muscle with lowered glycogen content.

Glycogen synthase activity. Glycogen synthase activity was significantly higher in the previously exercised muscle than in the rested muscle, expressed both as the %I-form (Fig. 3*A*) and as %FV (Fig. 3*B*), indicating a marked and sustained effect of the exercise bout performed 4 h earlier. Two hours of hyperinsulinemia significantly increased glycogen synthase activity in both rested and exercised muscle, and there was a significantly greater effect on glycogen synthase activity in the %I-form (Δ_{0-120} = 7.5 ± 1.5 vs. 1.6 ± 0.3) and the %FV (Δ_{0-120} = 17 ± 4.0 vs. 8.5 ± 2.1) in exercised muscle compared with rested muscle. In addition, glycogen synthase activity in the %I-form reached half activation faster ($t_{1/2}$ = 8 vs. 17 min) in exercised than in rested muscle. This was not the case for %FV, where half activation rates were similar. Glycogen synthase activity (%I-form or %FV) did not decrease significantly 30 min after insulin infusion in either rested or exercised muscle.

IRTK activity. Because we previously observed a significant time lag between the rise in plasma insulin concentrations and insulin action in skeletal muscle (9), we measured IRTK activity as an indicator of the arrival of insulin at the surface of the muscle fibers. IRTK activity was not different between exercised and rested muscle at any time point during the experiment (Fig. 4). Insulin significantly stimulated the IRTK activity with a half-maximal activation time of >50 min in both legs. In contrast to a range of metabolic processes stimulated by insulin (glucose infusion rate, glucose clearance, glycogen synthase activity), the IRTK activity did not reach a plateau during the 2 h of hyperinsulinemia. In addition, whereas the plasma insulin concentration returned to basal levels 30 min after insulin infusion (Fig. 1*A*), the IRTK activity was only partially reversed in both legs, which may be the mechanism for the partially sustained glucose clearance and glycogen synthase activity 30 min after insulin infusion (Figs. 2 and 3).

Akt phosphorylation. In vivo insulin-stimulated Akt activity in rodent skeletal muscle is highly correlated with phosphorylation of Akt on Ser^{473} ($r^2 = 0.89$, $P < 0.001$, $n = 42$) (J.F.P.W., L.J.G., unpublished observations). In the present study, we evaluated the degree of Akt activation by the degree of serine phosphorylation. Four hours after exercise, Akt phosphorylation was low and similar in the two legs (Fig. 5). Upon insulin infusion, there was a very rapid ($t_{1/2}$ = 1–2 min) increase in phosphorylation during the first 15 min, which was maintained during the next 105 min of insulin infusion. Akt phosphorylation significantly decreased 30 min after cessation of insulin infusion in muscles from both legs, but did not reach basal levels. Akt phosphorylation was not different between the exercised and rested muscle at any time.

GSK-3αSer^{21} phosphorylation and GSK-3 activity. GSK-3 is a serine/threonine kinase that is deactivated by insulin via serine phosphorylation (23,24). The deactivation of GSK-3 may lead to dephosphorylation and thus activation of glycogen synthase. In the present study, Ser^{21} phosphorylation of GSK-3α mimicked the degree of Akt Ser^{473} phosphorylation, with a significant and very rapid activation phase over the first few minutes ($t_{1/2}$ = 1–2 min) (Fig. 6*A* and *B*). This level of phosphorylation was maintained throughout the clamp. Thirty minutes after termination of the insulin infusion, GSK-3 serine phosphorylation was significantly decreased by ~50% in muscle from both legs. There were no differences between the exercised and the rested legs at any time during the experiments.

Because GSK-3 activity may also be regulated by mechanisms other than serine phosphorylation (e.g., by tyrosine

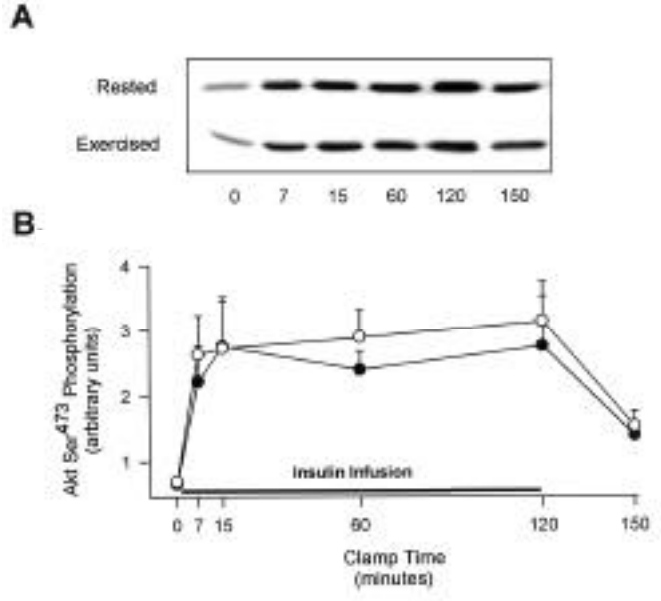

FIG. 5. Akt phosphorylation. Akt Ser^{473} phosphorylation (*A* and *B*) in rested (●) and exercised (○) thigh muscle. Akt phosphorylation was determined using a phospho-specific antibody that recognizes Akt only when phosphorylated on serine 473. A representative immunoblot from one subject is shown in *A*. Quantified data from all seven subjects are presented in *B* as arbitrary units obtained by densitometric measurements. Akt Ser^{473} phosphorylation was increased ($P < 0.001$) by insulin treatment and significantly decreased ($P < 0.05$) 30 min after termination of insulin infusion. Akt Ser^{473} phosphorylation was not different at any time point between the two legs. Data are means ± SE, $n = 7$.

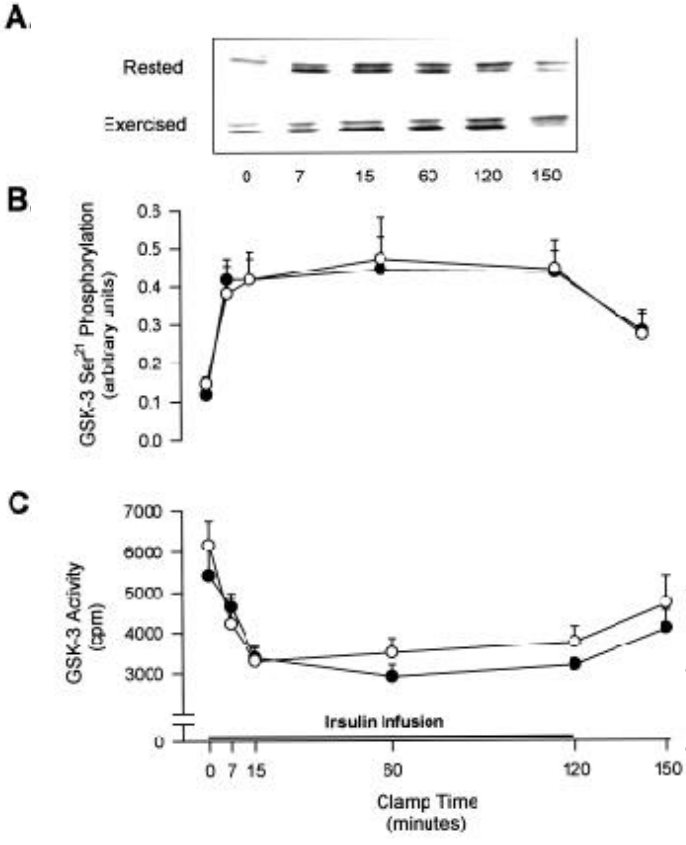

FIG. 6. GSK-3 phosphorylation and activity. GSK-3αSer^{21} phosphorylation (*A* and *B*) and GSK-3α activity (*C*) in rested (●) and exercised (○) thigh muscle. GSK-3 phosphorylation was determined using a phospho-specific antibody that recognizes GSK-3 only when phosphorylated on serine 21. *A* shows a representative immunoblot of muscle lysates from one subject, and quantified data from all seven subjects are shown in *B*, expressed as arbitrary scanning units obtained by densitometric measurements. Insulin infusion induced serine phosphorylation of GSK-3 and deactivation of the kinase activity ($P < 0.001$), and there was a significant reversal of both parameters 30 min after insulin infusion ($P < 0.05$). GSK-3 activity and phosphorylation were not different at any time point between the two legs. Data are means ± SE, $n = 7$.

phosphorylation [36–39]), we also performed GSK-3 kinase activity assays in GSK-3α immunoprecipitates using a glycogen synthase phosphopeptide as substrate. In muscle from both legs, GSK-3 activity rapidly decreased by 40–45% during the first 15 min of insulin infusion ($t_{1/2}$ = 3–8 min) (Fig. 6*C*). This degree of deactivation was maintained until the end of the insulin infusion, and there was partial reactivation 30 min after cessation of insulin infusion. There were no differences in GSK-3 activity between the muscles from the rested and exercised leg at any time.

Protein expression. Studies performed in rodent skeletal muscle have indicated that GLUT4 mRNA and protein expression are rapidly increased after intensive exercise (40,41). By immunoblotting, the insulin receptor (1.00 ± 0.10 vs. 1.11 ± 0.17), IRS-1 (2.68 ± 0.67 vs. 2.26 ± 0.62), p85 (6.3 ± 1.2 vs. 5.7 ± 0.8), Akt (0.20 ± 0.03 vs. 0.24 ± 0.03), and GLUT4 (4.4 ± 0.6 vs. 5.4 ± 0.5) protein expression were not significantly different between the rested and exercised muscle (data are means ± SE, $n = 7$).

DISCUSSION

In the present study, we show that exercise greatly enhances insulin-stimulated glucose uptake and glycogen synthase activity in human skeletal muscle. To our knowledge, we for the first time also demonstrate that physiological hyperinsulinemia regulates Akt and GSK-3 activities in human skeletal muscle. Interestingly, the increased insulin-stimulated glycogen synthase activity and glucose uptake in the exercised muscle was not associated with an upregulation of insulin signaling through the insulin receptor, Akt, and GSK-3, and it occurred without an increase in the expression of insulin-signaling proteins or the GLUT4 glucose transporter.

Together with our previous study (9), we have now described the activation time course of insulin signaling in human skeletal muscle to the level of GSK-3 in response to physiological hyperinsulinemia. Several characteristics of these time courses are noteworthy. First, physiological hyperinsulinemia promotes a sustained activation of all the signaling intermediaries we have investigated (IRTK, IRS-1, PI 3-kinase, Akt, GSK-3). This is in contrast to the transient activation of insulin-signaling molecules in rodent skeletal muscle studied with supraphysiological insulin concentrations and without the maintenance of euglycemia (42–44). Second, the activation time for the upstream signaling intermediates is relatively slow ($t_{1/2}$ for IRTK >50 min, IRS-1 tyrosine phosphorylation 26 min (9), PI 3-kinase activity 29 min [9]). In contrast, further downstream elements are activated very quickly (Akt serine phosphorylation $t_{1/2}$ = 1–2 min, GSK-3 serine phosphorylation $t_{1/2}$ = 1–2 min, GSK-3 activity $t_{1/2}$ = 3–8 min), and all reach steady-state levels within a few min-

utes. This important finding implies that the proximal signaling molecules possess spare kinase activity that results in amplification of signaling and/or that there are alternative mechanisms for insulin-stimulated regulation of Akt and GSK-3. The fact that the cessation of insulin infusion results in a rapid and pronounced decrease in Akt and GSK-3 signaling but only a small decrease in the proximal signaling events (Fig. 4) (9) supports the concept of alternative mechanisms for Akt and GSK-3 regulation by insulin in human muscle, rather than spare kinase activity. Third, all signaling events are reversed only when insulin infusion is withdrawn, but interestingly and similar to the onset, the offset of proximal signaling is markedly delayed compared with the decrease in the plasma insulin concentration. This is likely because the interstitial insulin concentration changes much more slowly than the plasma insulin concentration (45).

The very similar time course for the phosphorylation and dephosphorylation of Akt and GSK-3 supports the hypothesis that GSK-3 is a physiological substrate for Akt in human skeletal muscle. In addition, the time course of GSK-3 deactivation was slightly faster than the time course of glycogen synthase activation ($t_{1/2}$ = 3–8 vs. 12–17 min). Thus, it is conceivable that GSK-3 plays a role in insulin's action to increase glycogen synthase activity in human skeletal muscle. A role for Akt in insulin-stimulated glucose transport is controversial (15–22). The present data do not support the hypothesis that Akt is the link between PI 3-kinase and glucose transport. This is because the activation of leg glucose clearance was markedly slower ($t_{1/2}$ = 34 min) than Akt phosphorylation ($t_{1/2}$ = 1–2 min), but closely resembled the activation time for PI 3-kinase ($t_{1/2}$ = 29 min) (9). Nevertheless, the present data do not exclude a role for Akt in stimulation of muscle glucose transport, because even if Akt is activated rapidly, consequent steps in triggering translocation of GLUT4 might have longer half times for activation, more in agreement with the increase in glucose clearance.

Our novel finding that submaximal insulin-stimulated glycogen synthase activity is increased after exercise suggests a change in insulin sensitivity, as has been observed in rat skeletal muscle (6). Interestingly, despite the augmentation of insulin-stimulated glucose uptake and glycogen synthase activity after exercise, glycogen resynthesis in human muscles is very limited during 2 h of euglycemic hyperinsulinemia (Table 1). This contrasts with the hyperglycemic-hyperinsulinemic condition, in which glycogen synthesis is very rapid, with full glycogen repletion 2 h after exercise (46).

The similar level of expression and activation of insulin-signaling components and GLUT4 protein in the face of the different insulin sensitivity in rested and exercised muscle shows that increased GLUT4 protein expression is not a prerequisite for increased insulin sensitivity of glucose uptake, as recently suggested (40,41). In addition, the findings raise the possibility that exercise modulates insulin-signaling components further "downstream." It is also possible that contractile activity can directly activate or prime GLUT4 vesicular proteins or glycogen synthase associated proteins involved in regulation of the glucose transporter system and glycogen synthase activity, respectively. In rats, there is an inverse relationship between muscle glycogen content and exercise- and insulin-induced glucose transport and GLUT4 translocation as well as glycogen synthase activity (1,47–50). Therefore, the level of glycogen depletion in the muscle may also be a major determinant for the exercise-induced increase in insulin sensitivity for glucose transport, GLUT4 translocation (10), and glycogen synthase activation.

After exercise, glucose uptake is reversed relatively fast, typically within 2–4 h (current study and 9,30,51). Exercise is also a potent activator of glycogen synthase, but in contrast to glucose uptake, this effect is sustained for a prolonged period. There is some evidence that the reversal of glycogen synthase activation after exercise is related to the rate of glycogen resynthesis (1). Therefore, it is not surprising that we found that glycogen synthase activity was markedly elevated 4 h after exercise, because the muscles were still glycogen depleted. The standard assay of glycogen synthase activity as used in the present study reflects the phosphorylation status of the enzyme, suggesting that the high glycogen synthase activity observed 4 h after exercise is due to continuous dephosphorylation of the enzyme. We have recently shown that treadmill exercise deactivates GSK-3 in rat skeletal muscle concurrent with activation of glycogen synthase (33). Data from the present study demonstrate that GSK-3 deactivation is not the cause of the prolonged dephosphorylation and activation of glycogen synthase observed 4 h after exercise because GSK-3 activity was similar in rested and exercised muscle (Fig. 6).

In summary, physiological hyperinsulinemia leads to sustained activation of glucose uptake, glycogen synthase, and insulin-signaling intermediaries in human skeletal muscle. The time course of activation of the distal signaling components is much more rapid than that of the proximal components. Four hours after exercise, insulin's effects on muscle glucose uptake and glycogen synthase activity are enhanced, but this is not due to an augmentation of insulin receptor–mediated signaling to the level of Akt and GSK-3 or to an upregulation of protein expression of insulin-signaling molecules and GLUT4. The present findings suggest that enhanced insulin sensitivity in exercised muscle involves either insulin signaling downstream from Akt and GSK-3 or stimulation of processes directly involved in glycogen synthase activation and glucose transporter translocation. Finally, the sustained glycogen synthase activation after exercise is not associated with changes in GSK-3 activity.

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