

Decreased insulin action in skeletal muscle from patients with McArdle's disease

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Nielsen, Jakob N., John Vissing, Jørgen F. P. Wojtaszewski, Ronald G. Haller, Najma Begum, and Erik A. Richter. Decreased insulin action in skeletal muscle from patients with McArdle's disease. *Am J Physiol Endocrinol Metab* 282: E1267–E1275, 2002. First published February 19, 2002; 10.1152/ajpendo.00526.2001.—Insulin action is decreased by high muscle glycogen concentrations in skeletal muscle. Patients with McArdle's disease have chronic high muscle glycogen levels and might therefore be at risk of developing insulin resistance. In this study, six patients with McArdle's disease and six matched control subjects were subjected to an oral glucose tolerance test and a euglycemic-hyperinsulinemic clamp. The muscle glycogen concentration was $103 \pm 45\%$ higher in McArdle patients than in controls. Four of six McArdle patients, but none of the controls, had impaired glucose tolerance. The insulin-stimulated glucose utilization and the insulin-stimulated increase in glycogen synthase activity during the clamp were significantly lower in the patients than in controls (51.3 ± 6.0 vs. 72.6 ± 13.1 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg lean body mass}^{-1}$, $P < 0.05$, and 53 ± 15 vs. $79 \pm 9\%$, $P < 0.05$, $n = 6$, respectively). The difference in insulin-stimulated glycogen synthase activity between the pairs was significantly correlated ($r = 0.96$, $P < 0.002$) with the difference in muscle glycogen level. The insulin-stimulated increase in Akt phosphorylation was smaller in the McArdle patients than in controls (45 ± 13 vs. $76 \pm 13\%$, $P < 0.05$, respectively), whereas basal and insulin-stimulated glycogen synthase kinase 3 α and protein phosphatase-1 activities were similar in the two groups. Furthermore, the ability of insulin to decrease and increase fat and carbohydrate oxidation, respectively, was blunted in the patients. In conclusion, these data show that patients with McArdle's glycogen storage disease are insulin resistant in terms of glucose uptake, glycogen synthase activation, and alterations in fuel oxidation. The data further suggest that skeletal muscle glycogen levels play an important role in the regulation of insulin-stimulated glycogen synthase activity.

glycogen synthase; protein phosphatase; glycogen synthase kinase 3; glucose clamp technique; glycogen storage disease type V

SKELETAL MUSCLE serves as the major site of whole body insulin-mediated glucose disposal (10). The two main metabolic end points for insulin action are glucose uptake across the plasma membrane and glycogen synthesis, both of which can be dysfunctional in different pathophysiological conditions of insulin resistance (9). However, the cellular and molecular mechanisms responsible for impaired muscular insulin are not fully elucidated. Studies investigating the function of different insulin-signaling molecules as possible sites of dysfunction have so far been inconclusive (34, 43). It has, however, been shown that, in humans, glycogen exerts a negative effect on the ability of insulin to increase muscle glycogen synthase activity (7, 21, 28), but the effect of glycogen levels on insulin-stimulated muscle glucose uptake has not been studied. In rodent skeletal muscle studied *in vitro*, glycogen synthase activity and glucose transport are decreased by glycogen loading and increased by glycogen depletion. There is evidence that protein kinase B/Akt plays a role in this regulation (11, 16). Elucidating the mechanism by which glycogen affects insulin action in human skeletal muscle could eventually lead to novel targets of therapeutic drugs for the treatment of type 2 diabetes and related diseases, but to our knowledge no studies have addressed the question whether glycogen *per se* modulates insulin signaling in human skeletal muscle.

The production of transgenic animals is a powerful approach to reveal the role of single elements involved in regulation of metabolism by overexpressing or knocking out specific proteins in one or several tissues. McArdle's disease (glycogen storage disease type V), first described in 1951 (23), is a human "knockout experiment of nature," in which mutations in the gene encoding muscle glycogen phosphorylase cause loss of glycogen phosphorylase activity exclusively in skeletal muscle. Due to the blocked muscle glycogenolysis in this condition, McArdle patients have chronic high muscle glycogen stores. Because glycogen is known to

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modulate insulin action in muscle, we hypothesized that the insulin response in skeletal muscle in patients with McArdle's disease may be impaired.

In this study, we examined the possible effect of glycogen on insulin-stimulated glucose utilization, glycogen synthase activity, and activity of certain insulin-signaling intermediates in human skeletal muscle obtained from patients with McArdle's disease, a unique human experimental model of chronic high muscle glycogen levels.

METHODS

Subjects. A total of 12 volunteers (6 patients with McArdle's disease, 3 males and 3 females, matched to 6 control subjects, 3 males and 3 females) participated. The McArdle patients had a typical history of exercise intolerance and myoglobinuria. All patients had high intramuscular glycogen levels previously demonstrated by diagnostic histochemical methods and verified by biochemical analysis in the present study. Furthermore, muscle glycogen phosphorylase was absent in all patients (see RESULTS). A control subject was matched to each of the patients according to age, sex, body weight, body mass index, and lean body mass determined by hydrostatic weighing (36). Due to the nature of the disease, McArdle patients are limited in their ability to undertake even low-intensity physical activity. Thus evaluated by interview, the control subjects included in the study undertook very low levels of habitual physical exercise and did not participate in any physical exercise training activities. The purpose and nature of the study were explained to all subjects, and written consent was obtained before their participation. The Copenhagen Ethics Committee approved the experimental protocol.

Experimental protocol. All experiments were conducted in the Copenhagen Muscle Research Center at the Department of Human Physiology, Institute of Exercise and Sport Sciences, University of Copenhagen, and began at 0900 after a 10-h overnight fast. An antecubital vein was cannulated in each arm. One cannula was used for infusion of glucose, and the other cannula was used for infusion of insulin and a primed infusion of [6,6-²H₂]glucose (99% enriched, Cambridge Isotope Laboratories, Cambridge, MA). In addition, a retrogradely inserted hand vein cannula was used for obtaining arterialized blood samples by heating the hand to ~40°C. The subjects rested for 1.5 h before initiating a one-step, 2-h euglycemic-hyperinsulinemic [~75 μU insulin/ml (Actrapid) Novo Nordisk, Denmark] clamp procedure as previously described (32). Blood samples were obtained throughout the experiment at 20-min intervals and needle biopsies from vastus lateralis muscle were excised before and at the end of the clamp.

Measurement of whole body metabolism. Whole body glucose metabolism was evaluated during the experiment by means of a stable isotope technique (primed infusion of [6,6-²H₂]glucose) and indirect calorimetry and from the infusion rate of unlabeled glucose. From the enrichments of labeled glucose in deproteinized plasma samples (33), the rates of appearance and disappearance of glucose were calculated. The percentage of enrichment of plasma by [6,6-²H₂]glucose was determined with gas chromatography-mass spectrometry as previously described (6), with some modifications (33). The rate of appearance will always equal the rate of disappearance under steady-state condition as was present during the last 30 min of the clamp procedure. Endogenous glucose production was calculated by subtracting the rate of glucose

appearance from the glucose infusion rate. Nonoxidative glucose disposal was calculated by subtracting the oxidative glucose disposal from the rate of glucose disappearance. Oxidative glucose and fat disposal were evaluated by measurements of pulmonary exchange of oxygen uptake and carbon dioxide production (CPX, Medgraphics, Medical Graphics, St. Paul, MN) using a ventilated hood (Medgraphics), and calculations were done according to Mikines et al. (27), with the exception that measurements were not corrected for urinary nitrogen and glucose.

Analyses of plasma substrates and hormones. Glucose and lactate concentrations in plasma were determined in duplicates by using a dual-channel glucose-lactate analyzer (YSI-2700 Select, Yellow Springs Instrument, Yellow Springs, OH). Plasma insulin concentration was determined using a radioimmunoassay kit (Insulin RIA 100, Pharmacia Diagnostics, Uppsala, Sweden). Concentrations of plasma free fatty acid (FFA) and glycerol were determined in accordance with Refs. 29 and 35, respectively, by means of an automatic spectrophotometer (COBAS FARA 2, Roche Diagnostic, Basel, Switzerland). Plasma epinephrine and norepinephrine were analyzed by means of a radioimmunoassay (KatCombi, Immuno-Biological Laboratories, Hamburg, Germany).

Muscle glycogen. Muscle glycogen content was measured fluorometrically as glucose residues after acid hydrolysis, as described (29).

Preparation of muscle lysates. For studies of enzyme activity and Western blotting, muscle lysates were prepared as follows. Approximately 40 mg of frozen muscle tissue were homogenized (Omni model 2000, Omni International, Warrenton, VA) in 10 volumes of *buffer A* [in mM: 50 HEPES, 150 sodium chloride, 20 sodium pyrophosphate, 20 β-glycerophosphate, 10 sodium fluoride, 2 sodium orthovanadate, 2 EDTA, 2 phenylmethylsulfonyl fluoride (PMSF), 1 magnesium chloride, 1 calcium chloride, 1% Igepal CA630, 10% glycerol, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 3 mM benzamidine, pH 7.5], except for measurements of protein phosphatase activities, where *buffer B* was used (in mM: 50 Tris·HCl, 2 EDTA, 2 EGTA, 2 PMSF, 0.5% Triton X-100, 5% glycerol, 0.1% β-mercaptoethanol, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 3 mM benzamidine, pH 7.5). Homogenates were rotated end over end at 4°C for 60 min and centrifuged at 4°C for 30 min at 4,000 g. The supernatants were harvested, and total protein content was determined in the lysates by the bicinchoninic acid method (Pierce).

Glycogen synthase activity. Glycogen synthase activity was measured in triplicate on 5 μl of lysate in *buffer A* by a modification of the method by Thomas et al. (38) described previously (32). Synthase activity at a maximally stimulating glucose 6-phosphate concentration of 8 mM is defined as total activity, because the enzyme is fully activated regardless of its phosphorylation status. The activity at a glucose 6-phosphate concentration of 0.02 mM divided by total activity was defined as percent independent form (%I-form), whereas the activity measured at 0.17 mM glucose 6-phosphate divided by the total activity was defined as fractional velocity.

Glycogen synthase kinase 3 activity. Glycogen synthase kinase 3 (GSK3α and GSK3β) was immunoprecipitated from 100 μg of protein from muscle lysate in *buffer A* by use of an anti-GSK3α (Upstate Biotechnology, Lake Placid, NY) or an anti-GSK3β antibody (Transduction Laboratories, San Diego, CA). A P81 filter paper assay, with a phospho-GS2 peptide (Upstate Biotechnology) as substrate, was used to measure isoform-specific GSK3 activity, as previously described (22).

Protein phosphatase 1 and protein phosphatase 2A activity. Activities of protein phosphatase (PP)1 and PP2A were measured in muscle lysates in *buffer B* as glycogen phosphorylase phosphatase activity as previously described (3), by using a protein phosphatase assay system (GIBCO-BRL Life Technologies). In short, 1 μ g of lysate protein in *buffer B* was incubated with [32 P]phosphorylase A for 10 min at 30°C. The reaction was stopped by adding 180 μ l of trichloroacetic acid and 10 μ l of 3% bovine serum albumin as a carrier protein. The tubes were left on ice for 10 min followed by centrifugation at 14,000 *g* for 3 min. Aliquots of supernatant were counted to determine the released 32 P_i. Discrimination between PP1 and PP2A activity was done by the absence or presence of 1 nM okadaic acid in the assay. At 1 nM okadaic acid, PP2A is inhibited, and the remaining activity represents PP1 activity.

Western blotting. For measurements of Akt serine 473 phosphorylation and expression of glycogen phosphorylase and glycogen synthase, muscle lysates were subjected to SDS-PAGE (7.5% gel) and transferred to a polyvinylidene difluoride (PVDF) membrane by semi-dry blotting, as previously described (20). The membrane was probed with the primary antibody raised against the protein of interest [rabbit anti-phospho-Akt (Ser⁴⁷³), from New England Biolabs (Beverly, MA); sheep anti-glycogen phosphorylase (pan-A and -B-form reactive) from Biogenesis (Poole, UK); rabbit anti-glycogen synthase (donated by Henrik Vestergaard, Herlev Hospital, Herlev, Denmark)]. A goat anti-rabbit or anti-sheep secondary antibody conjugated to alkaline phosphatase (Zymed Laboratories, San Francisco, CA) was applied to the membrane before incubation with an enhanced chemifluorescence substrate (Vistra ECF, Amersham International, Uppsala, Sweden). Signal intensities were visualized and quantified using a Kodak image station 440CF and Kodak 1D software (Kodak Scientific Imaging Systems, New Haven, CT), respectively.

Statistical analyses. Data are expressed as means \pm SE. Two-tailed paired Student *t*-tests were applied for comparison of two normally distributed groups. Comparisons among more than two normally distributed groups were done by two-way ANOVA for repeated measures followed by a multiple comparison test (Student-Newman-Keuls method). The strength of association between parameters was analyzed by Pearson product moment correlation analysis. *P* < 0.05 was considered statistically significant.

RESULTS

Physical characteristics of the subjects. As seen in Table 1, matching of each McArdle patient to a control subject resulted in almost identical anthropometric parameters, except for body height, which was slightly,

Table 1. Physical characteristics of subjects

	McArdle	Control
Age, yr	39.3 \pm 4.2	39.5 \pm 3.7
Height, cm	172 \pm 4.4*	176 \pm 4.0
Body weight, kg	83.7 \pm 9.4	85.8 \pm 7.3
Body mass index	27.9 \pm 2.1	27.4 \pm 1.6
Body fat, %	37.4 \pm 4.5	35.8 \pm 3.5
Lean body mass, kg	51.1 \pm 4.4	54.7 \pm 6.2
Body fat/lean body mass ratio	0.75 \pm 0.10	0.71 \pm 0.13
Resting heart rate, beats/min	73 \pm 5	75 \pm 6

Data are presented as means \pm SE; *n* = 6 subjects in each group. **P* < 0.01 vs. control.

Table 2. Plasma glucose and insulin concentrations during a standard 2-h oral glucose tolerance test

Time, min	Plasma Glucose, mM		Plasma Insulin, μ U/ml	
	McArdle	Control	McArdle	Control
0	5.7 \pm 0.3	5.6 \pm 0.2	11.2 \pm 3.1	11.8 \pm 1.8
30	8.9 \pm 1.1	8.4 \pm 1.0	76.4 \pm 25.3	59.5 \pm 15.3
60	8.6 \pm 0.7	8.1 \pm 1.6	63.6 \pm 15.2	74.1 \pm 29.1
120	8.1 \pm 0.6	6.1 \pm 0.3	59.3 \pm 14.4	35.9 \pm 7.5

Data are presented as means \pm SE; *n* = 6. Plasma glucose and insulin are changed by time (*P* < 0.001 and *P* < 0.01, respectively), but there are no significant differences between McArdle patients and control subjects. Of the 6 McArdle patients, 4 were categorized as having impaired glucose tolerance (plasma glucose \geq 7.8 mM). All control subjects had normal glucose tolerance.

but significantly, lower in McArdle patients. The resting heart rate was not significantly different between the two groups.

Oral glucose tolerance test. McArdle patients had normal fasting plasma glucose and plasma insulin levels (Table 2). At the 2-h end point of the test, there was no statistical difference between either plasma insulin level or plasma glucose in the McArdle patients and the control subjects. However, due to their high plasma glucose concentration at the 2-h end point, four of the six McArdle patients were categorized as having impaired glucose tolerance (plasma glucose \geq 7.8 mM) (17). All control subjects had normal glucose tolerance.

Plasma substrates and hormones. Before initiation of the euglycemic-hyperinsulinemic clamp procedure, the basal (fasting) concentrations of plasma insulin, glycerol, epinephrine, norepinephrine, and blood glucose and lactate were similar in the two groups (Table 3). Plasma FFA was significantly lower in the patients than in the controls (Table 3). At the end of the clamp procedure, the blood glucose and plasma epinephrine and norepinephrine concentrations were unchanged compared with the basal values and did not differ between the two groups of subjects. Blood lactate increased (main effect) and plasma FFA decreased, but with no differences between McArdle patients and controls. Rise in plasma insulin was similar between McArdle patients and control subjects (Table 3).

Whole body metabolism. In the basal state, no differences in glucose disappearance, respiratory exchange ratio, or metabolism, as calculated from indirect calorimetry, could be observed between patients and controls. In the control subjects, insulin stimulation increased the rate of glucose disappearance, respiratory exchange ratio, and oxidative glucose disposal, whereas fat oxidation decreased. No significant changes in these parameters could be observed in the McArdle patients. Nonoxidative glucose disposal did not increase significantly in response to insulin (*P* < 0.09 for a main effect). During insulin stimulation, the patients with McArdle's disease had a significantly lower insulin-stimulated glucose clearance and glucose infusion rate than the controls (Table 4). Insulin-stimulated oxidative, but not nonoxidative, glucose disposal rate in the patients was lower than in the controls. Rate of glucose

Table 3. Blood and plasma substrates and hormones before and at the end of a 2-h euglycemic-hyperinsulinemic clamp procedure in McArdle patients and control subjects

	Basal		Insulin	
	McArdle	Control	McArdle	Control
Plasma insulin, $\mu\text{U/ml} \ddagger$	12.5 ± 3.3	9.8 ± 2.8	87.7 ± 7.9	76.2 ± 8.7
Blood glucose, mmol/l	5.0 ± 0.2	4.6 ± 0.3	4.9 ± 0.1	4.8 ± 0.3
Blood lactate, mmol/l \ddagger	0.76 ± 0.17	0.81 ± 0.15	1.07 ± 0.17	0.81 ± 0.10
Plasma FFA, $\mu\text{mol/l}$	$363 \pm 74^*$	601 ± 71	$95 \pm 11 \ddagger$	$126 \pm 13 \ddagger$
Plasma glycerol, $\mu\text{mol/l} \ddagger$	89 ± 16	68 ± 11	44 ± 7	25 ± 1
Plasma epinephrine, nmol/l	0.17 ± 0.03	0.16 ± 0.03	0.26 ± 0.06	0.20 ± 0.03
Plasma norepinephrine, nmol/l	1.56 ± 0.31	1.05 ± 0.30	2.01 ± 0.58	1.42 ± 0.43

Data are presented as means \pm SE; $n = 6$. * $P < 0.01$ vs. corresponding control value. $\ddagger P < 0.01$ vs. basal. $\ddagger P = 0.0007$ for basal vs. insulin (main effect).

disappearance, evaluated by stable glucose isotope infusion, was not significantly lower in the McArdle patients. Under steady-state condition, as present during the last 30 min of the clamp procedure, rate of appearance equals the rate of disappearance; therefore, only the latter is given in Table 4. Endogenous glucose production (e.g., hepatic glucose production) was shut down during insulin stimulation in both groups of subjects (Table 4). The slightly negative value for this parameter most likely reflects a methodological underestimation of the rate of glucose appearance, as encountered by others (24). As concluded previously (26), the actual error in rate of appearance measurements will be reflected by the magnitude of the negative hepatic glucose output. During the last 30 min of the 2-h euglycemic-hyperinsulinemic clamp procedure, there was no significant difference between the respiratory exchange ratio in the McArdle patients and the control subjects (Table 4).

Expression of glycogen phosphorylase and synthase. As expected, muscle glycogen phosphorylase was undetectable in all the McArdle patients, whereas the protein was present in the control subjects (Fig. 1). The amount of glycogen synthase was similar in the two groups, but a higher phosphorylation status of glycogen synthase in McArdle patients was indicated by the lower mobility in the electrophoresis gel (Fig. 1).

Muscle glycogen. Before the clamp procedure, the glycogen concentration was approximately twice as high in the McArdle patients compared with the con-

trols (Fig. 2). Glycogen was unchanged after the clamp in both groups (not shown).

Glycogen synthase activity. Fractional velocity of glycogen synthase was markedly lower in the McArdle patients than in the control subjects before and at the end of the insulin clamp (49 and 57% decreased, respectively; Fig. 3A). In response to insulin stimulation, the fractional velocity of glycogen synthase increased in the patients ($P < 0.05$) and in the control subjects ($P < 0.001$). However, the relative insulin-stimulated increase in fractional velocity was significantly smaller in the McArdle patients than in the controls (53 ± 15 vs. $79 \pm 9\%$, respectively, $P < 0.05$). The %I-form was also markedly decreased in the patients compared with controls, although statistically significant differences were present only during insulin stimulation (Fig. 3B). Insulin stimulation resulted in a 2.5-fold increase in the %I-form of glycogen synthase in controls, whereas no significant increase could be observed in the McArdle patients. The total activity of glycogen synthase was similar in patients and controls both before and at the end of the clamp procedure. Insulin did not alter total glycogen synthase activity in either group of subjects (not shown).

Akt phosphorylation (Ser⁴⁷³). The phosphorylation of Akt at Ser⁴⁷³ was increased in response to the insulin treatment ($P < 0.001$, main effect; Fig. 4). The relative increase in Akt phosphorylation was significantly smaller in the McArdle patients than in controls (45 ± 13 vs. $76 \pm 13\%$, respectively, $P < 0.05$). However,

Table 4. Whole body glucose metabolism before and during the final 30 min of a 2-h euglycemic-hyperinsulinemic clamp

	Basal		Insulin	
	McArdle	Control	McArdle	Control
Glucose clearance, $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg LBM}^{-1}$			$13.0 \pm 2.0^*$	20.2 ± 4.6
Glucose infusion rate, $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg LBM}^{-1}$			$51.3 \pm 6.0^*$	72.6 ± 13.1
Glucose R _d , $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg LBM}^{-1}$	27.4 ± 2.1	23.4 ± 3.8	45.3 ± 4.3	$65.6 \pm 14.8 \ddagger$
Hepatic glucose production, $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg LBM}^{-1}$			-6.1 ± 3.9	-7.0 ± 7.9
Respiratory exchange ratio	0.77 ± 0.01	0.78 ± 0.04	0.84 ± 0.02	$0.92 \pm 0.04 \ddagger$
Nonoxidative glucose disposal, $\text{J} \cdot \text{min}^{-1} \cdot \text{kg LBM}^{-1} \ddagger$	56 ± 8.0	43 ± 24	102 ± 18	121 ± 59
Oxidative glucose disposal, $\text{J} \cdot \text{min}^{-1} \cdot \text{kg LBM}^{-1}$	22 ± 5.4	31 ± 17	$48 \pm 5.4^*$	$89 \pm 16 \ddagger$
Fat oxidation, $\text{J} \cdot \text{min}^{-1} \cdot \text{kg LBM}^{-1}$	67 ± 2.0	71 ± 12	49 ± 6.7	$28 \pm 13 \ddagger$

Data are presented as means \pm SE; $n = 6$. R_d, rate of disappearance; LBM, lean body mass. * $P < 0.05$ vs. corresponding control. $\ddagger P < 0.05$ vs. corresponding basal. $\ddagger P = 0.09$ for basal vs. insulin (main effect). See text for further details.

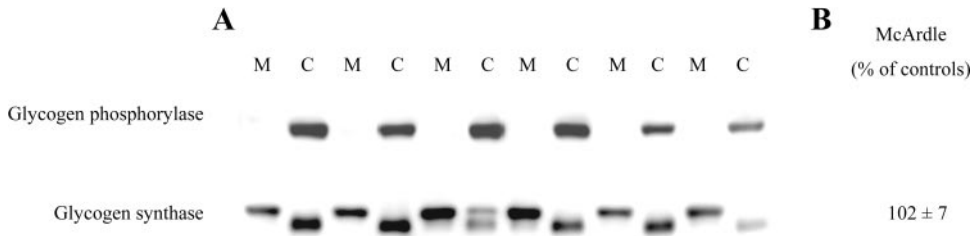


Fig. 1. Expression of glycogen phosphorylase and glycogen synthase protein in skeletal muscle of McArdle patients and control subjects, evaluated by immunoblotting. M and C in alternating lanes indicate samples from a McArdle patient and a control subject, respectively (A). Protein expression in McArdle patients is presented as percentage of the control values (B) ($n = 6$).

there were no significant differences in the absolute level of Akt phosphorylation between the McArdle patients and control subjects.

GSK3 activity. Insulin significantly decreased GSK3 α activity ($P < 0.001$, main effect; Fig. 5A). The level of activity was similar in the McArdle patients and controls in the basal state and during insulin infusion. The insulin-stimulated decrease in GSK3 α activity was not different between the groups. Insulin did not change GSK3 β activity, and there was no difference in GSK3 β activity between McArdle patients and controls (Fig. 5B).

PP1 and PP2A activity. PP1 activity was similar in the patients and controls both in the basal state and during insulin infusion (Fig. 6A). There was a tendency toward PP1 activity being lower in the patients than in the controls, although this did not reach statistical significance ($P = 0.07$ for a main effect). No significant changes in PP1 activity were observed in response to insulin; neither was there any significant difference between the groups of subjects when increase in insulin-stimulated PP1 activity was expressed as percent above basal. PP2A activity in the basal state was significantly lower in the McArdle patients than in the control subjects ($P = 0.055$; Fig. 6B). In response to insulin, PP2A activity increased in the patients ($P <$

0.01), whereas the activity was unchanged in controls. In addition, there was a significantly higher increase in insulin-stimulated PP2A activity in McArdle patients than in controls when PP2A activity was expressed as percent above basal.

Correlation analyses. To examine the strength of relationship between parameters, multiple correlation

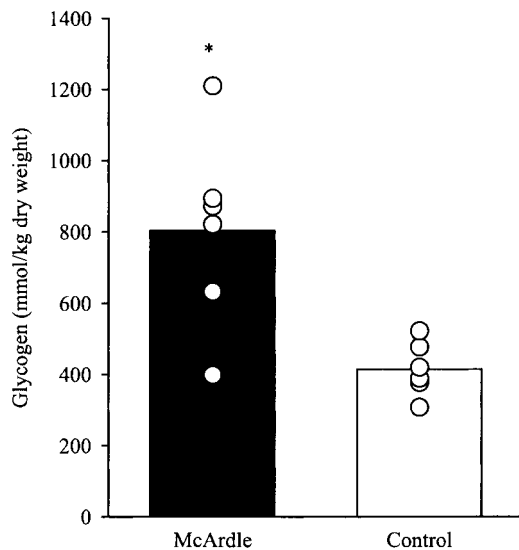


Fig. 2. Glycogen concentration in skeletal muscle of McArdle patients and controls before the insulin clamp procedure. Values are given as means ($n = 6$) and as individual values indicated by \circ . * $P < 0.05$ vs. control.

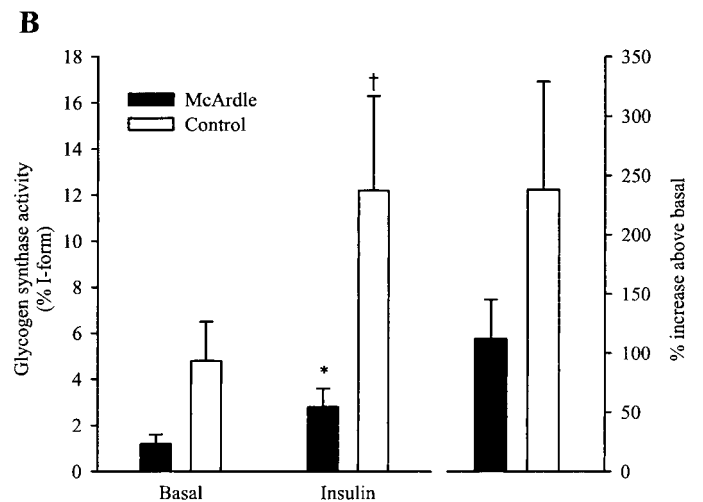
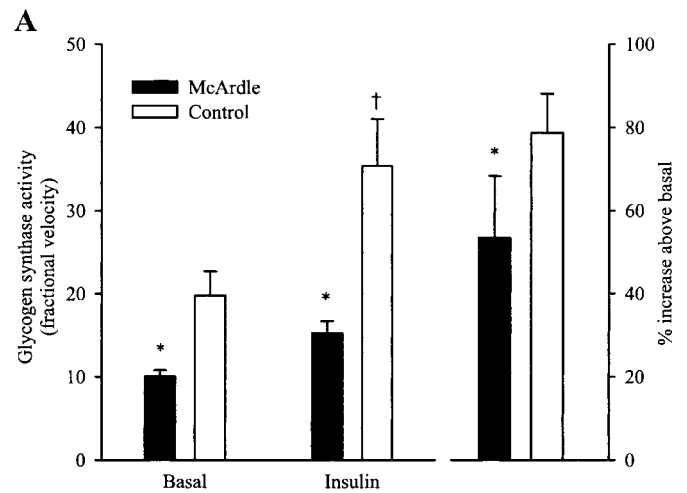


Fig. 3. Glycogen synthase activity expressed as fractional velocity (A) and %I-form (B) in McArdle patients and control subjects, measured before (basal) and at the end of a euglycemic-hyperinsulinemic clamp procedure (insulin) and expressed as %increase above basal ($n = 6$). Data are given as means \pm SE. * $P < 0.05$ vs. corresponding control; † $P < 0.05$ vs. corresponding basal.

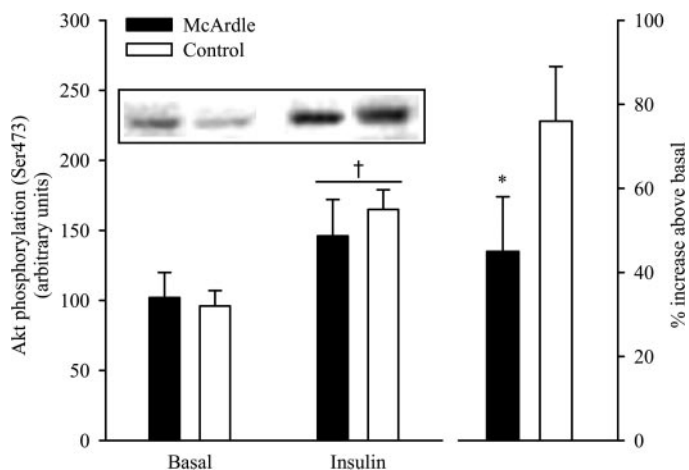


Fig. 4. Protein kinase B (Akt; Ser⁴⁷³) phosphorylation in McArdle patients and controls before (basal) and at the end of a euglycemic-hyperinsulinemic clamp procedure (insulin) and expressed as % increase above basal. A representative Western blot is shown in the inset. Data are presented as means \pm SE; $n = 6$ subjects/group. † $P < 0.001$ vs. basal (main effect); * $P < 0.05$ vs. corresponding control.

analyses were done. To avoid the analysis being biased by the large difference in glycogen between the two groups of subjects, the difference in preclamp glycogen concentration between McArdle patients and control subjects for each of the six matched pairs of subjects was compared with the difference in multiple parameters between McArdle patients and control subjects for each of the six matched pairs of subjects. In this way, glycogen correlated significantly with insulin-stimulated glycogen synthase activity, measured as both fractional velocity and %I-form (Table 5) as well as the insulin-stimulated increase in glycogen synthase activity (Δ -fractional velocity and Δ -I-form). Glucose infusion rate, clearance, and nonoxidative disposal were inversely related to the glycogen level, although not significantly correlated. Any measure of insulin signaling was uncorrelated to the glycogen level (not shown).

DISCUSSION

In this study, we observed that patients with muscle glycogen phosphorylase deficiency (McArdle's disease) have twofold higher muscle glycogen stores and that glucose clearance, glycogen synthase activity, and changes in fuel oxidation were impaired in response to a physiological insulin dose compared with matched control subjects. It has been suggested that exercise-induced depletion of the muscle glycogen stores plays an important role in enhanced insulin action (31). Conversely, in McArdle patients with a high muscle glycogen content, we found that glycogen synthase activity induced by euglycemic hyperinsulinemia was impaired and correlated tightly negatively with glycogen levels in skeletal muscle. A negative correlation between muscle glycogen content and insulin-stimulated glycogen synthase activity has also been reported in healthy subjects (7, 28). In some studies, insulin-induced whole body glucose utilization is independent of the prevailing glycogen concentration in human

skeletal muscle (7, 21), whereas in other studies examining leg glucose uptake, glycogen does play a role in insulin-induced muscle glucose utilization (31). In the present study, we observed that McArdle patients have reduced insulin-induced whole body glucose utilization. Although an inverse relationship between the glycogen level and glucose utilization was indicated, this did not reach the level of significance. This implies that other regulatory factors are also involved in decreasing insulin-stimulated glucose utilization in McArdle patients. Reduced physical activity is a natural implication of McArdle's disease and could play a role in the development of insulin resistance in this group of patients (14, 15, 19, 37). However, indicators of reduced physical activity (increased resting heart rate or altered body composition) were not present in the patients compared with the sedentary controls. Furthermore, although accurate measures of habitual

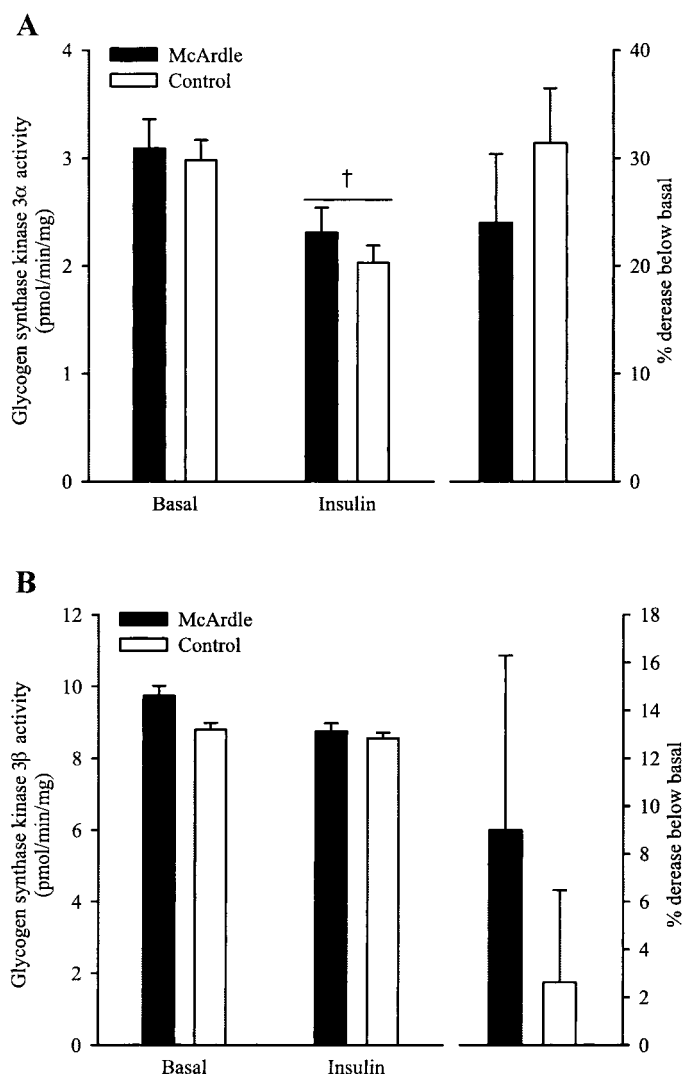


Fig. 5. Glycogen synthase kinase 3 α (GSK3 α ; A) and GSK3 β activity (B) in McArdle patients and control subjects before (basal) and at the end of a euglycemic-hyperinsulinemic clamp procedure (insulin) and expressed as % decrease below basal. Data are given as means \pm SE; $n = 6$ subjects/group. † $P < 0.001$, main effect of insulin.

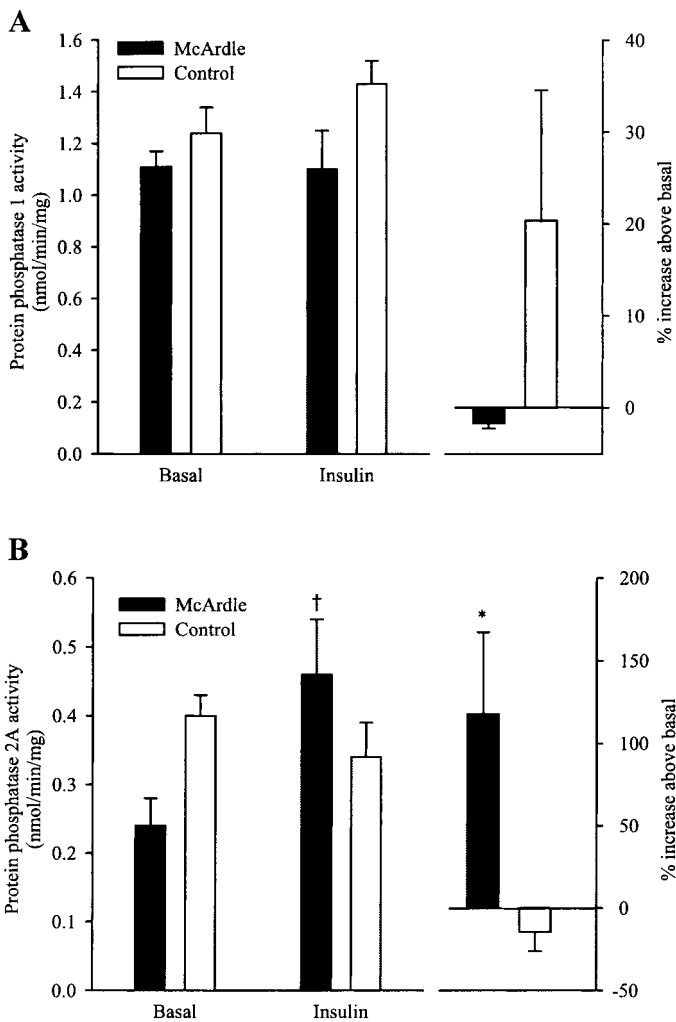


Fig. 6. Protein phosphatase (PP)1 (A) and PP2A activity (B) in McArdle patients and control subjects during basal condition and at the end of a euglycemic-hyperinsulinemic clamp procedure (insulin) and expressed as %increase above basal. Data are given as means \pm SE; $n = 6$ subjects/group. * $P < 0.05$ vs. corresponding control; † $P < 0.01$ vs. corresponding basal.

physical activity were not available, control subjects were selected only when, upon interview, they indicated minimal habitual physical activity.

The insulin-induced increase in Akt (Ser⁴⁷³) phosphorylation, which is known to reflect the activity status of the enzyme, was reduced in McArdle patients (Fig. 4), but Akt phosphorylation did not correlate with the glycogen concentration, although this has been observed in rodent studies (11). It remains controversial whether dysfunctional Akt regulation is involved in the pathology of type 2 diabetes mellitus or other states of insulin resistance (13), but it could be speculated that the reduced Akt phosphorylation in response to insulin may be linked to the impaired insulin-induced glucose clearance in the McArdle patients. The impaired glucose clearance during the insulin clamp procedure is in accord with the results from the oral glucose tolerance test (Table 2), where four of six McArdle patients and none of the controls were cate-

gorized as having impaired glucose tolerance. High levels of plasma FFA are known to inhibit insulin-induced glucose uptake. However, as plasma FFA concentrations were not higher in the patients, this does not explain the reduced glucose utilization.

Because insulin-stimulated glycogen synthase activity was significantly lower in the McArdle patients, but whole body nonoxidative glucose disposal was not, it could be argued that, despite the impaired glycogen synthase activity, muscle glycogen storage is uncompromised. However, it has previously been shown in healthy subjects that glycogen loading by an exercise and diet regimen decreased insulin-stimulated muscle glycogen synthesis measured by ¹³C/³¹P nuclear magnetic resonance spectroscopy concomitant with unchanged whole body nonoxidative glucose metabolism (21). This metabolic scenario also seems to take place in McArdle patients. It cannot be excluded that the normal whole body nonoxidative glucose disposal in the patients is due to some sort of compensatory mechanism augmenting glycogen storage in extramuscular tissue (e.g., liver).

A well described effect of insulin is to decrease whole body fat oxidation and increase carbohydrate oxidation. Thus, in the patients with McArdle's disease, the lack of a significant change in fat and carbohydrate oxidation is another indication of insulin resistance. It has been suggested that the effect of insulin to decrease fat oxidation in muscle is by activation of acetyl-CoA carboxylase (ACC) leading to an increase in muscle content of malonyl-CoA, in turn inhibiting carnitine palmitoyltransferase I (CPT I)(2). Interestingly, McArdle patients have significantly lower ACC protein content in their muscles than the controls (J. N. Nielsen, J. F. P. Wojtaszewski, R. G. Haller, D. G. Hardie, B. Kemp, E. A. Richter, and J. Vissing, unpublished observation). This might offer a mechanism for the reduced ability of insulin to decrease fat oxidation in the patients, because the increase in malonyl-CoA levels might also be diminished, leading to less inhibi-

Table 5. Relationships between glycogen level and end points of insulin action

	Glycogen, mmol/kg dry wt	
	r Value	P value
Glycogen synthase (fractional velocity)	-0.96	0.002
Glycogen synthase (%I-form)	-0.81	0.048
Glucose infusion rate, $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg LBM}^{-1}$	-0.66	0.15
Glucose clearance, $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg LBM}^{-1}$	-0.63	0.18
Nonoxidative glucose disposal, $\text{J} \cdot \text{min}^{-1} \cdot \text{kg LBM}^{-1}$	-0.63	0.18

The strength of association between parameters was analyzed by Pearson product-moment correlation analysis. To avoid the analysis being biased by the large difference in glycogen between the two groups of subjects, the difference in preclamp glycogen concentration between McArdle patients and control subjects for each of the 6 matched pairs of subjects was compared with the difference in multiple parameters between McArdle patients and control subjects for each of the 6 matched pairs of subjects. I-form, independent form.

tion of CPT I and, consequently, less decrease in fat oxidation.

One of the aims of this study was to elucidate whether differences in basal and insulin-induced glycogen synthase activity between McArdle patients and control subjects could be attributed to insulin-signaling intermediates known to affect glycogen synthase. Glycogen synthase activity is regulated by activating phosphatases and deactivating kinases but is also strongly correlated with the cellular glycogen level (7, 28, 42) by a yet unknown mechanism. Activity of GSK3 α is reduced by insulin stimulation (41), but as a novel finding in the present study, the basal level of GSK3 α and - β as well as the deactivation by insulin of GSK3 α were unaffected by glycogen level per se in human skeletal muscle (Fig. 5). Thus the reduction of glycogen synthase activity seen in McArdle patients in both the basal and insulin-stimulated conditions is unlikely to involve GSK3 α or - β as primary regulators. However, we did not look into possible differences in the time course of GSK3 activation. In vitro studies have shown that phosphatases acting as activators of glycogen synthase are inhibited by glycogen (25, 40), but one study including skeletal muscle from two healthy subjects and one McArdle patient failed to confirm the existence of such a relationship in vivo (39). Despite a higher muscle glycogen concentration in McArdle patients (Fig. 2), basal PP1 activity was not lower in the patients, although a tendency was present (Fig. 6A). Total cellular PP1 activity, as measured in this study, might be a crude measure for studying the regulation controlled by the subtle interaction of PP1 with its regulatory subunits and glycogen synthase on the glycogen particle (reviewed in Refs. 8 and 30). For the same reason, it is not surprising that PP1 activity is not stimulated significantly by a physiological concentration of insulin ($\sim 75 \mu\text{U/ml}$) in either control subjects or McArdle patients, in line with other studies in healthy and diabetic subjects (1, 12, 18).

Interestingly, PP2A activity increased in McArdle patients but was unchanged in controls in response to insulin. To our knowledge, no studies have addressed the regulation of PP2A by insulin in human skeletal muscle, but studies in muscle cells in culture show that insulin actually decreases the activity of PP2A (4, 5). The abnormal increase in insulin-stimulated PP2A activity in McArdle patients might be linked to a compensatory response to the deficient glycogenolysis in the patients, although the nature of such a mechanism remains elusive.

It is important to note that the present study is based on a human model of chronic high glycogen stores, thereby ruling out the possibility that our results are biased by any preexperimental regimen. Previous investigations studying the effect of glycogen level on insulin action have utilized experimental models involving glycogen manipulation by various combinations of exercise and diet (7, 21, 28). One may argue that the present study is based on a pathophysiological model and thus cannot be used to elucidate mechanisms operating in healthy skeletal muscle. Nonethe-

less, we were able to isolate the effect of glycogen by demonstrating that the difference in glycogen between patients with McArdle's disease and control subjects correlates strongly with the difference in insulin-stimulated glycogen synthase activity between the two groups (Table 5).

In conclusion, the present study shows that patients with McArdle's disease are insulin resistant with regard to glucose uptake, glycogen synthase activation, and changes in fuel oxidation. Our results also suggest that, in humans, skeletal muscle glycogen level plays an important role in insulin-stimulated glycogen synthase activity but less so in regulation of insulin-stimulated glucose utilization and in changes in fuel oxidation. The mechanism by which glycogen inhibits insulin-stimulated glycogen synthase activity remains unclear. Although the insulin-induced increase in Akt phosphorylation was smaller in the McArdle patients, this was not correlated with the glycogen level. The GSK3 and PP1 activities measured in this study could not explain the effect of glycogen on basal and insulin-stimulated glycogen synthase activity. The mediation of the glycogen effect could, however, involve GSK3 and/or PP1 through temporal regulation and spatial arrangement of these enzymes.

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