

Effect of Muscle Glycogen Depletion on In Vivo Insulin Action in Man

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ABSTRACT In rats, muscle glycogen depletion has been associated with increased insulin action. Whether this also occurs in man has not been reported. After 4 d rest, 13 males (E Group) had a percutaneous muscle biopsy of the vastus lateralis muscle followed by a euglycemic clamp at plasma insulin $\approx 100 \mu\text{U/ml}$ and $\approx 1,900 \mu\text{U/ml}$, with simultaneous indirect calorimetry. This was repeated 1 wk later, but after glycogen-depleting exercise the night before the euglycemic clamp. Seven subjects underwent the same protocol but were also re-fed 100 g carbohydrate (CHO) after the exercise (EF group).

In both groups, the mean muscle glycogen content was $\sim 40\%$ lower ($P < 0.01$) after exercise compared with the muscle glycogen content measured after rest. In the E group, the mean muscle glycogen synthase activity (percent independent of glucose-6-phosphate) increased threefold ($P < 0.001$) after exercise, but increased only twofold in the EF group ($P < 0.02$ between groups). In both groups, the mean basal and insulin-stimulated CHO oxidation rates were lower in the post-exercise, glycogen-depleted condition compared with the rested, glycogen-replete condition. The mean insulin-stimulated CHO storage rate increased significantly in the E group after exercise but not in the EF group. In the E group, the total insulin-stimulated CHO disposal rate (M) was 17 ($P < 0.04$) and 10% ($P < 0.03$) higher after exercise during the low and high dose insulin infusion, respectively. No significant changes in M were observed in the EF group.

For all subjects, after rest and exercise, the M correlated with the CHO storage rates during the low ($r = 0.80$, $P < 0.001$) and high dose ($r = 0.77$, $P < 0.001$) insulin infusions. After exercise, the muscle glycogen synthase activity correlated with the CHO

storage rate ($r = 0.73$, $P < 0.002$; $r = 0.75$, $P < 0.002$) during the low and high dose insulin infusions, respectively, and also with M ($r = 0.64$, $P < 0.008$; $r = 0.57$; $P < 0.02$).

INTRODUCTION

Recent in vitro animal experiments have demonstrated increased insulin action in exercised, glycogen-depleted muscles, compared with rested, glycogen-replete muscles (1, 2). The mechanism of the effect was not causally related to the glycogen depletion per se since the enhanced insulin action was still observed after the muscle glycogen content had returned to preexercise concentrations (1). The enhanced insulin action was also not correlated with increased glycogen synthase activity as measured by standard techniques (1). However, Richter et al. (1) suggested that changes in the activity of an intermediate form of the enzyme, as described by Kochan et al. (3), might correlate with the observed increases in insulin action.

Some authors have suggested that, in man, muscle glycogen depletion may be responsible for the apparent increased insulin action after exercise (4); but there are no reports describing the relationship between muscle glycogen content and in vivo insulin action. This study was designed to determine the effect of muscle glycogen depletion on in vivo insulin action in man as measured by the euglycemic clamp technique. Simultaneous indirect calorimetric measurements were made to estimate basal and insulin-stimulated carbohydrate oxidation and storage rates. A muscle biopsy was also done to determine the relationship between muscle glycogen content, muscle glycogen synthase activity, and in vivo insulin action. The results showed that muscle glycogen depletion was associated with decreases in glucose oxidation rates, increases in glucose storage rates, and variable changes in total insulin-

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stimulated carbohydrate disposal rates (M).¹ In the glycogen-depleted condition, the glucose storage rate and M were well correlated with the muscle glycogen synthase activity.

METHODS

Subjects and experimental protocol. 20 caucasian males were studied. All subjects were in good health, had no family history of diabetes mellitus, and were not taking any medications known to affect carbohydrate metabolism. None of them were engaged in collegiate or professional sports. Seven subjects jogged two to three times per week but the others were sedentary. Written informed consent was obtained from all subjects. They were physically examined and after an overnight fast had blood drawn for complete blood count, liver function tests, blood-urea nitrogen, creatinine, electrolytes, calcium, total protein, and albumin. After obtaining a urine for routine urinalysis, each subject had an oral glucose tolerance test to document normal glucose tolerance (5). If all these were within normal limits, on a separate day each subject's body composition was estimated by underwater weighing (6) with simultaneous determination of residual lung volume.

13 subjects, the E group, were admitted to the Phoenix Clinical Research Section for 4 d of restricted activity and a weight-maintaining diet containing at least 250 g of carbohydrate/d. The next morning, after a 15-h fast (except for water) each subject underwent a percutaneous muscle biopsy before and after a euglycemic clamp procedure (see below). At the end of the day the subjects were discharged and resumed their normal activities. After 2–3 d they were readmitted. On the evening of the 4th day, 2 h after the evening meal, fed at 1530–1600 h, the glycogen-depleting exercise was performed (see below). The next morning, again after 15 h of fasting, the muscle biopsy and euglycemic clamp procedure were repeated (see below). The subjects were randomly assigned to perform the glycogen-depleting exercise during the first or second admission.

To obtain a range of glycogen depletion, seven subjects, the EF group, went through the same protocol except that 3 h after the glycogen-depleting exercise, and at the same time during the no-exercise admission, they were given 100 g of an oral glucose solution. The characteristics of the subjects in E group and EF group are shown in Table I.

Glycogen-depleting exercise. The exercise was begun with a 20–25-min walk/jog as warm up and initial exercise. This was followed by intermittent cycle ergometry, at 60 rpm for 2 min, then 3 min of rest. The workload was adjusted to maintain the heart rate at 80–90% of the maximum heart rate predicted for their age (7), which approximates an equivalent percent of the maximum oxygen uptake. When the subject could no longer cycle at 60 rpm the exercise was stopped. The average duration of the exercise session was ~45 min.

Euglycemic clamp. At 0600 h, an intravenous catheter was placed in an antecubital vein for infusion of insulin, glucose, and [3-H³]glucose. Another catheter was placed retrograde in a dorsal vein of the contralateral hand for blood withdrawal. The hand was kept in a warming box at 70°C.

¹ *Abbreviations used in this paper:* GSI, glycogen synthase %I; %I, percent independent of glucose-6-phosphate; M, total insulin-stimulated carbohydrate disposal rate; Ra, appearance rate.

TABLE I
Subject Characteristics

	E group (Exercised, not re-fed)	EF group (Exercised, re-fed)
n	13	7
Age (yr)	24±1	25±3
Body weight (kg)	68±3	72±6
Body fat (%) [*]	13±1	14±2

^{*} Body fat was determined by underwater weighing.

A primed (30 μCi)-continuous (0.30 μCi/min) infusion of [3-H³]glucose (2–5 Ci/mmol) was then begun into the antecubital catheter and continued throughout the procedure. After 2.5 h, four blood samples were drawn over 30 min for [3-H³]glucose specific activity determination. After 3 h, a primed continuous infusion of insulin (40 mU/m² per min) was started. This resulted in a mean plasma insulin concentration of 103±4 μU/ml for all subjects. After 100 min another primed infusion was given and a new constant insulin infusion rate begun (400 mU/m² per min) for 100 min. This produced a mean plasma insulin concentration of 1,876±60 μU/ml for all subjects. The mean plasma insulin concentrations of the E and EF groups were not different from the mean for the group, either before or after exercise, at the low or high plasma insulin concentrations. 4 min after the start of the insulin, a variable 20% glucose infusion was started to maintain the plasma glucose concentration at ~90 mg/100 ml for the entire 200 min of hyperinsulinemia. Blood for plasma glucose concentration was drawn every 5 min throughout the test. Blood for plasma insulin and [3-H³]glucose specific activity was drawn every 10 min from 60–100 min and 160–200 min.

Muscle biopsy. 2 h after the start of the [3-H³]glucose infusion, a percutaneous biopsy of the vastus lateralis muscle was done within 5–7 min as previously described (8). Two specimens were obtained. The first specimen was immediately weighed and processed for muscle glycogen content determination (see below). The second specimen was immediately placed in liquid nitrogen, then stored at –80°C for later determination of glycogen synthase activities (see below). At the end of the euglycemic clamp the muscle biopsy was repeated in the opposite thigh.

Indirect calorimetry. Immediately after the completion of the initial muscle biopsy, a clear, plastic, ventilated hood was placed over the subject's head. Room air was drawn through the hood and the flow rate measured by a gasometer (American Meter Div. of the Singer Co., Philadelphia, PA) with an adapted potentiometer. A constant fraction of expired air was withdrawn and analyzed for oxygen and carbon dioxide content. The oxygen analyzer was a zirconium cell analyzer (Applied Electrochemistry, Sunnyvale, CA) and the carbon dioxide analyzer was an infrared analyzer (Applied Electrochemistry, Sunnyvale, CA). The analyzers and flowmeter outputs were connected to a desktop computer (Hewlett-Packard, Co., Palo Alto, CA). This recorded continuous, integrated calorimetric measurements every 5 min for the hour before and for the duration of the euglycemic clamp. The protein oxidation during the test was estimated from the urinary urea production rate. The nonprotein respiratory quotient was then calculated and the substrate oxidation rates determined from the tables of Lusk (9).

Calculations. The appearance rate (Ra) of glucose in the plasma was calculated from the blood [3-H³]glucose specific

activities using the equations of Steele (10), in their steady state form for the basal period and the non-steady state form for the *Ra* during the euglycemic clamp. During the basal period the *Ra* equals the endogenous glucose production rate. During the euglycemic clamp, the endogenous glucose production rate equals the difference between the rate of exogenously infused glucose and the *Ra* determined from Steele's equations. The total glucose disposal rate is then equal to the sum of the rate of exogenously infused glucose plus the rate of endogenous glucose production. In these experiments, these data were calculated for each 20 min between 60–100 and 160–200 min during the euglycemic clamp and then averaged to calculate the total glucose disposal or M-value during the low and high dose insulin infusion. The basal and insulin-stimulated carbohydrate oxidation rates were calculated from the indirect calorimetric data by averaging the data for 40 min before the beginning of the insulin infusion and for the last 40 min during the low and high dose insulin infusion. The carbohydrate storage rate was estimated by subtracting the carbohydrate oxidation rate from the total glucose disposal rate. The carbohydrate oxidation and storage rates tend to increase with increasing duration of the insulin infusion as does the net carbohydrate disposal rate, or M-value. However, this does not invalidate comparisons between individuals or the results of multiple tests of one individual as long as the time of the insulin infusion is held constant.

Analytical methods. The plasma glucose concentration was measured by the glucose oxidase method using a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, CA). Plasma insulin concentrations were determined by the Herbert et al. modification (11) of the radioimmunoassay of Berson and Yalow (12). The determination of tritiated glucose specific activity in blood samples was performed as described by others (13) using perchloric acid to precipitate the protein. Muscle glycogen content was determined by first solubilizing muscle tissue in KOH and then precipitating the glycogen with iced ethanol, and hydrolyzing in 6 N H₂SO₄ (14).

Glycogen synthase was assayed by a modification of the procedure of Thomas et al. (15), and the results were expressed as the percent active in the absence of glucose-6-phosphate (percent independent [%I] activity) relative to the maximum activity (– glucose-6-phosphate/+ glucose-6-phosphate).

Statistics. All data are expressed as the means ± the standard error of the means. Statistical evaluation of the data was done using the *t* test for paired and unpaired data when appropriate.

RESULTS

Muscle glycogen content and glycogen synthase activity. (Table II). In both the E (exercised, not re-fed) group and the EF (exercised, re-fed) group, the mean muscle glycogen content was significantly lower the morning after exercise compared with the concentration observed after rest. There also was a significant increase in the mean muscle glycogen content after the euglycemic clamp procedure in E group, both in the rested, glycogen-replete condition and in the postexercise, glycogen-depleted condition. In the EF group, the mean muscle glycogen content increased significantly after the euglycemic clamp procedure only in the glycogen-depleted condition. In both groups, there was no statistical difference between the change in the mean muscle glycogen content during the euglycemic clamp observed at rest, compared with the change after exercise.

The mean muscle glycogen synthase %I (GSI) activity was similar in the two groups in the rested condition, both before and after the euglycemic clamp procedure. After exercise, in the glycogen-depleted

TABLE II
Muscle Glycogen Content and GSI Activity of the Vastus Lateralis Muscle after Rest and after Exercise before and after the Euglycemic Clamp Procedure

	Rest		Postexercise	
	Before clamp	After clamp	Before clamp	After clamp
E group (exercised, not re-fed)				
Muscle glycogen content (g/100 g wet tissue)	1.89±0.07	2.14±0.09*	0.97±0.08†	1.45±0.18*†
GSI activity (%)	13±1	32±3*	40±3†	57±4*†
EF group (exercised and re-fed)				
Muscle glycogen content (g/100 g wet tissue)	1.77±0.17	1.84±0.18	1.13±0.13†	1.40±0.18*
GSI activity (%)	13±1	32±6*	25±1†	50±3*†

The muscle glycogen content determination was for *n* = 13 in the E group and *n* = 6 in the EF group. The glycogen synthase measurements are for *n* = 10 in the E group and *n* = 5 in the EF group before the clamp; and *n* = 8, *n* = 4 for these groups, respectively, after the clamp.

* *P* < 0.05 for the before and after clamp comparison.

† *P* < 0.05 for the rest vs. postexercise comparison.

condition, the mean GSI activity was higher in both groups compared with the mean activity observed after rest. However, after exercise and before the euglycemic clamp, the mean GSI activity of the E group ($40 \pm 3\%$) was higher than that of the EF group ($25 \pm 1\%$) ($P < 0.02$). After the euglycemic clamp, the mean GSI activity of the two groups were similar ($57 \pm 4\%$ and $50 \pm 3\%$).

The relationship between the muscle glycogen content and the GSI activity of the vastus lateralis muscle for all subjects, in the rested, glycogen-replete condition and the postexercise, glycogen-depleted condition, is shown in Fig. 1.

Insulin action (Fig. 2). The mean basal endogenous glucose production rate did not change in either group after the glycogen-depleting exercise compared with the rate observed after 4 d of rest.

In the E group, the mean M increased significantly after exercise, both during the low and high dose insulin infusions. However, no change in the mean M was observed in the EF group, comparing the post exercise with the rested condition.

The mean basal and insulin-stimulated carbohydrate oxidation rates were lower in the E group in the postexercise, glycogen-depleted condition compared with the rate observed in the rested, glycogen-replete condition. In the EF group, the mean carbohydrate oxidation rate was also significantly lower after exercise in the basal state and during the low dose insulin infusion. However, after exercise and during the high dose insulin infusion, the mean carbohydrate oxidation rate was not significantly lower than the rate observed in the rested condition.

In the E group, the mean basal and insulin-stimulated carbohydrate storage rates were higher in the postexercise, glycogen-depleted condition compared with the rates observed in the rested, glycogen-replete condition. In the EF group, the mean basal carbohydrate

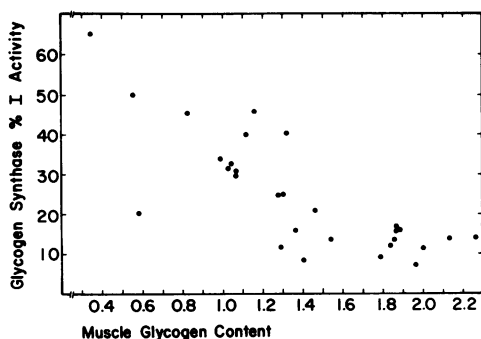


FIGURE 1 The relationship between the muscle glycogen content (grams per 100 grams of wet tissue) of the vastus lateralis muscle and the glycogen synthase %I activity.

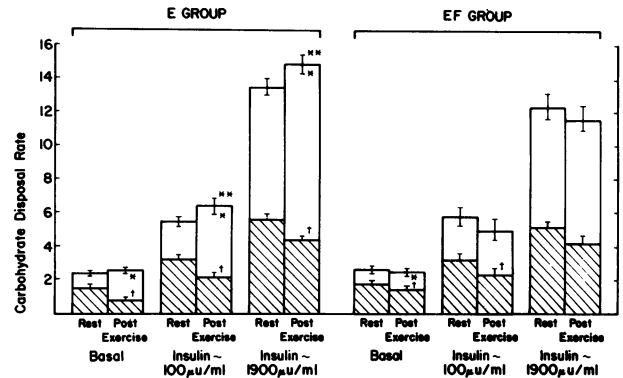


FIGURE 2 The carbohydrate disposal rate (expressed as milligrams per kilogram of fat free mass per min) in the E group (exercised, not re-fed), $n = 13$, and in the EF group (exercised, re-fed), $n = 7$, after rest (left-hand bars) and after exercise (right-hand bars). The results are for the basal state and in response to the insulin infusions during the euglycemic clamp procedures. The hatched areas represent carbohydrate oxidation; the open areas represent carbohydrate storage. *, significant ($P < 0.05$) increase in carbohydrate storage, comparing the rested with the postexercise condition. †, significant ($P < 0.05$) decrease in carbohydrate oxidation comparing the rested with the postexercise condition. **, significant ($P < 0.05$) increase in M, comparing the rested with post-exercise condition.

rate was higher after exercise, but no significant changes were observed in the mean insulin-stimulated carbohydrate storage rates.

Combining the data from both the E and EF groups in the rested and postexercise conditions, there was a good correlation between the glucose storage rates and M values, during both the low ($r = 0.80$, $P < 0.0001$) and high dose ($r = 0.77$, $P < 0.0001$) insulin infusions. The glucose oxidation rates were less well correlated with the M values ($r = 0.40$, $P < 0.01$; $r = 0.41$, $P < 0.01$) during the low and high dose insulin infusion, respectively.

In vivo and in vitro comparisons. Combining the results from both groups, there was no correlation between the muscle glycogen content and the carbohydrate storage rate or the total carbohydrate disposal rate. This was true in either the rested or the postexercise condition, or when the data from both conditions were analyzed together. When the data from the rested and postexercise condition were combined, the muscle GSI activity (before the insulin infusions) correlated with the basal glucose storage rates ($r = 0.62$, $P < 0.0001$) and with the insulin-stimulated glucose storage rates during the low ($r = 0.66$, $P < 0.0001$) and high ($r = 0.61$, $P < 0.001$) dose insulin infusion. However, this was primarily because of the correlation between these parameters in the postexercise glycogen-depleted condition. In the glycogen-depleted condi-

tion, the muscle GSI activity (before the infusion of insulin) correlated with the basal ($r = 0.53$, $P < 0.05$) and insulin-stimulated glucose storage rates ($r = 0.72$, $P < 0.002$; $r = 0.75$, $P < 0.002$) during the low (Fig. 3) and high (Fig. 4) dose insulin infusion, respectively. However, no significant correlation between these parameters are observed in the rested, glycogen-replete condition.

Also, after exercise, the muscle GSI activity, (before the insulin infusion) correlated with the M values ($r = 0.64$, $P < 0.008$; $r = 0.57$, $P < 0.02$) during the low and high dose insulin infusion, respectively. No correlation between the muscle GSI activity and M was observed in the rested, glycogen-replete condition.

There was no significant correlation between any of the in vivo measures of insulin action and the muscle GSI activity measured at the end of the insulin infusions.

DISCUSSION

This study was designed to determine the effect of muscle glycogen depletion on in vivo insulin action in man. The results show that muscle glycogen depletion after exercise is associated with reduced basal and insulin-stimulated carbohydrate oxidation rates, even after re-feeding 100 g of carbohydrate after the exercise. Insulin-stimulated carbohydrate storage rates are increased after glycogen-depleting exercise if there is no re-feeding of carbohydrate. Changes in M appear to depend on the extent of the increase in the carbohydrate storage rate. Thus, in this study, the E group (exercised, not re-fed) had significantly increased carbohydrate storage rates after exercise, and an increase in the M-value was also observed. The total insulin-stimulated, carbohydrate disposal rate can apparently increase even though the carbohydrate oxidation rate

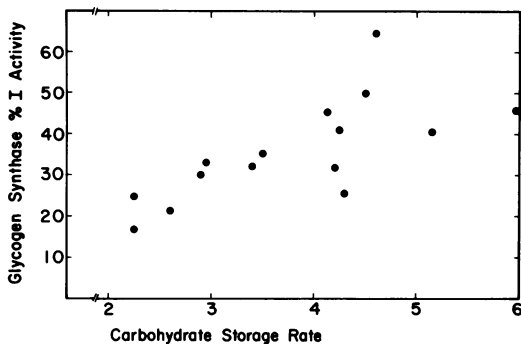


FIGURE 3 In the postexercise condition, the relationship between the resting muscle GSI activity and the carbohydrate storage rate (expressed as milligrams per kilogram of fat-free mass per min), as estimated by indirect calorimetry, during the euglycemic clamp at a plasma insulin concentration of $103 \pm 4 \mu\text{U/ml}$. $r = 0.73$; $P < 0.002$.

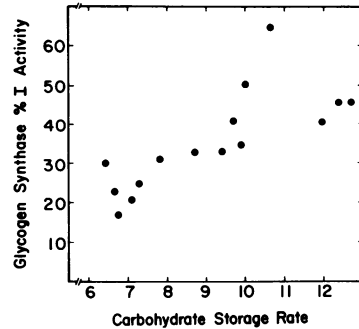


FIGURE 4 In the postexercise condition, the relationship between the resting muscle GSI activity and the carbohydrate storage rate (expressed as milligrams per kilogram of fat-free mass per min), as estimated by indirect calorimetry, during the euglycemic clamp at a plasma insulin concentration of $1,876 \pm 60 \mu\text{U/ml}$. $r = 0.75$; $P < 0.002$.

may be decreased. In addition, the M is well correlated with the carbohydrate storage rate at rest and after exercise. This emphasizes the relatively greater importance of carbohydrate storage rates, compared with oxidation rates, in determining the total carbohydrate disposal rates in glucose tolerant subjects. Similar conclusions have been drawn by Rouselle et al. (16) who suggested that changes in glucose oxidation rates have little effect on glucose tolerance.

DeFronzo et al. (17) have previously reported that 85% of the infused glucose during a euglycemic clamp procedure is disposed of by muscle. The primary effect of insulin on the muscle tissue appeared to be to enhance glucose storage, presumably as glycogen. However, actual muscle glycogen measurements were not made.

The predicted change in the mean total muscle glycogen content after the insulin infusion in all our 20 subjects, in the rested condition, was 38 g. This assumes that: (a) 85% of the infused glucose was disposed of by muscle; (b) in the rested condition the glucose was distributed to all the muscles; (c) the muscle mass is equal to 37.4% of the fat-free mass (18), and (d) all the glucose not oxidized was stored as glycogen. The observed change in the mean muscle glycogen content for the group was 42 g. In the postexercise, glycogen-depleted condition, assuming that the infused glucose was distributed only to the glycogen-depleted muscle mass of the legs, and that the muscle mass of the legs is $\sim 60\%$ of the total body muscle mass, then the predicted change in the mean total muscle glycogen content was 52 g, and the observed change was, by coincidence, 52 g. These calculations are only estimates and assume that the muscle glycogen content is evenly distributed in the muscle mass of the body, which is not completely accurate (19). However, they do support the previous observations of DeFronzo et al. (17),

and further underline the importance of glucose storage in muscle to overall glucose disposal in man.

The muscle glycogen depletion observed in this study was associated with an increased muscle GSI activity below a muscle glycogen content of ~ 1.2 g/100 g of wet tissue (Fig. 1). Hultman et al. (19) have previously reported a similar relationship. There appears to be a threshold muscle glycogen content below which the GSI activity rises, rather than a linear relationship between these two parameters, over the entire range of muscle glycogen content. This probably is the explanation why re-feeding carbohydrate after exercise (EF group), with little resultant difference in muscle glycogen content compared with the E group (1.13 ± 0.13 vs. 0.97 ± 0.08 , respectively, $P = \text{NS}$), resulted in significant difference in the mean GSI activity, 25 ± 1 and $40 \pm 3\%$ ($P < 0.02$) between the groups. The re-feeding increased the muscle glycogen content in several subjects in the EF group above this threshold glycogen concentration such that significant decreases in the GSI activity occurred.

The comparisons between the in vitro and in vivo results show that the muscle GSI activity is well correlated with the glucose storage rate, and the total glucose disposal rate, in the glycogen-depleted condition. Although a causal relationship cannot be conclusively inferred from these correlations, we believe it is worth considering the possibility that muscle glycogen synthase activity may contribute to the control of glucose storage rates and, hence, the total glucose disposal rate in vivo in man. Previous authors (1, 2), working with experimental animals, have suggested that glucose transport is rate limiting for insulin action in postexercise, glycogen-depleted muscle in vitro. In man in vivo, however, the relative role of insulin binding, glucose transport and/or subsequent steps in insulin action in determining glucose disposal rates remains poorly defined. We believe the present results can be used to form a new, testable hypothesis that in the glycogen-depleted condition muscle glycogen synthase activity may contribute to the regulation of insulin action in vivo.

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