Lack of defect in insulin action on hepatic glycogen synthase and phosphorylase in insulin-resistant monkeys

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Ortmeyer, Heidi K., and Noni L. Bodkin. Lack of defect in insulin action on hepatic glycogen synthase and phosphorylase in insulin-resistant monkeys. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G1005–G1010, 1998.—It is well known that an alteration in insulin activation of skeletal muscle glycogen synthase is associated with insulin resistance. To determine whether this defect in insulin action is specific to skeletal muscle, or also present in liver, simultaneous biopsies of these tissues were obtained before and during a euglycemic hyperinsulinemic clamp in spontaneously obese insulin-resistant male rhesus monkeys. The activities of glycogen synthase and glycogen phosphorylase and the concentrations of glucose 6-phosphate and glycogen were measured. There were no differences between basal and insulin-stimulated glycogen synthase and glycogen phosphorylase activities or in glucose 6-phosphate and glycogen contents in muscle. Insulin increased the activities of liver glycogen synthase (P < 0.05) and decreased the activities of liver glycogen phosphorylase (P < 0.001). Insulin also caused a reduction in liver glucose 6-phosphate (P = 0.05). We conclude that insulin-resistant monkeys do not have a defect in insulin action on liver glycogen synthase, although a defect in insulin action on muscle glycogen synthase is present. Therefore, tissue-specific alterations in insulin action on glycogen synthase are present in the development of insulin resistance in rhesus monkeys.

METHODS

Animals. Seven male rhesus monkeys (Macaca mulatta) housed at the Obesity and Diabetes Research Center of the University of Maryland were studied. All monkeys were housed in individual steel cages and maintained under consistent laboratory conditions in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892]. All protocols were reviewed and approved by the Institutional Animal Care and Use Committee. The monkeys were provided monkey chow (Purina, St. Louis, MO; 17% protein, 70% carbohydrate, and 13% fat) and water ad libitum. The monkeys were not receiving medication at the time of the study.

In the present study, glycogen synthase and glycogen phosphorylase activities were measured in liver, skeletal muscle, and subcutaneous and omental adipose tissue obtained before and during a euglycemic hyperinsulinemic clamp in five (liver) to seven (all other tissues) insulin-resistant rhesus monkeys. The purpose of the study was to examine whether, with whole body insulin resistance, there is an alteration in in vivo insulin action on liver enzyme activities. We hypothesized that although insulin action on rate-limiting enzymes of glycogen metabolism is aberrant in skeletal muscle and subcutaneous adipose tissue (as we have previously shown [11, 12]), insulin action might be normal on these enzymes in livers of insulin-resistant primates.

Although a defect in insulin action at the liver is present in type 2 diabetes mellitus, e.g., decreased suppression of hepatic glucose production resulting in hyperglycemia (4), it is not clear whether a defect in insulin action at the liver is involved in the development of peripheral insulin resistance. In spontaneously obese rhesus monkeys, in which the natural history of type 2 diabetes has been well characterized, peripheral insulin resistance is evident well before the onset of increased hepatic glucose production and hyperglycemia (1). In the rhesus monkey insulin resistance develops concurrently with decreased insulin action on glycogen synthase in skeletal muscle and subcutaneous adipose tissue (11, 12). The activity of liver glycogen synthase in insulin-resistant rhesus monkeys under insulin-stimulated conditions has not been reported.

We recently reported liver glycogen synthase and glycogen phosphorylase activities in healthy lean young adult rhesus monkeys before and during a euglycemic hyperinsulinemic clamp (13). In vivo insulin significantly increased the activity of liver glycogen synthase and significantly decreased the activity of liver glycogen phosphorylase in these lean animals. We have also recently reported a study of basal fasting liver enzyme activities in obese monkeys ranging in insulin sensitivity (10). There were no significant differences in basal activities of glycogen synthase, glycogen phosphorylase, protein phosphatase 1/2A and 2C, and cAMP-dependent protein kinase in the three groups of monkeys studied (normal obese, hyperinsulinemic obese, and type 2 diabetic obese rhesus monkeys).

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Table 1 shows the characteristics of the seven monkeys. The monkeys were between 8.4 and 16.9 yr old, between 11.3 and 22.1 kg body wt, and had between 22.1 and 45.1% body fat. Six of the seven animals were hyperinsulinemic with normal fasting glucose concentrations (insulin resistant). One monkey (J-8) was hyperinsglycemic with a low-fasting insulin concentration (type 2 diabetic). The mean whole body glucose disposal rate [M; 5.59 ± 0.30 mg·kg fat-free mass (FFM)·min⁻¹] of the monkeys in the present study was similar to the mean M previously published (5.73 ± 0.8 mg·kg FFM⁻¹·min⁻¹) for a group of obese insulin-resistant monkeys (phases 5–7) (1). This M was significantly lower than the mean M from noninsulin-resistant obese monkeys (phase 2) (11.39 ± 0.1 mg·kg FFM⁻¹·min⁻¹).

In vivo characterization procedures. After a 16-h fast, plasma was obtained for the determination of fasting plasma glucose, insulin, and C-peptide concentrations before the basal fasting tissue biopsies were obtained. Plasma glucose concen-
trations were determined using the glucose oxidase method and a glucose autoanalyzer (Beckman Instruments, Fullerton, CA). Plasma insulin concentrations were measured by a modification of the double antibody RIA (9), and C-peptide concentrations were determined by the method of Kuzuya et al. (8). Basal hepatic glucose production measurements were performed on a separate day from the euglycemic hyperinsulinemic clamp as described previously (1). Body fat was determined using the tritiated water dilution method of Pace et al. (15).

Tissue biopsies and euglycemic hyperinsulinemic clamp procedures. Skeletal muscle (vastus lateralis), adipose tissue (subcutaneous and omental), and liver samples were obtained before and once during the euglycemic hyperinsulinemic clamp. Approximately 1-g biopsies of tissue were excised under sterile conditions and immediately freeze-clamped (within 3 s) with aluminum clamps cooled in liquid nitrogen. The order of biopsies was skeletal muscle, subcutaneous adipose tissue, omental adipose tissue, and liver. The frozen samples were kept in liquid nitrogen until further processing.

For the infusion of insulin, glucose, lactated Ringer solution (fluid replacement), and intravenous drugs a peripheral intravenous catheter was placed in each animal. A second catheter for blood withdrawal was placed contralaterally with an arterial catheter (within 3 s) with aluminum clamps cooled in liquid nitrogen. The order of biopsies was skeletal muscle, subcutaneous adipose tissue, omental adipose tissue, and liver. The frozen samples were kept in liquid nitrogen until further processing.

Immediately after the basal liver biopsy, a priming infusion of regular pork insulin was administered over 10 min followed by a continuous insulin infusion (400 mU·m² body surface area⁻¹·min⁻¹) for ~200 min. Blood samples were obtained every 5 min, and a 20% glucose infusion was administered, as needed, to maintain plasma glucose between 4.4 and 5.0 mmol/l. Total body insulin-mediated glucose disposal rate (M) was calculated when the glucose was stable between 4.4 and 5.0 mmol/l. The steady-state plasma glucose and insulin values were 4.7 ± 0.01 mmol/l and 25,056 ± 1,885 pmol/l, respectively. Glucose disposal rate was corrected for FFM.

The second set of tissue biopsies was obtained under conditions of euglycemia and hyperinsulinemia after 99 min (skeletal muscle), 108 min (subcutaneous adipose tissue), 112 min (omentum adipose tissue), and 133 min (liver) and immediately freeze-clamped. Enzyme and substrate assays. The glycogen synthase and glycogen phosphorylase independent activities (GPI) before (basal) and during (insulin) the euglycemic hyperinsulinemic clamp. Basal vs. insulin activities were significant at P = 0.05.

RESULTS

Liver. The individual data for liver glycogen synthase and glycogen phosphorylase independent activity are shown in Fig. 1. Liver glycogen synthase and glycogen phosphorylase total activity are shown in Fig. 2. Liver
glycogen synthase and glycogen phosphorylase fractional activity were significant at P < 0.005. B: liver glycogen phosphorylase total activities (GPT) before (basal) and during (insulin) the euglycemic hyperinsulinemic clamp. Basal vs. insulin activities were significant at P < 0.0005.

In vivo insulin during a euglycemic hyperinsulinemic clamp caused significant increases in glycogen synthase independent activity (0.15 ± 0.08 vs. 0.21 ± 0.05, P < 0.05), total activity (1.55 ± 0.10 vs. 2.9 ± 1.21 nmol·min⁻¹·mg protein⁻¹, P = 0.0005), and fractional activity (15.8 ± 4.1 vs. 30.6 ± 6.4%, P < 0.05). In vivo insulin caused significant decreases in glycogen phosphorylase independent activity (0.21 ± 0.02 vs. 0.09 ± 0.01 µmol·min⁻¹·mg protein⁻¹, P = 0.001), total activity (0.28 ± 0.02 vs. 0.18 ± 0.01 µmol·min⁻¹·mg protein⁻¹, P < 0.001), and fractional activity (74.2 ± 2.9 vs. 48.6 ± 2.0%, P < 0.0005). There was also a significant decrease in glucose 6-phosphate content during the clamp (0.24 ± 0.06 vs. 0.07 ± 0.01 nmol/mg dry wt, P = 0.05). There was no significant change in glycogen content during the clamp (43.2 ± 20.7 vs. 64.5 ± 16.2 mg/g dry wt).

Whole body insulin-mediated glucose disposal rate (M) was significantly inversely related to basal and insulin-stimulated glycogen phosphorylase total activities (r = −0.94, P = 0.005, and r = −0.96, P = 0.002, respectively), to basal and insulin-stimulated glycogen phosphorylase independent activities (r = −0.99, P < 0.001, and r = −0.89, P < 0.02, respectively), and to basal glycogen phosphorylase fractional activity (r = −0.85, P < 0.02) and was positively related to the insulin-stimulated minus basal change in 1) glycogen phosphorylase total activity (r = 0.92, P < 0.01), 2) glycogen phosphorylase independent activity (r = 0.98, P < 0.001), and 3) glycogen phosphorylase fractional activity (r = 0.92, P < 0.005).

Skeletal muscle. There were no significant differences between basal fasting and insulin-stimulated skeletal muscle glycogen synthase activities, glycogen phosphorylase activities, glucose 6-phosphate content, or glycogen content. The basal and insulin-stimulated glycogen synthase activities were as follows: independent 1.46 ± 0.51 vs. 2.35 ± 1.09 nmol·min⁻¹·mg protein⁻¹, total 12.85 ± 2.65 vs. 11.76 ± 2.75 nmol·min⁻¹·mg protein⁻¹, and fractional 11.0 ± 2.6 vs. 16.1 ± 4.0%. The basal and insulin-stimulated glycogen phosphorylase activities were as follows: independent 0.60 ± 0.16 vs. 0.48 ± 0.08 µmol·min⁻¹·mg protein⁻¹, total 1.95 ± 0.21 vs. 1.74 ± 0.21 µmol·min⁻¹·mg protein⁻¹, and fractional 29.1 ± 6.3 vs. 28.3 ± 4.8%. The basal and insulin-stimulated substrate contents were as follows: glucose 6-phosphate 0.37 ± 0.10 vs. 0.32 ± 0.07 nmol/mg dry wt and glycogen 28.9 ± 5.8 vs. 29.2 ± 5.4 mg/g dry wt.

Subcutaneous adipose tissue. The only significant difference between basal and insulin-stimulated subcutaneous adipose tissue enzyme activities was seen in glycogen synthase independent activity (0.15 ± 0.08 vs.
0.31 ± 0.08 nmol·min⁻¹·mg protein⁻¹, P < 0.05). The other activities were as follows: glycogen synthase total activity 6.57 ± 3.05 vs. 6.51 ± 1.71 nmol·min⁻¹·mg protein⁻¹, glycogen synthase fractional activity 2.4 ± 0.4 vs. 4.7 ± 0.8%, glycogen phosphorylase independent activity 0.03 ± 0.01 vs. 0.03 ± 0.005 µmol·min⁻¹·mg protein⁻¹, glycogen phosphorylase total activity 0.06 ± 0.02 vs. 0.06 ± 0.01 µmol·min⁻¹·mg protein⁻¹, and glycogen phosphorylase fractional activity 39.6 ± 1.6 vs. 44.1 ± 2.6%.

Omental adipose tissue. Each of the glycogen synthase activities was significantly higher under insulin-stimulated conditions compared with basal conditions. The basal vs. insulin-stimulated glycogen synthase activities were as follows: independent 0.10 ± 0.05 vs. 0.29 ± 0.06 nmol·min⁻¹·mg protein⁻¹, P = 0.01; total 1.86 ± 0.44 vs. 3.06 ± 0.33 nmol·min⁻¹·mg protein⁻¹, P = 0.01; and fractional 4.0 ± 1.1 vs. 9.6 ± 2.1%, P < 0.05. There were no significant differences between basal fasting and insulin-stimulated omental adipose tissue glycogen phosphorylase activities. The basal vs. insulin-stimulated glycogen phosphorylase activities were as follows: independent 0.03 ± 0.005 vs. 0.02 ± 0.002 µmol·min⁻¹·mg protein⁻¹, total 0.06 ± 0.01 vs. 0.05 ± 0.04 µmol·min⁻¹·mg protein⁻¹, and fractional 45.1 ± 3.5 vs. 41.7 ± 2.5%.

Glucacon. The glucacon concentration under insulin-stimulated conditions (334 ± 40 ng/l) was significantly lower than the glucacon concentration under basal fasting conditions (575 ± 62 ng/l, P < 0.05).

Cortisol. The cortisol concentration was not significantly different between the basal fasting and euglycemic hyperinsulinemic clamp conditions (29 ± 3 vs. 36 ± 2 ng/ml).

Catecholamines. The epinephrine (594 ± 12 vs. 839 ± 144 pg/ml) and norepinephrine (1,657 ± 172 vs. 1,709 ± 104 pg/ml) concentrations were not significantly different between the basal fasting and euglycemic hyperinsulinemic clamp conditions.

DISCUSSION

This study is the third in a series aimed at understanding liver glycogen metabolism in rhesus monkeys. The first study examined the effects of in vivo insulin during a euglycemic hyperinsulinemic clamp in lean young adult rhesus monkeys. In vivo insulin significantly increased liver glycogen synthase independent, total, and fractional activities and decreased liver glycogen phosphorylase independent, total, and fractional activities (13). Liver glucose 6-phosphate content was also significantly reduced by in vivo insulin during the clamp (13). The second study examined basal liver enzymes and substrates in obese normal, hyperinsulinemic, and type 2 diabetic rhesus monkeys (10). The enzymes glycogen synthase, glycogen phosphorylase, protein phosphatase 1 and 2C, and cAMP-dependent protein kinase and the substrates glucose 6-phosphate and glycogen were measured in the three obese groups (10). Neither the enzymes nor the substrates were significantly different between the three groups; there were, however, several significant relationships noted between the enzymes and substrates when the monkeys were grouped into one set (10). The present study was designed to examine whether insulin action on liver glycogen synthase and glycogen phosphorylase from obese insulin-resistant monkeys was defective.

There is no question that defects in skeletal muscle, liver, adipose tissue, and pancreas all contribute to clinically overt type 2 diabetes mellitus (4). What is not as clear is whether defects in all of these tissues also contribute to the prediabetic state or whether a defect in one tissue predominates during the preceding insulin-resistant condition. Based on the results of the present study, we can suggest, at least as far as insulin action on glycogen synthase activity is concerned, that there is not a defect in the liver in the insulin-resistant (prediabetic) state.

In vivo insulin significantly increased all of the liver glycogen synthase activities and significantly decreased all of the glycogen phosphorylase activities in the insulin-resistant monkeys. Liver glucose 6-phosphate content was decreased by in vivo insulin. Hence the liver of the obese insulin-resistant monkeys responds to in vivo insulin in a manner that is similar to the liver from young adult lean rhesus monkeys (13).

Remarkably, whole body insulin-mediated glucose disposal rates were positively related to the change in glycogen phosphorylase activities with insulin. This suggests that the most insulin-resistant monkeys (lowest glucose disposal rates) have the greatest decrease in glycogen phosphorylase activity with insulin.
In vivo insulin did not significantly affect skeletal muscle glycogen synthase or glycogen phosphorylase activities or glucose 6-phosphate or glycogen contents in these insulin-resistant monkeys. This is consistent with the suggestion that insulin resistance at the skeletal muscle is an early event in the progression of type 2 diabetes (7).

In vivo insulin significantly increased the activity of glycogen synthase independent activity in subcutaneous and omental adipose tissue and the fractional activity and total activity in omental adipose tissue. In hyperinsulinemic monkeys (11). One explanation for this may be that the previous group of hyperinsulinemic monkeys were slightly older than the current group of prediabetic monkeys (12). In the previous study, age was shown to be inversely related to insulin action on subcutaneous adipose tissue glycogen synthase independent activity (11).

It is clear from the present study and from our previous study that in vivo insulin significantly increases liver glycogen synthase activities and significantly decreases liver glycogen phosphorylase activities, in both healthy young adult lean monkeys (13) and in older obese insulin-resistant monkeys. In ad libitum fed rabbits, it was previously shown that in vivo insulin caused a rapid decrease in both liver glycogen phosphorylase independent activity and in phosphorylase kinase activity (17). Liver glycogen synthase independent activity was increased only when the independent activity of glycogen phosphorylase reached a very low value in those rabbits (17). In the present study, all five monkeys had an increase in liver glycogen synthase independent activity and all five monkeys had a decrease in liver glycogen phosphorylase independent activity with in vivo insulin. The mechanism of insulin action on liver glycogen metabolism of rabbits and other mammals, including rhesus monkeys (3) has been proposed to be by inactivation of glycogen phosphorylase, which in turn would increase the activity of glycogen synthase phosphatase, and thus result in increased glycogen synthase activity (17). We are not able to confirm or refute this theory of the mechanism of insulin action on liver glycogen metabolism because the first liver biopsies of our animals under insulin-stimulated conditions in both the present and previous studies were not sampled until after ~130 min of insulin administration (13). However, in the present study in lean young adult monkeys (13), glycogen synthase fractional activity and independent activity were significantly inversely related to glycogen phosphorylase fractional activity and independent activity only at the 195-min time point (195 min after the onset of insulin administration during the euglycemic hyperinsulinemic clamp). In the present study, the activities of glycogen synthase and glycogen phosphorylase were not significantly inversely related at the 130-min time point. These results suggest that glycogen synthase may be activated at a different rate by insulin than glycogen phosphorylase is inactivated. Another group has suggested that the increase in activity of glycogen synthase may not be coupled to the decrease in activity of glycogen phosphorylase (2). In hepatocytes isolated from rats, glucose 6-phosphate was shown to increase the activity of glycogen synthase, whereas the glucose molecule itself was shown to inactivate glycogen phosphorylase (2). However, it is not possible to extrapolate the findings of the hepatocyte study to the present study because the hepatocyte study was not done in the presence of insulin.

Among the earliest measurable alterations in rhesus monkeys as they develop type 2 diabetes are an increase in the β-cell response to a glucose bolus (β-cell hyperresponsiveness), a decrease in peripheral insulin sensitivity as measured by the euglycemic hyperinsulinemic clamp (1), and an increase in fasting plasma insulin concentration (1). An increased hepatic glucose production was found concurrent with hyperglycemia in these monkeys (1). These findings suggest that alterations at the pancreas and at the skeletal muscle precede alterations at the liver. A recent study in lean, mild noninsulin-dependent diabetic humans demonstrated that there was little or no impairment in hepatic insulin sensitivity (as measured by hepatic glucose production), although these same subjects had alterations in extrahepatic insulin sensitivity (as measured by glucose utilization) (16). In the present study, the individual values for hepatic glucose production were all lower than the mean value reported for diabetic monkeys (1).

The monkeys in the present study had between very low and very high C-peptide to insulin ratios. Monkeys similar to those in the present study have been shown to have significantly higher fasting plasma C-peptide concentrations and significantly lower molar ratios of C-peptide to insulin than normal young monkeys, indicating a decrease in the apparent hepatic insulin extraction rate (6). The mechanism of decreased hepatic insulin extraction is likely to be saturation of mechanisms responsible for hepatic insulin clearance in response to the hyperinsulinemia rather than hepatic insulin resistance.

In summary, in obese insulin-resistant monkeys, in vivo insulin during a euglycemic hyperinsulinemic clamp increases the activities of liver and omental adipose tissue glycogen synthase and decreases the activities of liver glycogen phosphorylase and the content of liver glucose 6-phosphate. Hence the effect of insulin on liver glycogen metabolism appears to be normal in insulin-resistant monkeys, although skeletal muscle glycogen metabolism appears to be altered. We conclude that there are tissue-specific defects in insulin action on the rate-limiting enzymes of glycogen synthesis and breakdown in obese insulin-resistant monkeys.
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