In Vivo Regulation of Liver and Skeletal Muscle Glycogen Synthase Activity by Glucose and Insulin

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SUMMARY

The regulation of liver and skeletal muscle glycogen synthase by plasma insulin and glucose has not been investigated in vivo at physiological blood glucose concentrations. We have, therefore, used the glucose clamp technique to investigate the effects of these variables independently in rats. Short-term streptozocin- (0.15 g/kg) diabetic animals were used in addition to normal rats to avoid endogenous insulin secretion during hyperglycemic clamps. In normal and diabetic animals, 3 h of hyperinsulinemia without change in blood glucose concentrations caused only a small increase in liver glycogen synthase activity (+34%), whereas hyperglycemic clamps at 6.0 and 10.0 mmol/L resulted in marked increases (+268 and +394% of basal, $P < 0.001$). Liver glycogen concentrations at the end of the clamps reflected these changes. In skeletal muscle, glycogen synthase was increased by +58% by the euglycemic hyperinsulinemic clamp and was not increased significantly further by hyperglycemia. Similarly, muscle glycogen concentration increased with the 4.0-mmol/L clamp but during the hyperglycemic clamps was only raised more in direct proportion to blood glucose concentrations. The results confirm that blood glucose concentration is the major short-term regulator of glycogen synthase activity in the liver but that insulin is of prime importance in skeletal muscle.

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Glycogen synthase is believed to be the rate-limiting enzyme for glycogen synthesis in both liver and muscle.[1] Short-term, hormonally mediated changes in activity are widely believed to be due to changes in the phosphorylation state of the enzyme, with the less phosphorylated species physiologically active.[2,3] There is good evidence that insulin plays a primary role in the regulation of the skeletal muscle enzyme[4–6] through its ability to promote dephosphorylation of the enzyme.[7,8]

By contrast, the physiological regulation of glycogen synthase in liver is less well understood. It is well established from in vitro studies, in particular by liver perfusion, that glucose concentration has a major effect on activation of the enzyme.[9–13] In many of these studies, however, the glucose concentrations used were supraphysiological.[10–13] Insulin, by contrast, has little effect on glycogen synthase activity in this type of study.[9] In vivo studies of the action of glucose have been complicated by the ensuing disturbance of the metabolic state, including stimulation of insulin secretion.[14] For this reason, experiments were limited to very short periods (1–5 min), the results being consistent with in vitro studies.[15]

Insulin has, however, been shown to be important for the maintenance of hepatic glycogen synthase phosphatase activity.[16] Furthermore, livers from diabetic animals show a decreased capacity for glycogen synthesis,[17–20] and perfusion with high glucose concentrations fails to activate glycogen synthase to the expected extent.[13,21]

To define further the roles of insulin and glucose on hepatic and skeletal muscle glycogen synthesis in vivo, we used the hyperinsulinemic glucose clamp technique to vary their concentrations independently. To avoid endogenous insulin secretion during the hyperglycemic clamps, the studies were performed on normoglycemic short-term streptozocin diabetic rats. An insulin infusion rate was chosen that would result in peripheral insulin concentrations greater than peak levels after a standard meal to ensure adequate hepatic insulinization. Blood glucose was clamped at basal and hyperglycemic levels to assess the role of glucose in the regulation of glycogen synthase in liver and muscle.

MATERIALS AND METHODS

Animals. Syngeneic male Wistar *ab* Boots rats (Nottingham University, Nottingham, UK) weighing 265–280 g were injected intravenously (i.v.) with 150 mg/kg streptozocin under ether anesthesia. This dose of streptozocin completely, consistently, and irreversibly destroys all pancreatic islet β-cells.[22,23] Miniosmotic pumps (Alzet 2002, Palo Alto, CA) filled

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with soluble insulin (Hoechst 21PH, Hoechst, Frankfurt, FRG) were implanted subcutaneously (s.c.) at the time of streptozocin injection. The dose of insulin (3 U/day) was chosen on the basis of previous observations of insulin requirements in this strain of rats. Drinking water was replaced with a solution of 50 g/L glucose in water for the first 15 h. Rats were fed ad libitum on laboratory chow unless otherwise stated. Blood glucose was measured daily at 0800 h from tail vein blood samples taken under ether anesthesia from ad libitum–fed rats. Only animals with blood glucose concentrations between 3.5 and 7.0 mmol/L were used for metabolic studies, but data are reported on all animals studied. All animals used for metabolic studies had been handled daily for 6–8 wk before induction of diabetes. Because it could be argued that the streptozocin insulin-treated animals might respond differently from normal rats as a result of decreased portal vein insulin concentrations with peripheral insulin administration, a group of normal littermates was included in the fasting and euglycemic (4.0 mmol/L) glucose clamp studies.

Studies in fasting animals. Five days after induction of diabetes, jugular venous and femoral venous cannulas (Bard-I-Cath, Sunderland, UK) were implanted under ether anesthesia 24 h before study. The miniosmotic pumps were removed that evening, and insulin (Actrapid; Novo, Bagsvaerd, Denmark) diluted in Haemaccel (Hoechst) was infused i.v. overnight to maintain blood glucose between 3.5 and 4.0 mmol/L. Normal rats received an infusion of Haemaccel alone at the same rate (0.04 ml/h). Animals were fasted for 18 h before the study. All blood samples were taken from unanesthetized, unrestrained rats.

After a basal blood sample (400 μl) for glucose and insulin determination, six diabetic and six normal rats were anesthetized with ether, and liver and quadriceps muscle were freeze-clamped for determination of glycogen synthase activity and glycogen content. Tissue was ground in liquid nitrogen and stored at −70°C. Glycogen synthase activity was measured on freeze-clamped tissue within 48 h.

Hyperinsulinemic glucose clamp studies. Rats for the clamp studies were prepared as described above up to the time of the basal blood sample at 0900 h. Then the insulin infusion rate was increased to 85 mU/h and given through one limb of a double-lumen cannula connected to the femoral venous cannula. This dose was chosen to obtain concentrations in the portal vein above those measured in the fed state.[23a] In these circumstances, hepatic glucose output will be suppressed, and glucose disposal rate will equal the glucose infusion rate. Blood samples (30 μl) for glucose were taken at 5- to 15-min intervals from the jugular venous cannula and replaced with 0.15 mol/L saline. Blood glucose concentration was measured by the glucose oxidase method (Yellow Springs Glucose Analyser, Clandon Scientific, London, UK) within 2 min of obtaining the sample. A solution of 500 g/L glucose in water was infused through the other limb of the double-lumen cannula and the rate adjusted to maintain blood glucose at 4.0, 6.0, or 10.0 mmol/L in three separate studies. Six animals were included in each study. Blood samples (400 μl) for insulin were taken at 30, 60, 90, 120, and 180 min and replaced with fresh washed rat erythrocytes in 0.15 mol/L saline. At 180 min the animals were anesthetized with ether, and liver and quadriceps muscle were freeze-clamped, ground in liquid nitrogen, and stored at −70°C for determination of glycogen content and the activity of glycogen synthase within 48 h.

Glycogen synthase activity. Glycogen synthase activity was measured essentially as described by Golden et al.[24] Briefly, samples of muscle (~0.2 g) or liver (0.2–0.4 g) were homogenized (Polytron Kinematica, Lucerne, Switzerland) in 2.0 ml of Tris-HCl buffer, pH 7.8, containing 10 mmol/L EDTA, 5 mmol/L dithiothreitol, 50 mmol/L NaF, and 2.5 g/L rabbit liver glycogen type III (Sigma, Poole, UK). The homogenate was centrifuged at 10,000 × *g* for 30 s in an MSE microcentrifuge and the supernatant used for glycogen synthase assay by measuring the incorporation of UDP-U-[^{14}C]glucose into glycogen at 30°C. The final concentration of UDPglucose

TABLE 1
Blood glucose and plasma insulin concentrations and the glucose requirement from +90 to +180 min of the glucose clamp studies in the experimental animals

	Clamp blood glucose concentration (mmol/L)		
	4.0	6.0	10.0
Blood glucose (±SD; mmol/L)			
Normal rats	3.9 ± 0.1		
Diabetic rats	4.0 ± 0.0	6.0 ± 0.1	10.1 ± 0.2
CV of blood glucose (±SD; %)			
Normal rats	5.6 ± 1.2		
Diabetic rats	5.7 ± 1.0	4.9 ± 1.3	3.7 ± 2.7
Plasma insulin (mU/L)			
Normal rats	131 ± 14		
Diabetic rats	144 ± 15	148 ± 14	141 ± 13
Glucose requirement ($\mu mol \cdot min^{-1} \cdot kg^{-1}$)			
Normal rats	170 ± 5		
Diabetic rats	176 ± 9	254 ± 13	397 ± 17
Glucose infused during 3-h clamp (mmol/rat)			
Normal rats	7.42 ± 0.24		
Diabetic rats	7.54 ± 0.31	10.87 ± 0.32	18.48 ± 0.35

Mean ± SEM (or SD where indicated). N = 6 for each group.
CV, coefficient of variation.

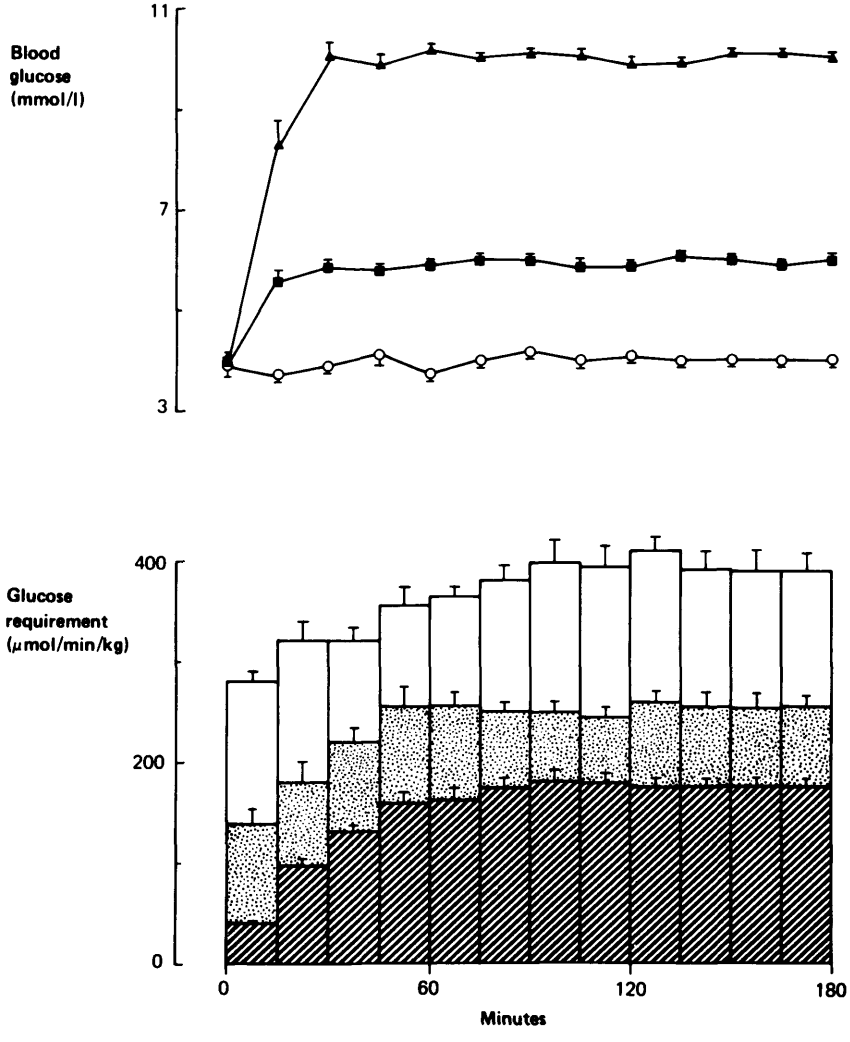

FIGURE 1. Blood glucose and glucose infused during hyperinsulinemic glucose clamps in experimental diabetic rats. Target blood glucose levels were 4.0, 6.0, and 10.0 mmol/L. Mean ± SE.

in the assay was 6.7 mmol/L. Total glycogen synthase activity was measured in the presence of 10 mmol/L glucose-6-phosphate. One unit of enzyme activity is defined as the amount of enzyme catalyzing the transfer of 1 μmol/min of glucose from UDPglucose into glycogen at 30°C.

Other analyses. Insulin was measured by radioimmunoassay with a porcine insulin standard (Novo).[25] The intra-assay coefficient of variation was 7%. For glycogen determination, samples of muscle or liver were homogenized (Polytron Kinematica, setting No. 4, 20 s) in perchloric acid (0.6 mol/L) and the supernatant incubated with amyloglucosidase (Sigma).[26] Liberated glucose was assayed by an automated hexokinase method.[27] Tissue glycogen as glucosyl residues was calculated after subtraction of the glucose concentration in unhydrolyzed samples.

Statistical analysis. Results are presented as means ± SEM. Significant differences between the groups were assessed by Student's *t*-test. The significant difference within groups was assessed by analysis of variance.

RESULTS

Fasting blood glucose concentrations in the overnight insulin-infused diabetic rats [3.9 ± 0.3 (±SD) mmol/L] were not different from those of normal control rats [4.0 ± 0.2 (±SD) mmol/L], and similar blood glucose concentrations were achieved during the glucose clamps (Table 1). Portal venous blood glucose concentrations were identical to superior vena cava (SVC) concentrations during clamps in separate groups of animals under ether anesthesia at 4.0 mmol/L (portal 4.2 ± 0.2, SVC 4.2 ± 0.2 mmol/L) and 6.0 mmol/L (6.1 ± 0.1 vs. 6.1 ± 0.1 mmol/L). Arterial blood glucose concentrations were, however, higher (6.1 ± 0.2 vs. 4.2 ± 0.2 mmol/L). Insulin-treated diabetic rats had normal fasting activities of liver and muscle glycogen synthase (Tables 2 and 4), and fasting liver and muscle glycogen concentrations were similar to those of normal control animals (Table 3). At the same insulin infusion rate (0.85 mU/h), similar plasma insulin concentrations were achieved in the diabetic and control rats during the clamp studies (Table 1). Insulin sensitivity as assessed by the glucose requirement for maintenance of normoglycemia during the clamp study was also unchanged in these short-term insulin-treated rats (Table 1). The glucose concentration profile and glucose requirement during the euglycemic and hyperglycemic clamps is shown in Figure 1.

Liver glycogen synthase activity and liver glycogen at end of glucose clamps. After a 3-h infusion of insulin at 85 mU/h with maintenance of blood glucose at 4.0 mmol/L, the increase in liver glycogen synthase activity (glucose-6-phosphate–independent form) did not reach statistical significance compared with the fasting state in control or diabetic

TABLE 2
Liver glycogen synthase activity (U/g wet wt) in normal and insulin-treated diabetic animals in the fasting state and at the end of a 3-h hyperinsulinemic glucose clamp

		Clamp blood glucose concentration (mmol/L)		
	Fasting	4.0	6.0	10.0
Active				
Normal rats	0.19 ± 0.02	0.26 ± 0.03*		
Diabetic rats	0.19 ± 0.02	0.25 ± 0.03*	0.70 ± 0.11	0.94 ± 0.06†
Total				
Normal rats	0.63 ± 0.04	0.74 ± 0.06		
Diabetic rats	0.60 ± 0.05	0.71 ± 0.06	1.15 ± 0.14	1.22 ± 0.03†
Percent active				
Normal rats	31 ± 3	36 ± 4		
Diabetic rats	32 ± 2	35 ± 3	50 ± 10	77 ± 4†

An insulin infusion rate of 85 mU/h was used. Percent active was calculated on the basis of individual observations before calculation of the mean value.
Mean ± SEM. N = 6 for each group.
*0.1 > P > 0.05 compared with fasting animals.
†P < 0.001 for different clamp blood glucose concentrations by analysis of variance.

animals considered separately (Table 2). Analysis of variance, however, confirmed the change when the treatments were considered together (fasting vs. clamp, $F = 7.10$, $P < 0.02$). A modest increase in hepatic glycogen content was apparent by the end of the 3-h euglycemic clamp [diabetic rats, 16 ± 1 to 59 ± 6 μmol/g wet wt; normal rats, 17 ± 3 to 54 ± 6 μmol/g wet wt; $P < 0.001$ for both groups (Table 3)].

Plasma insulin concentrations were comparable during the clamps at different blood glucose levels (Table 1). At the end of the 6.0-mmol/L and 10.0-mmol/L glucose clamps, both active and "total" liver glycogen synthase activities were significantly increased compared with the end of the 4.0-mmol/L clamp (Table 2). The increase in the active fraction was proportionately greater so that the percent in the active form was significantly increased during the 6.0-mmol/L and 10.0-mmol/L glucose clamps (Table 2). This activation of the enzyme was accompanied by a marked increase in liver glycogen deposition (Table 3, $P < 0.001$).

Muscle glycogen synthase and muscle glycogen at end of glucose clamp studies. Total muscle glycogen synthase activity in the insulin-treated diabetic rats did not change during the glucose clamp studies (Table 4). The activity of the glucose-6-phosphate–independent form was, however, significantly increased at the end of the 3-h euglycemic glucose clamp compared with the activity in fasting animals (Table 4). The response of the insulin-treated diabetic rats was similar to that of normal rats. There was no significant increase in the activity of the enzyme with increasing glucose concentrations (Table 4). The amount of glycogen deposited in skeletal muscle during the clamp was greater during the hyperglycemic glucose clamps (Table 3).

DISCUSSION

Our knowledge of the control of glycogen synthesis in liver is derived principally from in vitro studies with the isolated perfused organ preparation, or isolated hepatocytes, and short-term in vivo studies generally on anesthetized animals. In the perfused rat liver, high concentrations (11–28 mmol/L) of glucose activate glycogen synthase independently of insulin,[9,10] although in isolated hepatocytes activation only occurs above 12–15 mmol/L.[12] The possibility of a role for insulin is, however, suggested by the observation of an absence of this glucose effect in livers or hepatocytes taken from diabetic animals[21,28] but restoration of this effect with insulin.[13,28,29]

In the whole animal, the roles of glucose and insulin are difficult to separate because of the integrated nature of metabolism. To circumvent these problems, studies have been short (1–5 min), although even in this time insulin concentrations rise after a bolus dose of glucose.[14] In these circumstances, a large i.v. bolus of glucose giving blood concentrations >15 mmol/L appears to activate hepatic glycogen synthase, but insulin has no effect.[14,15,30–32]

The glucose clamp technique[33] allows the effects of insulin and glucose concentrations to be studied independently and at levels closer to those found physiologically. The long duration of the stimulus is also more physiological and in the current study allowed the measurement of the functional correlate of glycogen synthase activity, namely glycogen deposition. When insulin concentrations are raised but blood glucose concentration is held constant, we found a small rise in active glycogen synthase activity in the liver of both diabetic and normal control rats (Table 2). This change would appear to be due to insulin itself. The glucose clamps were

TABLE 3
Liver and skeletal muscle glycogen concentrations in the experimental animals in the fasting state and at the end of a hyperinsulinemic glucose clamp

Glycogen (μmol/g wet wt)	Fasting	Clamp blood glucose concentration (mmol/L) 4.0	6.0	10.0
Liver				
Normal rats	17 ± 3	54 ± 6*		
Diabetic rats	16 ± 2	59 ± 7*	140 ± 12	233 ± 11†
Muscle				
Normal rats	16 ± 1	29 ± 1*		
Diabetic rats	16 ± 1	30 ± 2*	47 ± 3	78 ± 4†

Mean ± SEM. N = 6 for each group.
*P < 0.001 compared with fasting animals.
†P < 0.001 for increasing clamp blood glucose concentration by analysis of variance.

TABLE 4
Muscle glycogen synthase activity in normal and insulin-treated diabetic rats in the fasting state and at the end of a 3-h hyperinsulinemic glucose clamp

Glycogen synthase (U/g wet wt)	Fasting	Blood glucose concentration after 3 h (mmol/L)		
		4.0	6.0	10.0
Active				
Normal rats	0.40 ± 0.01	0.63 ± 0.07*		
Diabetic rats	0.39 ± 0.02	0.62 ± 0.09†	0.67 ± 0.04	0.80 ± 0.04
Total				
Normal rats	2.08 ± 0.14	2.05 ± 0.12		
Diabetic rats	2.01 ± 0.11	2.03 ± 0.10	2.26 ± 0.15	2.26 ± 0.06
Percent active				
Normal rats	20 ± 1	31 ± 3*		
Diabetic rats	19 ± 2	30 ± 3*	30 ± 1	36 ± 3

An insulin infusion rate of 85 mU/h was used.
Mean ± SEM. N = 6 in each group.
*P < 0.01 compared with fasting animals; †P < 0.05.

performed with SVC cannulas, but portal venous blood glucose concentrations were identical. Arterial blood glucose concentrations were, however, considerably higher (arterial 6.1 ± 0.2 mmol/L, portal vein 4.2 ± 0.2 mmol/L), and it is possible that the small contribution of the hepatic artery to total liver blood flow might have caused a small activation of glycogen synthase during the 4.0-mmol/L clamps (Table 2).

For the hyperglycemic clamps, high-dose- (0.15 g/kg) streptozocin–diabetic rats were used to avoid the confounding effect of endogenous insulin secretion. This dose causes total and irreversible islet β-cell destruction.[23] These short-term diabetic animals, in good metabolic control, had identical insulin sensitivity to the normal control animals and identical liver and muscle glycogen synthase activities in the fasting state and during the euglycemic clamps. They would, therefore, appear to be a valid model for studying physiological changes at higher blood glucose concentrations.

Clamping the blood glucose at higher levels resulted in a three- to fivefold increase in expressed glycogen synthase activity (Table 2), with the greatest effect occurring with the change from 4 to 6 mmol/L. Consistent with this was the increase in glycogen deposition, which was disproportionately greater than the change in blood glucose concentration between 4 and 6 mmol/L but only changed proportionately to glucose concentration between 6 and 10 mmol/L (Table 5). The evidence of Table 5 strongly suggests that once activation of the synthase has occurred by insulin or glucose, the major determinant of the rate of glycogen storage is substrate concentration, in this case with a direct relationship to plasma glucose concentration.

In liver the increase in glycogen synthase activity between the 4.0- and 6.0-mmol/L clamps was much greater (2.8×) than the increase in glycogen concentration (1.6×, Table 5) when adjusted for blood glucose concentration. This suggests that the "pull" mechanism for increasing flux through glycogen synthase is relatively inefficient. This assumption would be consistent with kinetic analysis, which, because of the large concentration difference between UDPglucose and glucose-1-phosphate, indicates that activation of the synthase will have a comparatively small effect on flux through the pathway.[34] The opposing influence, a decrease in product inhibition of UDPglucose pyrophosphorylase[35] through a fall in the UDPglucose concentration,[30] would not provide a compensatory effect of adequate magnitude.

The regulation of glycogen synthase in muscle is less controversial. Studies on isolated mouse soleus muscle show no activation of glycogen synthase by high concentrations of glucose (20 mmol/L) alone, but supraphysiological insulin concentrations (200–2000 mU/L) caused rapid activation.[5] Other preparations and hindlimb perfusion studies have given similar results.[4,8,36] Thus, the glucose-6-phosphate–independent glycogen synthase activity increased significantly

TABLE 5
Relationship of blood glucose concentration and glucose requirement to glycogen concentration at the end of the 3-h clamp

	Glucose concentration (mmol/L)		
	4.0	6.0	10.0
Liver			
Glycogen/glucose concentration	14.7 ± 1.7	22.2 ± 2.0*	23.2 ± 1.2*
Glycogen/glucose requirement	0.34 ± 0.03	0.56 ± 0.06*	0.59 ± 0.03*
Muscle			
Glycogen/glucose concentration	7.5 ± 0.5	7.7 ± 0.6	7.3 ± 0.4
Glycogen/glucose requirement	0.17 ± 0.02	0.18 ± 0.01	0.18 ± 0.01

*P < 0.02 compared with 4.0-mmol/L clamp.

in both normal and diabetic animals during our 4.0-mmol/L glucose clamp in response to plasma insulin concentrations of 120–150 mU/L (Table 1). Further increases in activity were not found at higher glucose concentrations. This is confirmed by muscle glycogen concentrations at the end of the clamps (Table 4), which increase in simple proportion to the glucose concentration (Table 5).

It remains possible that the activation mechanisms of liver and muscle glycogen synthase are similar and that the difference in physiological regulation merely reflects the need for insulin in raising the intracellular concentrations of glucose-6-phosphate and derivatives in skeletal muscle. However, this assumption is inconsistent because of the marked activation in liver by quite small changes in plasma glucose concentration, although even very high ambient concentrations are reported to have no effect in muscle.[5]

Although this study comes closer to the physiological milieu than previous experimental work on glycogen synthase, caution should still be exercised in adopting the results without reference to the abnormal route of delivery of glucose and insulin in the clamp technique. Although the livers of the experimental animals were not weighed in our study to reduce warm ischemia time, it may be assumed that they were 8–10 g. This would imply that <7% of the infused glucose was stored as hepatic glycogen during the 4.0-mmol/L clamp and <15% after activation of glycogen synthase at 10.0 mmol/L. Nevertheless, we have previously noted that rats fed a similar carbohydrate load (1.8 g) deposited equally small amounts in the liver as glycogen (<10%).[23a]

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