Defective activation by glucose of hepatic glycogen synthesis in the obese hyperglycaemic mouse

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(Received 4 May 1983/Accepted 20 September 1983)

The administration of an oral glucose load to 24 h-starved lean (+/?) male C57BL/6 mice produced a rapid, 7-fold increase in the rate of hepatic glycogen synthesis and a sustained activation of glycogen synthase. In contrast, glucose produced only a small (4.5-fold), short-lived increase in hepatic glycogen synthesis in genetically obese (*ob/ob*) mice and no activation of glycogen synthase.

The obese hyperglycaemic (*ob/ob*) mouse is now well established as an animal model for the study of obesity and maturity-onset (non-insulin-dependent) diabetes in man. This animal displays many of the abnormalities of carbohydrate metabolism that are characteristic of the human diabetic. The most notable of these are hyperglycaemia after starvation for 24 h and impaired glucose tolerance.

In both normal man and normal lean animals, the administration of a glucose load produces a rapid suppression of hepatic glucose output followed by an activation of glycogen synthesis, which results in the storage of a significant fraction of the glucose load as liver glycogen (Felig *et al.*, 1975; Stalmans, 1976). Although a possible failure of the liver both to suppress glucose output and to store dietary glucose as glycogen has been implicated in the post-prandial hyperglycaemia of diabetic man (Felig *et al.*, 1978), the contribution of the liver to the hyperglycaemia and to the impaired glucose tolerance of the obese mouse has not been clearly delineated.

Previous studies have provided equivocal evidence as to whether basal rates of hepatic glucose output of the obese mouse differ from those of the lean mouse. Rates of glycogenolysis are increased in perfused livers from fed obese mice (Elliott *et al.*, 1971), although rates of hepatic gluconeogenesis have been reported variously to be increased (Mobley *et al.*, 1977) or to be not different from those of lean mice (Elliott *et al.*, 1971).

The studies of Kreutner *et al.* (1975) indicate that the impaired glucose tolerance of the obese mouse may, at least in part, arise from an abnormal response of the liver to the glucose load. Measurements of incorporation of [^{14}C]alanine into blood glucose suggest that hepatic gluconeogenesis may not be suppressed by glucose in the obese mouse. In

addition, glucose does not stimulate the incorporation of [^{14}C]alanine into glycogen, the accumulation of glycogen or the activation of glycogen synthase.

The use of ^{14}C -labelled precursors to determine rates of product synthesis possesses a number of inherent limitations (Katz *et al.*, 1979) and cannot therefore be used to give net rates either of glucose or glycogen formation. To overcome this problem, we now report the use of a ^3H -labelled water procedure (Postle & Bloxham, 1980) for the measurement of absolute rates of hepatic glycogen synthesis to study the response to an oral glucose load of glycogen synthesis in the livers of lean and obese mice.

Materials and methods

Animals

Male, obese-hyperglycaemic mice (C57BL/6 *ob/ob*) and their lean littermates (+/?) were from Olac (1976) Ltd., Bicester, Oxon, U.K., and were received at 4–5 weeks of age. The mice were maintained on Oxoid pelleted breeder diet (H. C. Styles, Bewdley, Worcs., U.K.) and water *ad libitum* for 8 weeks before use. At this time the obese mice weighed between 45 and 55 g and the lean mice 19–21 g. All animals were starved for 24 h before experiment.

Oral glucose tolerance

Changes in concentrations of blood glucose at intervals after the oral (intragastric) administration of glucose to 24 h-starved lean (1.0 g/kg body wt.) and obese (0.6 g/kg body wt.) mice were measured. (These doses are approximately equivalent on a lean-body-mass basis.) Blood samples (10 μl) were taken serially from the cut end of the tail and

haemolysed with 1 ml of 1.0 mM-maleimide containing 0.04 mM-digitonin. Glucose in the haemolysate was determined enzymically with hexokinase and glucose-6-phosphate dehydrogenase using a LKB-Corona Reaction Rate Autoanalyser (Schmidt, 1961).

Determination of rates of hepatic glycogen synthesis in vivo

Rates of hepatic glycogen synthesis were determined at intervals after the oral administration of glucose to lean (1 g of glucose/kg body wt.) and obese (0.6 g of glucose/kg body wt.) mice. Incorporations of ^3H into liver glycogen were measured in mice that had been injected intraperitoneally with $^3\text{H}_2\text{O}$ [0.5 mCi/mouse in 0.2 ml of 0.9% (w/v) NaCl] and killed by cervical dislocation 30 min later. Samples of liver were freeze-clamped within 10 s of death and stored in liquid N_2 until analysis.

Glycogen content and ^3H radioactivity in glycogen glucose were determined by the method described by Postle & Bloxham (1980). Although studies with isolated liver cells show that the amount of ^3H incorporated per unit of glycogen glucose is dependent upon the nature of the precursors (Rognstad *et al.*, 1974), whole animal experiments reveal that ^3H incorporation into glycogen glucose is largely independent of the carbon source for glycogen synthesis (Postle & Bloxham, 1980). This allows, therefore, absolute rates of synthesis to be calculated.

Measurement of glycogen synthase activities

Frozen samples of liver were extracted in ice-cold 50 mM-triethanolamine / HCl / 25 mM-NaF / 5 mM-EDTA, pH 7.5 (3 ml/g of tissue) with an Ultra-Turrax tissue homogenizer. The homogenates were centrifuged at 10 000 *g* for 10 min at 4°C (Eppendorf 5413). Samples of supernatant were stored at 0°C and were used for the determination of glycogen synthase activity (Thomas *et al.*, 1968).

Results

Effect of administration of glucose on rates of hepatic glycogen synthesis in lean and obese mice

Blood glucose concentrations of starved obese mice were significantly greater than those of starved lean mice ($P < 0.001$) and obese mice displayed a significantly exaggerated hyperglycaemia ($P < 0.05$) for 4 h in response to an oral load of glucose (results not shown).

Fig. 1 shows the time course of changes in rates of hepatic glycogen synthesis and of liver glycogen content of 24 h-starved lean and obese mice after the oral administration of glucose. In the absence of a glucose load, rates of incorporation of ^3H into liver glycogen were significantly lower in obese mice than

Fig. 1. Effect of administration of glucose on hepatic glycogen synthesis and glycogen content of 24 h-starved lean and obese mice

Hepatic glycogen content and rates of glycogen synthesis were determined in groups of 24 h-starved lean and obese mice at intervals after oral administration of glucose as described in the Materials and methods section. Results are presented as means $\pm$ S.E.M. of six mice per group. P (versus time-zero value for lean mice): $\dagger < 0.01$; $\dagger\dagger < 0.001$, by Student's t test. P , significance of difference of means between lean and obese mice: $* < 0.05$, $** < 0.001$, by Student's t test. ●, Lean mice; ○, obese mice. The open columns on the histogram represent rates of glycogen synthesis of lean mice; the stippled columns represent rates of glycogen synthesis of obese mice.

in lean mice. The administration of an oral glucose load to lean mice produced a rapid (within 30 min) 7-fold increase in the incorporation of ^3H into liver glycogen. The rate of glycogen synthesis then declined gradually until about 1½–2 h after glucose administration, at which time the rate was equal to that of non-glucose-loaded mice. The increase in ^3H incorporation into liver glycogen in the 60 min after glucose administration was accompanied by a 9-fold increase in the content of liver glycogen from 6.8 ± 1.7 μmol of glycogen-glucose/g wet wt. of liver

Table 1. *Effect of administration of glucose on glycogen synthase activities of 24 h-starved lean and obese mice*

Groups of 24 h-starved lean and obese mice were killed at intervals after the oral administration of glucose (1 g/kg body wt., lean mice; 0.6 g/kg body wt., obese mice). Samples of liver were freeze-clamped for subsequent determination of glycogen synthase activities as described in the Materials and methods section. Glycogen synthase activities are expressed as activity ratios [ratio of the activity of glycogen synthase in the absence of added glucose 6-phosphate (synthase *a*) to the activity of glycogen synthase in the presence of 10 mM-glucose 6-phosphate (synthase *a* + *b*)]. Results are means $\pm$ S.E.M. of six mice per group. The significance of differences of means between groups was: $\dagger P < 0.05$; $\dagger\dagger P < 0.01$, versus appropriate time-zero group by Student's *t* test.

Time after administration of glucose (min)	Glycogen synthase activity ratio	
	Lean mice	Obese mice
0	0.24 $\pm$ 0.03	0.19 $\pm$ 0.01
30	0.63 $\pm$ 0.09 $\dagger\dagger$	0.32 $\pm$ 0.07
60	0.46 $\pm$ 0.06 $\dagger$	0.21 $\pm$ 0.05
90	0.37 $\pm$ 0.02 $\dagger\dagger$	0.25 $\pm$ 0.04
120	0.42 $\pm$ 0.10	0.38 $\pm$ 0.05 $\dagger\dagger$
180	0.55 $\pm$ 0.09 $\dagger\dagger$	0.21 $\pm$ 0.03
240	0.65 $\pm$ 0.11 $\dagger\dagger$	0.19 $\pm$ 0.02

(mean $\pm$ S.E.M., *n* = 6) to 57.8 $\pm$ 3.6 μ mol of glycogen-glucose/g wet wt. of liver (mean $\pm$ S.E.M., *n* = 6). Assuming an average incorporation rate of 2.8 3 H atoms per glycogen-glucose moiety (Postle & Bloxham, 1980), rates of isotopically determined glycogen synthesis (net, above basal, rate of 140 μ mol of 3 H/h per g of liver) during the 60 min after administration of glucose agree well with the measured net accumulation of liver glycogen (57 μ mol/g wet wt. of liver) over this time.

In contrast with the rapid 7-fold increase in the rate of hepatic glycogen synthesis observed in lean mice, the administration of glucose to obese mice produced a smaller (4.5-fold) stimulation of glycogen synthesis (Fig. 1). The activation by glucose of hepatic glycogen synthesis in the obese mouse was of shorter duration than that observed in lean mice. In addition, this smaller, transient activation of glycogen synthesis was not accompanied by a net, statistically significant (*P* > 0.05), accumulation of liver glycogen.

The hepatic glycogen content of 24 h-starved obese mice was 14 times greater than that of 24 h-starved lean mice [96 μ mol/g of liver (obese) compared with 7 μ mol/g of liver (lean)]. This maintenance of a high liver glycogen content under acute starvation conditions has been noted previously (Loten *et al.*, 1974).

The relatively low rate of incorporation of 3 H into the large store of liver glycogen suggests that the turnover rate of glycogen in the livers of 24 h-starved obese mice is considerably lower than that of their lean littermates.

Effect of administration of glucose on the hepatic glycogen synthase activity of lean and obese mice

To study further the defective activation by glucose of hepatic glycogen synthesis in obese mice, the time course of changes in the activity of glycogen synthase was measured after administration of glucose to lean and obese mice (Table 1).

The basal (non-glucose-loaded) total activity of glycogen synthase (results not shown) and activity ratios of glycogen synthase in the livers of lean and obese mice were similar. These findings are in agreement with previous reports (Das & Hems, 1974).

Administration of glucose to lean mice produced a rapid and sustained increase in the activity of glycogen synthase *a*. This activation by glucose of glycogen synthase activity, measured *in vitro*, correlated well for the first 2 h after glucose both with isotopically determined rates *in vivo* of glycogen synthesis and net glycogen accumulation. At 3–4 h after the administration of glucose, glycogen synthase remained activated despite the fact that glycogen synthesis had returned to the starved pre-glucose rate.

In the obese mouse, however, administration of glucose failed to produce a rapid or a significant sustained activation of glycogen synthase. These results support the earlier observations that showed that glucose did not strongly stimulate either glycogen synthesis or glycogen accumulation in the livers of obese mice (Fig. 1).

Discussion

The role of the liver in the regulation of blood glucose concentrations after the administration of glucose to the normal animal is now well established (Stalmans, 1976). Both glucose and insulin, released in response to glucose ingestion, suppress hepatic glucose output and stimulate glycogen synthesis. A significant proportion (about 50%) of the ingested glucose load is stored as liver glycogen.

The results reported in the present paper demonstrate that the ability of the obese mouse to dispose of an oral load of glucose is impaired compared with that of its lean littermate. That the impaired glucose tolerance of the obese mouse derives, at least in part, from a failure of the liver to store an appropriate fraction of the administered glucose as glycogen is supported by the findings that glucose did not produce either a net accumulation of liver glycogen or a sustained activation of hepatic glycogen synthesis.

Activation of glycogen synthesis is dependent upon conversion of inactive glycogen synthase *b* into the active *a* form. This reaction is catalysed by glycogen synthase phosphatase (Stalmans *et al.*, 1974). It is unlikely that defective activation of glycogen synthesis in livers of obese mice is a consequence of decreased activities of either glycogen synthase (see the Results section) or of glycogen synthase phosphatase, since the tissue content and catalytic properties of these two enzymes are the same as those of lean mice (Kreutner *et al.*, 1975). Activation of glycogen synthase by glycogen synthase phosphatase only occurs after phosphorylase *a* activity is almost completely inactivated in response to elevated hepatic glucose concentrations (Stalmans *et al.*, 1974). The possibility that phosphorylase *a* in the liver of obese mice may be resistant to inactivation by glucose merits investigation.

Activation of glycogen synthase is also dependent upon the glycogen content of the liver since glycogen inhibits glycogen synthase phosphatase (De Wulf & Hers, 1968). The obese mouse is noteworthy for its ability to maintain a high liver glycogen content even under starvation conditions. This observation suggests that the high liver glycogen content of the obese mouse may be important in the defective activation of glycogen synthesis by preventing the normal expression of glycogen synthase phosphatase activity. Sestoft & Hue (1980) have suggested that a similar excess of liver glycogen reserves may contribute to the impaired hepatic glucose uptake characteristic of the human maturity-onset diabetic.

We are grateful to Mr. M.V. Sennitt for assistance during the experimental procedures.

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