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Research Article

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Insulin Resistance Is Associated with Reduced Fasting and Insulin-stimulated Glycogen Synthase Phosphatase Activity in Human Skeletal Muscle

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Abstract

Insulin-stimulated glycogen synthase activity in human skeletal muscle correlates with insulin-mediated glucose disposal rate (M) and is reduced in insulin-resistant subjects. We have previously reported reduced insulin-stimulated glycogen synthase activity associated with reduced fasting glycogen synthase phosphatase activity in skeletal muscle of insulin-resistant Pima Indians. In this study we investigated the time course for insulin stimulation of glycogen synthase and synthase phosphatase during a 2-h high-dose insulin infusion (600 mU/min per m²) in six insulin-sensitive caucasians (group S) and in five insulin-resistant Pima Indians (group R). Percutaneous muscle biopsies were obtained from the quadriceps femoris muscle after insulin infusion for 0, 10, 20, 40, and 120 min.

In group S, insulin-stimulated glycogen synthase activity increased with time and was significantly higher than in group R. In group S, synthase phosphatase activity increased significantly by 25% at 10 min and then decreased gradually. No significant change in synthase phosphatase was seen in group R and activity was lower than group S at 0 to 20 min.

These data suggest that a low basal synthase phosphatase activity and a defect in its response to insulin explain, at least in part, reduced insulin stimulation of skeletal muscle glycogen synthase associated with insulin resistance. (*J. Clin. Invest.* 1990. 85:476–481.) insulin • glycogen synthase • protein phosphatase • muscle

Introduction

Glucose storage via glycogen synthesis in skeletal muscle is a major determinant of whole-body insulin-mediated glucose disposal in man (1–3). A rate-limiting enzyme in glycogen synthesis is glycogen synthase, which is regulated by both covalent phosphorylation-dephosphorylation and allosteric modifications (4–7). Phosphorylation (inactivation) of glycogen synthase is catalyzed by several different protein kinases acting on different phosphorylation sites (8–10), while the reverse reaction (activation) is catalyzed by protein phosphatases (11, 12). Insulin administration results in a rapid increase in the percentage of synthase in the dephosphorylated (glucose-6-phosphate, G6P¹-independent) form and stimulates glycogen synthesis in both animal and human tissues (1, 3, 14–18).

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1. Abbreviations used in this paper: G6P, glucose-6-phosphate; M, insulin-stimulated glucose disposal rate.

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However, the mechanism by which insulin stimulates the conversion of synthase to the dephosphorylated form is unclear in human skeletal muscle. It could result from a decrease in protein kinase activity and/or an increase in synthase phosphatase activity (5–8, 11). Several animal studies have suggested that insulin stimulation of glycogen synthase is associated with activation of synthase phosphatase (16, 19). However, insulin-mediated changes of synthase phosphatase activity have not been demonstrated in human tissues.

In this study, we investigated the time course for insulin-mediated regulation of both glycogen synthase and synthase phosphatase during a hyperinsulinemic, euglycemic clamp in human skeletal muscle from insulin-sensitive and insulin-resistant subjects.

Methods

Subjects. Six insulin-sensitive caucasians (group S) and five insulin-resistant Pima Indians (group R) participated in this study. These subjects were selected to have a glucose disposal rate that was significantly lower in group R than in group S (Table I). Age, body weight, percent body fat, fasting plasma glucose, and fasting plasma insulin concentrations of the two groups are also shown in Table I. Body weight, body fat, and fasting insulin were significantly higher in group R than in group S (each $P < 0.001$).

Informed consent was obtained and fitness for the study was determined by medical history, physical examination, electrocardiography, and routine blood biochemical and hematological testing. None of the subjects were taking any medication and no subject had any abnormalities on these examinations. After consuming a weight maintaining diet (20% protein, 50% carbohydrate, and 30% fat) for at least 2 d, each subject had a 75-g oral glucose tolerance test. None of the subjects had diabetes mellitus but three in group R had impaired glucose tolerance according to the criteria established by the National Diabetes Data Group (20). After at least 3 d on a weight maintenance diet, a hyperinsulinemic, euglycemic clamp (1) was performed. Body fat was estimated by underwater weighing with simultaneous measurement of residual lung volume (21).

Euglycemic clamp (Fig. 1). After an overnight fast, an intravenous catheter was placed in the antecubital vein for infusion of insulin and glucose. Another catheter was inserted retrograde in a dorsal hand vein of the contralateral hand for blood withdrawal and this hand was kept in a warming box (70°C) during the entire clamp procedure for venous blood arterialization. The clamp was initiated by a primed-continuous high-dose insulin infusion (600 mU/min per m²) for 120 min. After the start of the insulin infusion, a variable infusion of 20% glucose was given as necessary to maintain the plasma glucose concentration at 100 mg/dl for all subjects. The plasma insulin concentration was determined before the start of the insulin infusion and at 10, 20, 40, 60, 80, and 120 min during the clamp. The plasma glucose was determined before the start of insulin infusion and every 2.5–5 min through the end of the clamp. Insulin-stimulated glucose disposal rate (M) was determined during the 80- to 120-min period. The steady-state plasma insulin and glucose concentrations were ~2,700 μ U/ml and 100 mg/dl, respectively, in both groups (Table II). Plasma glucose concentration was measured by the glucose oxidase method using a glucose analyzer (Beckman Instruments, Inc., Fullerton, CA). Plasma insulin

Table 1. Subjects' Characteristics

No.	Sex	Age yr	Body weight kg	Body fat %	Glucose disposal rate mg/kg-FFM/min	Fasting plasma	
						Glucose mg/dl	Insulin μ U/ml
1	M	25	70.5	6	14.2	84	7
2	F	28	56.1	20	9.3	89	4
3	M	21	82.4	18	13.4	88	3
4	M	27	68.0	15	11.6	90	6
5	M	19	65.8	11	13.5	77	4
6	M	21	67.6	13	12.8	91	6
Group S							
mean $\pm$ SE		24 $\pm$ 2	68.4 $\pm$ 3.5*	14 $\pm$ 2*	12.5 $\pm$ 0.7*	87 $\pm$ 2	5 $\pm$ 1*
1	F	19	123.3	42	5.5	88	37
2	M	26	113.8	36	6.7	92	30
3	M	43	147.9	42	5.3	106	43
4	F	37	111.0	47	7.5	99	30
5	F	33	117.6	41	4.6	128	35
Group R							
mean $\pm$ SE		32 $\pm$ 4	122.7 $\pm$ 6.6	42 $\pm$ 2	5.9 $\pm$ 0.5	103 $\pm$ 7	35 $\pm$ 2

Three subjects (3, 4, and 5) in group R were impaired glucose tolerant. *T* test: * $P < 0.001$ between groups.

concentrations were determined using a radioassay analyzer (Concept 4; ICN, Horsham, PA).

Muscle biopsy. Before the start of insulin infusion and at the indicated timepoints (Fig. 1), percutaneous muscle biopsies were taken from the quadriceps femoris muscle after local anesthesia of skin and fascia. Specimens (80–120 mg) were collected within 5 min using the Bergström needle (Depuy, Phoenix, AZ). The first biopsy site was 35% of the distance from the superior margin of the patella to the anterior superior iliac spine in the right thigh. The second through fifth biopsies were taken at 10, 20, 40, and 120 min during the clamp. Biopsy sites for the second through fifth biopsies were as follows: the same location relative to the patella in the left thigh, 2 cm caudal from the first biopsy, 2 cm caudal from the second biopsy, and 4 cm caudal from the first biopsy, respectively. The biopsy specimens were frozen in liquid nitrogen within 15 s and stored at -70°C .

Enzyme assay. The biopsies were lyophilized, dissected free of blood, fat, and all visible connective tissue, and powdered. The powder was thoroughly mixed and stored at -70°C until analysis.

Glycogen synthase activity was determined with the following modification of the method of Guinovart (22) and Thomas (23). Briefly, dry muscle powder was weighed and homogenized in a 30% glycerol, 10 mM EDTA, and 50 mM potassium fluoride (KF) (pH 7) solution (200 μ l/mg dry wt) using a ground glass tissue grinder (Rad-

noti Glass Technology, Inc., Monrovia, CA) at 4°C . The homogenate was centrifuged at 10,000 g for 20 min at 4°C . 100 μ l supernatant was diluted with 500 μ l of a buffer containing 50 mM Tris, 20 mM EDTA, and 130 mM KF (pH 7.8) at 4°C and used for glycogen synthase assay. The active form of glycogen synthase was assayed at 0.17 mM G6P and total glycogen synthase activity was assayed at a 7.2 mM G6P. Fractional activity of glycogen synthase was expressed as the activity ratio of active form to total glycogen synthase. The assay used 0.13 mM UDP-glucose and 4.7 mg/ml glycogen. Activities are expressed as units per gram of tissue dry weight. 1 U equals micromoles of [^{14}C]glucose incorporation into glycogen per minute at 30°C . G6P and UDP-glucose were purchased from Sigma Chemical Co. and [^{14}C]UDP-glucose from New England Nuclear (Boston, MA).

Glycogen synthase phosphatase was assayed according to the method of Miller (24) with some modification. The homogenate was prepared with 16 μ l buffer/mg dry wt according to the method of Chang and Huang (16). Briefly, powder was homogenized with 50 mM Tris, 10 mM EDTA, and 50 mM 2-mercaptoethanol (pH 7.8) using a glass tissue grinder at 4°C . The homogenate was centrifuged at 17,000 g for 10 min at 4°C . The supernatant was incubated for 5 min at 30°C with purified (13) rabbit glycogen synthase D (44 mU GSD/assay; Sigma Chemical Co., St. Louis, MO) in 10 mM Tris, 1 mM EDTA, and 5 mM dithiothreitol buffer, which was preincubated for 5 min at 30°C . Synthase phosphatase activity was determined from the change in the active form of glycogen synthase per minute and expressed as units per gram dry weight.

Both glycogen synthase and synthase phosphatase activities were linear with time within the limits described for each assay. Preliminary studies demonstrated that the activities of these enzymes were not altered by the lyophilization procedure. The average (range) of dry muscle weight was 23% (22–24%) of wet weight. The contents of non-muscle constituents were variable from sample to sample with an average of 7% (2–25%) of dry weight. Interassay variations of glycogen synthase and synthase phosphatase activities were 5% and 8%, respectively, and were smaller than those using wet muscle (data not shown).

Muscle G6P and ATP were determined for five subjects in group S and four in group R using the change in NADP-NADPH associated fluorescence after the addition of G6P dehydrogenase and hexokinase (G5760 and H1131 from Sigma Chemical Co.) after 0.5 M perchloric acid extractions (25). Interassay variations of G6P and ATP were both 5%.

Statistics. Significance ($P < 0.05$) of the differences were analyzed with Student's paired or nonpaired *t* test. All data were expressed as mean $\pm$ SE unless otherwise indicated.

Results

The activities of glycogen synthase and synthase phosphatase during insulin infusion are shown in Fig. 2. Total glycogen synthase activity was significantly higher in group S than in group R at time 0 and 120 min (both $P < 0.05$). In group S, total glycogen synthase activity significantly increased at 120 min ($P < 0.05$), whereas no significant change occurred in group R (Fig. 2 A). Before the insulin infusion, glycogen synthase fractional activity was not significantly different between the two groups. However, in group S the 10-min fractional activity was significantly higher than in group R ($P < 0.05$) and remained significantly stimulated above basal from 10 min to the end of the clamp ($P < 0.01$ at 10 and 20 min, $P < 0.001$ at 40 and 120 min). In group R, a significant increase in fractional activity was not observed before 20 min, but remained significantly stimulated above basal for the duration of insulin infusion (Fig. 2 B). The active form of glycogen synthase showed similar results. In group S, glycogen synthase activity at all timepoints after insulin infusion was significantly higher than in group R (Fig. 2 C).

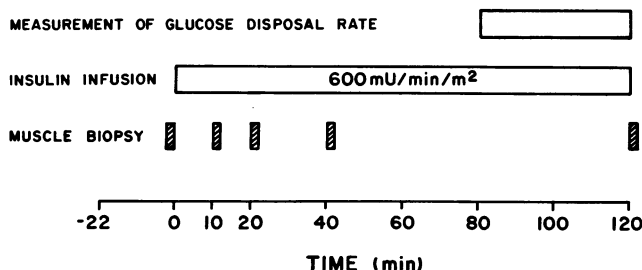


Figure 1. Synoptic diagram of the hyperinsulinemic, euglycemic clamp. Muscle biopsies were taken at 0, 10, 20, 40, and 120 min during the clamp. Glucose disposal rates were determined for the 40-min period between 80 and 120 min.

Table II. Plasma Glucose and Insulin Concentrations during Clamp

Time (min)	0	10	20	40	60	80	100	120
Plasma glucose (mg/dl)*								
Group S	100±1	102±2	95±3	99±3	101±3	102±2	102±3	99±2
Group R	102±2	105±3	106±9	111±10	99±1	100±2	99±1	99±2
Plasma insulin (μU/ml)†								
Group S	5±1§	1,517±65	1,748±112	2,043±169	2,410±229	2,669±282	—	2,781±383
Group R	34±2	1,598±39	1,721±51	2,092±148	2,265±66	2,584±154	—	2,942±137

* Mean±SE of samples drawn at 2.5- to 5-min intervals using a 20-min interval before indicated times. † Mean±SE of samples drawn at indicated times. § *t* test: $P < 0.001$ between groups.

Synthase phosphatase activity was significantly higher in group S than in group R at 0, 10, and 20 min ($P < 0.01$). In group S, synthase phosphatase activity increased by 25% at 10 min ($P < 0.01$) and then returned to the basal level by 40 min. At the end of the clamp, the activity was significantly lower than the basal ($P < 0.01$). In group R no significant change in mean synthase phosphatase activity was observed during the clamp (Fig. 2 D).

The changes of synthase phosphatase activities during the clamp are shown for each subject in Fig. 3. In group S, all six

subjects showed an increase in activity at 10 min and five were maximally activated at 10 min (Fig. 3 A). In group R the results were variable (Fig. 3 B). Only two of the five subjects showed activation at 10 min and two subjects did not show any increase in the activity during the clamp. The maximum-stimulated activity of each subject in group R fell below the range for stimulated activity in group S. In group S, only the phosphatase activity at 0 min was significantly correlated with insulin-mediated glucose disposal rates ($r = 0.9$, $P < 0.05$). Phosphatase activity at 0 min was not significantly correlated

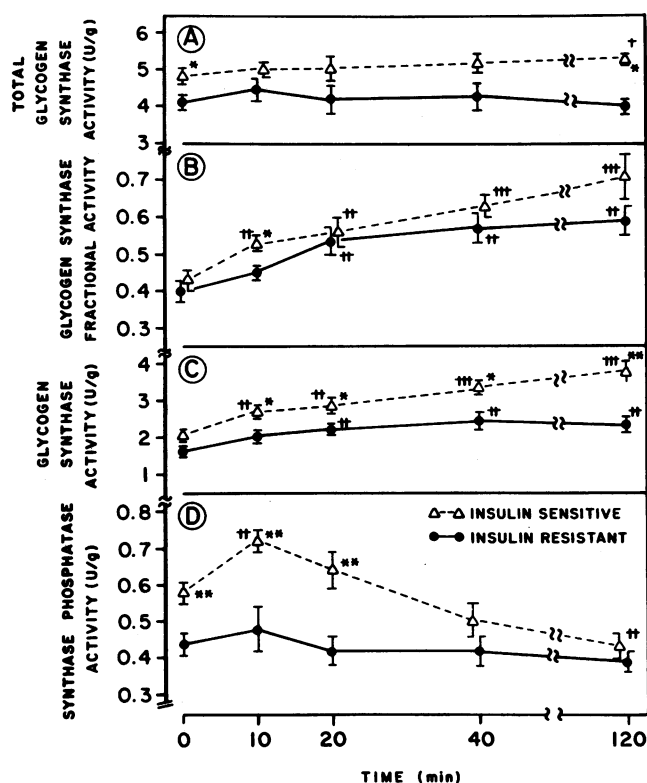


Figure 2. Change of total glycogen synthase activity (A); glycogen synthase fractional activity (B); active form of glycogen synthase activity (C); and synthase phosphatase activity (D) in group S (Δ) and group R (●). Glycogen synthase and synthase phosphatase activities were measured as described in Methods. Results are expressed as means±SE. *T*-test: * $P < 0.05$, ** $P < 0.01$ between groups. Paired *t*-test: + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$ vs. 0 min.

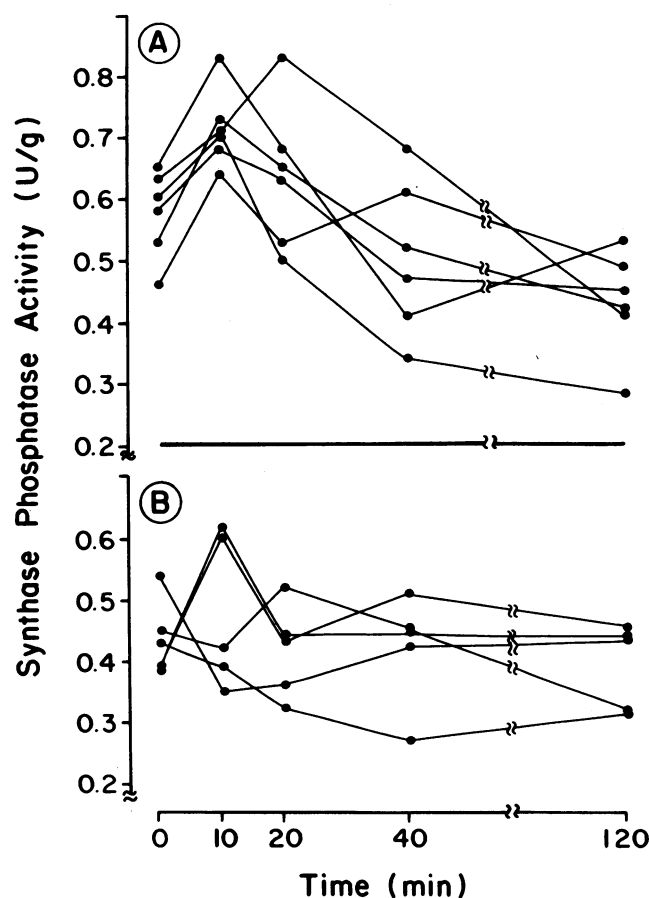


Figure 3. Individual synthase phosphatase activities at each time-point in group S (A) and group R (B).

with percent body fat ($r = 0.7$, $P = 0.09$). In group R, phosphatase activity was not correlated with glucose disposal or with percent body fat.

Mean G6P concentrations (micromoles per gram) from 0, 10, 20, 40, and 120 min were 1.03 ± 0.16 , 1.14 ± 0.21 , 1.45 ± 0.45 , 1.30 ± 0.25 , and 1.89 ± 0.52 in group S and 1.53 ± 0.34 , 1.20 ± 0.16 , 1.18 ± 0.29 , 1.36 ± 0.52 , and 1.82 ± 0.29 in group R. Mean ATP concentrations (micromoles per gram) at each timepoint ranged from 25.5 ± 2.1 to 27.0 ± 3.5 in group S and 21.1 ± 3.9 to 22.2 ± 3.8 in group R. There was no significant change in either G6P or ATP concentrations during insulin infusion in these subjects and there was no significant difference in both basal and insulin-stimulated G6P and ATP between groups.

Discussion

We have reported a positive correlation between insulin-stimulated glucose disposal rate (M) and insulin-stimulated skeletal muscle glycogen synthase activity in vivo in man and suggested that the regulation of glycogen synthase may contribute to the reduced glucose disposal associated with insulin resistance in Southwestern American Indians (1, 3). A similar conclusion was reached in an in vivo study of normal glucose tolerant caucasians (26).

Glycogen synthase data in the present study reconfirmed the association of impaired activation of insulin-stimulated glycogen synthase with insulin resistance in Southwestern American Indians. Although the basal glycogen synthase activities (Fig. 2 C) between the two groups did not differ significantly, basal total glycogen synthase activities (Fig. 2 A) were significantly lower in group R and there was no increase in the mean total activity during the 120-min clamp. This is in contrast to the significant increase in total glycogen synthase activities after 120 min of insulin infusion in group S. Freymond et al. (14) also reported a significant increase in total glycogen synthase activity at the end of a 460-min insulin infusion in man. Pain et al. (27) reported decreased protein synthesis in skeletal muscle using streptozotocin-diabetic rats. Goheer et al. (28) also reported a significant increase of total glycogen synthase in H4 cells after 1 h incubation with physiological concentrations of insulin. Therefore, lower total glycogen synthase activity and a defective insulin-mediated increase of total activity in insulin-resistant subjects may be reflecting an impairment of insulin-stimulated protein synthesis in skeletal muscle. These observations suggest that reduced synthesis of glycogen synthase may contribute to the mechanisms for insulin resistance observed under these conditions.

We assayed the active form of glycogen synthase under physiological concentrations (0.17 mM) of G6P in an effort to more closely approximate in vivo activity in skeletal muscle. After insulin infusion, both the fractional (Fig. 2 B) and active form (Fig. 2 C) of glycogen synthase activities in group S increased by 10 min and appeared to increase through 120 min. On the other hand, in group R significant changes occurred later than in group S and the activities appeared to plateau after 40 min. This is in spite of significant increase in plasma insulin concentrations in group R between 40 and 120 min ($P < 0.001$) (Table II). Therefore, lower insulin-stimulated glycogen synthase activities in group R were not simply due to the lower total activity but also a result of lower fractional activity.

Glycogen synthase activity is regulated by both noncovalent allosteric interaction and covalent phosphorylation/dephosphorylation (5–7). Phosphorylation states of several specific sites in the glycogen synthase molecule alter its activity (8, 9). It is known that insulin is a potent activator of glycogen synthase in liver, fat, and muscle, but the sequence of events leading to insulin-stimulated intracellular glycogenesis initiated by insulin is not clear in human skeletal muscle. Inhibition of phosphorylation by inactivation of protein kinases (9, 10) and/or stimulation of dephosphorylation by protein phosphatase activation (11, 16) have been considered as mechanisms of insulin action.

We have recently reported on synthase phosphatase activity measured before and at the end of a 460-min insulin infusion on 30 subjects with a wide range of M values (14). Although the glycogen synthase activity increased after insulin infusion, the synthase phosphatase activity did not change. It was possible, however, that synthase phosphatase activation was not observed because dilution during the assay might have resulted in dissociation of presumed insulin-induced noncovalently bound regulators of phosphatase activity. Toth et al. (18) reported that insulin activated rat liver synthase phosphatase within 5 min using concentrated homogenates, but no hormonal effects were observed when the homogenates were diluted. They suggested that the insulin effect was mediated by a transferable cytosolic effector. In addition, we are not aware of any report on synthase phosphatase stimulation beyond 10–30 min after insulin administration. Therefore, it is possible that in our previous study (14) we missed an early change of the phosphatase activity that occurred before 460 min.

In an attempt to answer these questions, we determined the time course of synthase phosphatase activity after a high-dose (600 mU/min per m^2) insulin infusion using a homogenate that was five times more concentrated (protein concentration in assay ~ 10 g/liter) than in the previous study (13, 14). This concentration of muscle extract was previously used by Chang and Huang (16). They demonstrated a significant increase of both glycogen synthase and synthase phosphatase at 8 and 10 min after insulin administration using rat skeletal muscle. As shown in Fig. 2, basal synthase phosphatase activity was significantly higher in group S than in group R and a significant increase of the activity was observed at 10 min only in group S followed by a loss of activity in five of the six subjects (Fig. 3 A). Individual activities during the clamp plotted in Fig. 3 B indicate that although two subjects in group R showed stimulation, the resulting phosphatase activity failed to reach the range of stimulated activity observed for the six group S subjects (Fig. 3 A). When the individual data from both groups were considered, seven of the eight subjects showing stimulation at 10 min appeared to peak at 10 min. A transient insulin-induced increase of protein phosphatase activity has also been observed in an in vitro study using 3T3 cells (29).

Several mechanisms for insulin-induced activation of synthase phosphatase can be considered: (a) G6P stimulates and ATP inhibits glycogen synthase phosphatase activity (13, 30). G6P has been proposed as a second messenger for insulin by Larner et al. (31); (b) inhibition of a cyclic-AMP dependent protein kinase may decrease phosphorylation of inhibitor 1 or the G-component of protein phosphatase-1 resulting in increased phosphatase activity (32–34); (c) phosphorylation of inhibitor 2, a modulator protein of cytosolic protein phosphatase-1, by glycogen synthase kinase 3 and casein kinase 2

(35–37). The first mechanism is not likely to be important. G6P determined in seven subjects (five in group S and two in group R) who demonstrated an activation of synthase phosphatase at 10 min shows no significant change during insulin infusion. We also determined muscle ATP concentrations in nine subjects (five in group S and four in group R). Muscle ATP did not change significantly during insulin infusion. The remaining mechanisms for insulin stimulation of glycogen synthase phosphatase activity may be important in explaining the reduction in both fasting and insulin-stimulated phosphatase activity in group R. A failure of insulin to inhibit cAMP-dependent protein kinase or activate casein kinase 2 or glycogen synthase kinase 3 could reduce the action of both fasting and infused insulin on phosphatase activity. Alternatively, the abnormality in fasting and insulin-stimulated total glycogen synthase activity (Fig. 2) discussed above suggests that reduced synthesis of the phosphatase could also lead to both basal and insulin-stimulated abnormalities in phosphatase activity.

Phosphatase activation was only a transient phenomenon. Activation of glycogen synthase, however, appeared to be continuous over 120 min of insulin infusion in insulin-sensitive subjects. This observation suggests that additional mechanism(s) exist for glycogen synthase activation during long-term insulin infusion. Insulin-induced inhibition of protein kinase activity may become important, as phosphatase activity begins to decrease. Although the mechanism that maintains the active form of glycogen synthase at 120 min is not clear, it is apparent that this mechanism is also abnormal in group R subjects. The decrease in phosphatase activity for the S group at 120 min compared to 0 min may be caused by increased glycogen in the 120-min homogenates. Although glycogen was not measured in these samples, glycogen synthesis has been demonstrated in insulin-sensitive subjects during similar insulin infusion protocols (1, 38) and would be expected to maintain concentrations ranging close to 0.08% (wt/vol) after dilution into the phosphatase assay. Glycogen at 0.1% has been shown to inhibit human muscle glycogen synthase phosphatase (13).

The unique characteristics of the R group, in addition to low insulin-mediated glucose disposal rates, are obesity and Southwestern American Indian heritage. Because the numbers of subjects in each group are small, the failure to find a significant relationship between obesity and phosphatase activity does not rule out possible effects of obesity on the activity of glycogen synthase phosphatase. Similarly, more extensive studies would be required to examine possible racial differences in phosphatase activity. Regardless of the importance of race or obesity to the insulin-resistant state studied here, it is clear that low insulin-mediated glucose uptake is also associated with abnormal activities of both synthase and phosphatase enzymes.

This is the first report that demonstrates insulin-induced activation of synthase phosphatase in human skeletal muscle. Both basal and activated synthase phosphatase activity appear to be reduced in subjects with reductions in both insulin-stimulated glycogen synthase activity and insulin-mediated glucose disposal. These data suggest that a low basal synthase phosphatase activity and a defect in its response to insulin explain, at least in part, reduced insulin stimulation of glycogen synthase in skeletal muscle associated with insulin resistance in Southwestern American Indians.

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