Hepatic glycogen synthetase deficiency

Definition of syndrome from metabolic and enzyme studies on a 9-year-old girl

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SUMMARY In the 13 years since hepatic glycogen synthetase deficiency was first described in identical twins no further cases seem to have been observed. We report a child who had suffered from occasional morning convulsions since the age of 7. Three 24-hour metabolic profiles showed fasting hypoglycaemia, hyperketonaemia, but normal lactate. Hyperglycaemia and hyperlactataemia occurred after meals. Glucagon caused a rise in glucose 3 hours after a meal with a fall in lactate and alanine; no effect of glucagon was seen after a 12-hour fast. Normal increments in glucose followed oral galactose or alanine. Liver and abdominal wall muscle biopsies were taken. Glycogen content was subnormal in liver but normal in muscle. Glycogen synthetase (EC 2.4.1.11) was virtually absent from liver but fully active in muscle. Hepatic glycogen synthetase deficiency causing fasting hypoglycaemia has been confirmed. It is postulated that some children with 'ketotic hypoglycaemia' may suffer from this disorder.

Hepatic glycogen synthetase deficiency leading to an inability to synthesize glycogen is a rare cause of hypoglycaemia in childhood. Identical twins with the defect were reported in 1963 (Lewis *et al.*) but no further cases have been detected since then and some doubts have been expressed on the existence of the defect. We report the results of metabolic and enzyme studies on a 9-year-old girl which prove the existence of the enzyme defect and allow a definition of the clinical syndrome.

Case report

The child was born in 1966 after an uneventful pregnancy of 37 weeks, weighing 2.0 kg. Her parents noted early morning behaviour changes, drowsiness, and lack of attention with the establishment of daytime feeding, all of which were reversed by food. Apart from these features, she remained well until aged 7 when she first developed occasional morning convulsions. She was admitted to hospital at the age of 7½ years for investigation.

She was 105 cm tall, weight 17.3 kg (both below the 3rd centile). No other abnormal physical signs were detected; in particular, the liver was not enlarged. The morning after admission she was

noted to be disorientated and drowsy, with a fasting blood glucose of 1.1 mmol/l (20 mg/100 ml). Her urine was strongly positive for ketone bodies. Glycosuria was not present in the fasting urine, but it developed consistently during the day after meals.

The investigations performed at that time, detailed below, showed a defect in glucose homeostasis characterized by hypoglycaemia and hyperketonaemia without hyperlactataemia when starved, and hyperglycaemia and hyperlactataemia when fed. Though a presumptive diagnosis of glycogen synthetase deficiency was made, further investigations were declined by the parents, and she was discharged with a recommendation to avoid starvation, and to take frequent carbohydrate meals.

During the next 18 months further morning convulsions occurred and she was readmitted. Her height was 120 cm and weight 21 kg (aged 9.3 years). The liver was palpable 1 cm below the costal margin, but no other abnormal signs were detected. Formal testing at the age of 9.3 years yielded an IQ of 77 with a reading and spelling age of 9 years.

Additional metabolic studies were performed and liver and abdominal wall muscle biopsies were obtained. They confirmed the diagnosis of hepatic glycogen synthetase deficiency.

In view of hyperlactataemia after carbohydrate meals, she was discharged with a recommendation

to take protein-rich meals at 4-hourly intervals by day and night. At follow-up 6 months later she had not had any convulsions, she was alert and active on waking. Her height velocity increased from a pretreatment value of 4.8 cm per year to 8.8 cm per year during this period.

Methods

Blood samples for assay of hormones and metabolites were drawn through an indwelling venous cannula without venoconstriction. Immediately on withdrawing, the blood (0.5–1.0 ml) was added to 5 ml ice-cold 10% perchloric acid; the mixture was centrifuged in the cold to remove protein and the supernatant fluid was neutralized with 20% KOH. The precipitate of potassium perchlorate was removed by centrifugation and the supernatant was used for assay of metabolites. Blood (0.5 ml) was also added to a heparinized tube for assay of plasma insulin and growth hormone; 0.9 ml was added to 0.1 ml aprotinin (Trasylol) containing 50 mmol/l ethylene diamine tetra-acetic acid (EDTA) for assay of plasma glucagon; 2.5 ml was added to an ice-cold heparinized tube containing solid reduced glutathione for assay of plasma catecholamines. Plasma samples were obtained by immediate centrifugation of the heparinized blood, and stored at -20°C until assayed. Glucose (Slein, 1963), lactate (Hohorst *et al.*, 1959), pyruvate (Hohorst *et al.*, 1959), acetoacetate (Williamson *et al.*, 1962), hydroxybutyrate (Williamson *et al.*, 1962), and alanine (Williamson, 1974) were determined by standard enzymatic techniques. Plasma free fatty acids were determined colorimetrically (Itaya and Ui, 1965) and whole blood amino acids were determined in the neutral deproteinized supernatant (see above) on an automatic amino acid analyser. Plasma insulin, growth hormone, and glucagon were measured by radioimmunoassay techniques (Albano *et al.*, 1972; Turner *et al.*, 1973; Bloom, 1974). Plasma catecholamines were determined by a radioenzymatic assay (Hortnagel *et al.*, 1977) and plasma cortisol by a competitive protein binding method (V. L. McVittie, M. Ryan and J. D. McVittie, unpublished method).

Biopsies of liver and of abdominal wall muscle were obtained at laparotomy. The child was given a 10% glucose infusion during this procedure, which occurred 4 hours after a meal. Biopsy specimens were frozen immediately in liquid nitrogen and kept at -20°C until the enzyme assays were performed. Control tissue specimens were obtained from donors of organ transplants. Tissues were thawed and analysed for glycogen (Hers, 1964), protein (Lowry *et al.*, 1951), α -glucosidase (Hers, 1964), glucose-6-phosphatase (Hers, 1964), glycogen phosphorylase

(Hers, 1964), fructose-1-phosphate and fructose-1,6-diphosphate aldolase (Gitzelmann, 1974), and fructose-1,6-diphosphatase (Gitzelmann, 1974). Glycogen structure was shown by measuring maximum absorbance of the polysaccharide-iodine complex (Krisman, 1962) using twin cuvettes; biopsy specimens from the child were compared with control specimens and with hot alkali-extracted (Somogyi, 1934) liver glycogen of man and rat. Assays of glucose-6-phosphate dependent and independent glycogen synthetase (EC 2.4.1.11) were performed with liver and muscle according to the procedure of Thomas *et al.* (1968). Homogenates were prepared in glass homogenizers by mixing one part of tissue with 19 parts of buffer (50 mmol/l Tris-HCl, 5 mmol/l EDTA, 250 mmol/l sucrose, pH 7.8). Assays were performed in the presence and absence of 6.7 mmol/l glucose-6-phosphate, at 30°C and pH 7.8 and completed within 30 minutes after preparation of homogenates. The final reaction mixture contained 4.7 mmol/l UDP-glucose and 6.7 mg/ml glycogen. One unit of glycogen synthetase catalysed the incorporation of one μmol of glucose from UDP-glucose into glycogen per minute. UDP-glucose- ^{14}C was purchased from New England Nuclear Corporation, rat liver glycogen was isolated by hot alkali-extraction (Somogyi, 1934). Glycogen synthetase of control liver and muscle was found to be stable at -20°C for at least 3 months. The first assay of glycogen synthetase on the child's liver was carried out 22 hours after the biopsy, and the biochemical analysis of the specimens was completed within 10 days. In some experiments equal volumes of the child's liver homogenate were added to homogenates of control liver or muscle, and in others her liver and muscle were combined. The mixtures were allowed to react at 30°C for 10 minutes prior to the synthetase assay.

Results

Plasma electrolytes, liver function tests, and haematological studies were normal. The clue to the metabolic derangement in this child is the dramatic change in circulating concentrations of glucose, lactate, and ketone bodies during a 24-hour period (Fig. 1). Before breakfast the blood glucose was 1.5 mmol/l (27 mg/100 ml) and the total ketone bodies (acetoacetate + 3-hydroxybutyrate) 8.5 mmol/l (87 mg/100 ml). At 12 noon blood glucose was 11.8 mmol/l (212 mg/100 ml) and ketone bodies 0.36 mmol/l (4 mg/100 ml), but by 4 a.m. blood glucose had fallen to 2.0 mmol/l (36 mg/100 ml) and the ketone bodies had risen to 6.3 mmol/l (65 mg/100 ml). Blood lactate concentrations were normal after an overnight fast but rose rapidly after

Fig. 1 24-hour metabolite profile. Each point represents the mean value from three profiles. Conversion: SI to traditional units—Glucose: 1 mmol/l $\approx$ 18 mg/100 ml. Lactate: 1 mmol/l $\approx$ 9 mg/100 ml. Ketones: 1 mmol/l $\approx$ 10.2 mg/100 ml. Alanine: 1 mmol/l $\approx$ 8.91 mg/100 ml.

breakfast (consisting of cereal, milk, sugar, and toast with a glass of milk) to levels above 5 mmol/l (45 mg/100 ml) and this was accompanied by a rise in pyruvate from 75 μ mol/l to 390 μ mol/l (0.66–3.4 mg/100 ml). The fasting concentration of alanine,

an important gluconeogenic precursor, was low (0.14 mmol/l; 1.2 mg/100 ml) but increased about threefold on feeding. The fasting free fatty acid concentration was 3.2 mmol/l.

At the time of maximum hypoglycaemia, plasma cortisol and catecholamines were raised (cortisol 40 μ g/100 ml, 1104 nmol/l; adrenaline and noradrenaline 0.3 and 0.64 μ g/l respectively) with low plasma insulin (0.8 mU/l). Interestingly, plasma glucagon (5.0 pmol/l) and growth hormone (4.4 μ g/l) were not above normal fasting adult values.

Administration of glucagon (0.03 mg/kg IM) after an overnight fast (12 hours) resulted in no significant change in blood glucose or any other metabolite measured (Fig. 2). In contrast, glucagon caused a definite increase in blood glucose of 2.0 mmol/l (36 mg/100 ml) when administered 3 hours after breakfast (Fig. 2b). This increase in glycaemia was accompanied by a marked fall (of 2.5 mmol/l; 22.5 mg/100 ml) in blood lactate (Fig. 2b) with concomitant changes in the concentrations of alanine and pyruvate (not shown). Blood ketone bodies remained low.

Oral administration of galactose (1 g/kg) after an overnight fast rapidly reversed the hypoglycaemia and the ketosis, but these changes were accompanied by a rise in lactate (Fig. 3). Oral alanine (500 mg/kg), however, produced a 2.0 mmol/l increase in blood glucose within 1 hour, without an increase in blood lactate (Fig. 4).

Fig. 2 Effects of glucagon (0.03 mg/kg IM) on metabolite concentrations in blood.

Fig. 3 Changes in blood glucose, ketone bodies, and lactate after oral galactose (1 g/kg).

Table 1 Changes in concentrations of certain whole blood amino acids in response to breakfast

Amino acid (mmol/l)	Time (min) after breakfast			
	-10	0	120	150
Glutamate	0.219	0.202	0.189	0.192
Glycine	0.128	0.130	0.110	0.120
Alanine	0.134	0.135	0.319	0.244
Valine	0.329	0.350	0.124	0.133
Isoleucine	0.127	0.113	0.057	0.029
Leucine	0.154	0.222	0.075	0.066

Conversion: SI to traditional units—Glutamate: 1 mmol/l $\approx$ 14.71 mg/100 ml. Glycine: 1 mmol/l $\approx$ 7.51 mg/100 ml. Alanine: 1 mmol/l $\approx$ 8.91 mg/100 ml. Valine: 1 mmol/l $\approx$ 11.71 mg/100 ml. Isoleucine: 1 mmol/l $\approx$ 13.12 mg/100 ml. Leucine: 1 mmol/l $\approx$ 13.12 mg/100 ml.

Comparison of whole blood amino acid patterns in the fasting and fed states showed marked and reciprocal changes in the concentrations of the branched-chain amino acids and alanine (Table 1).

The results of assays of enzyme activity and of determination of glycogen and protein in liver and muscle biopsies are given in Tables 2 and 3. In liver, all the enzymes measured had activities within the normal range with the exception of glycogen synthetase, which was only a few per cent of the control

Fig. 4 Changes in blood glucose, ketone bodies, and lactate after oral alanine (500 mg/kg).

Table 3 Protein content and activity of certain enzymes in liver biopsy

	Patient	Normal range
Protein (mg/g)	205	170-220
Glucose-6-phosphatase (U/g)	8.1	2-10
α -Glucosidase (U/g)	1.2	
Phosphorylase (U/g)		
+ AMP	10.5	15-30
- AMP	6.4	
Fructose-1,6-diphosphatase (U/g)	3.3	3-5
Aldolase (U/g)		
F-1,6-P ₂	4.9	2.5-4.3
F-1-P	4.5	1.4-3.0

values. Glycogen content in liver was only 0.65% which was below the normal range, but the light absorption maximum of the glycogen-iodine complex was 468 nm and thus normal (Steinitz, 1967) ($\lambda_{\max}$ of human liver glycogen 461 nm, rat liver glycogen 460 nm). The child's muscle glycogen synthetase activity and glycogen content were similar to those of controls.

In the mixing experiments, glycogen synthetase in the homogenate of the child's liver could not be activated by reacting it with homogenates of her muscle nor with control liver or muscle. On the other hand, her liver did not inhibit the glycogen synthetase activity of the controls.

Table 2 Comparison of glycogen content and glycogen synthetase activity in biopsies of the patient's liver and muscle

	Glycogen content (g/100 g)			Glycogen synthetase activity (U/g)	
	Patient	Controls		Patient	Controls
Liver	0.65	1-6	+ G-6-P	0.04	1.99-3.60 (n = 6)
			- G-6-P	<0.01	0.03-0.16 (n = 6)
Muscle	0.76	0.70	+ G-6-P	1.71	1.52
		0.72	- G-6-P	0.11	1.24
					0.11
					0.16

Discussion

Previous cases. In 1963, Lewis *et al.* described a pair of identical twins who developed fasting hypoglycaemia during the first few months of life. The other salient points from this report were the occurrence of day-time glycosuria without ketonuria and a poor glycaemic response to glucagon when fasted. There was also a poor urinary catecholamine excretion during insulin-induced hypoglycaemia. Hepatic glycogen synthetase was absent, but no measurement of this enzyme was made in muscle. In later reports on the same family (Dykes and Spencer-Peet, 1972; Ranald *et al.*, 1972), an increase in blood lactate after oral glucose or galactose was noted. No measurements of blood ketone bodies were made in these patients, nor were full details of the enzyme methods given.

A further case of apparent glycogen synthetase deficiency was reported by Parr *et al.* (1965), but as the measurements of glycogen synthetase were performed on necropsy material, the validity of the diagnosis is doubtful. No further cases have been reported since 1963.

Definition of the clinical and biochemical syndrome. The findings in the present case, together with those described by Lewis *et al.* (1963) and Dykes and Spencer-Peet (1972), allow a definition of the syndrome of hepatic glycogen synthetase deficiency.

The clinical characteristics are: a small-for-gestational age infant who develops morning symptoms of hypoglycaemia with cessation of nocturnal feeding and who later develops convulsions. The liver is not enlarged. Marked fasting ketonuria and daytime glycosuria are present. Mental retardation may not be evident.

The biochemical abnormalities include severe hypoglycaemia in the morning, followed by hyperlactataemia on feeding. Oral administration of galactose results in an exaggerated and prolonged rise in blood glucose with hyperlactataemia. We interpret the increase in blood lactate as being the result of the inability of carbohydrate to be stored as glycogen and consequently increased hepatic glycolysis occurs. Glucagon in the *fed state* causes a slight rise in blood glucose with a concomitant fall in lactate and alanine, whereas after *fasting* it causes no change in the concentrations of any of these metabolites. The decrease in the blood concentrations of gluconeogenic precursors in the *fed state* on administration of glucagon suggests that the hormone has activated glucose synthesis and/or inhibited glycolysis. Oral administration of alanine is followed by a rise in blood glucose, but there is no significant change in blood lactate concentration.

The striking new findings in our case are the severe hyperketonaemia which accompanies the hypoglycaemia and the rapidity and reproducibility with which the blood metabolite profile alters during the day. The condition can be suspected from the characteristic diurnal metabolite profile, but final confirmation of the condition requires enzyme measurements on a liver biopsy.

Glycogen synthetase activity in liver is extremely low and cannot be corrected by the addition of control liver. The defect appears to be in glycogen synthetase itself and not in its activation system (Segal, 1973, 1975). Glycogen synthetase deficiency is restricted to liver, since muscle synthetase (our case) and red cell synthetase (Spencer-Peet, 1964) are normal. This fact is not so surprising since it is well established that in mammals hepatic glycogen synthetase differs from that of muscle and other tissues (Segal, 1975; Huijing, 1975).

Some glycogen is synthesized in liver in the absence of glycogen synthetase and this has a normal degree of branching and normal exterior chain length. These findings support the observation (Mordoh *et al.*, 1965) that glycogen synthesized *in vitro* by phosphorylase and branching enzyme in the absence of glycogen synthetase has a normal maximum light absorbance when complexed with iodine.

Mechanism of the hypoglycaemia. A feature of this syndrome is the failure of gluconeogenesis to maintain the blood glucose in the fasting state. The appropriate rise in blood glucose after oral alanine or galactose suggests that there is no defect in the metabolic pathway of glucose synthesis. A possible clue to the origin of the hypoglycaemia is the low blood alanine concentration, an important gluconeogenic precursor (Exton, 1972). This level is similar to that reported in children with 'ketotic hypoglycaemia' (Hamond *et al.*, 1974). In both conditions the onset of hypoglycaemia and hyperketonaemia is more rapid during a fast than in normal children (Hamond *et al.*, 1974). It is of interest that on short-term starvation children with 'ketotic hypoglycaemia' also show a rise in branched-chain amino acid concentrations (Hamond *et al.*, 1974) as in the child we studied (Table 1). Presumably the low circulating alanine is due to a decreased mobilization of the amino acid, which may be explained either by a failure of catecholamine-stimulated release (Sizonenko *et al.*, 1973) or an inhibition of alanine formation by the high concentrations of ketone bodies (Sherwin *et al.*, 1975). We prefer the latter explanation because this child had raised plasma catecholamines during hypoglycaemia. We suggest therefore that the rapid onset

of hypoglycaemia during a fast is due to a combination of an inadequate hepatic glycogen store and a failure to release alanine from peripheral muscle.

Despite severe hypoglycaemia, this child showed few clinical symptoms for the first 7 years of life, and has maintained a reasonable level of intelligence. Presumably this is a reflection of the availability of ketone bodies for cerebral energy supply during hypoglycaemia (Williamson, 1971).

Treatment. Our metabolic information suggests a basis for effective treatment. It is necessary to prevent periods of starvation. It is clear that glucose alone is unsatisfactory since glycogen cannot be synthesized and the excess glucose is converted to lactate. On the other hand, oral alanine causes no change in blood lactate. This suggests that protein-containing meals are more satisfactory than those rich in carbohydrate. Small meals rich in protein and given at 3–4 hourly intervals have had a dramatic effect on improving both the symptoms of this child and her height velocity.

Possible connection with 'ketotic hypoglycaemia of childhood'. The parents were not concerned by this child's symptoms in early infancy because her elder brother, now aged 12, had similar ones for the first 3 years of life, but subsequently became asymptomatic. A recent fasting blood sample, however, has shown a similar metabolic profile, but despite this his growth and development have been entirely normal. Further biochemical studies are in progress, but it seems likely that he has a similar enzyme defect. The findings of fasting hypoglycaemia and ketonuria with a tendency to symptomatic improvement in later childhood are characteristic of the so-called 'ketotic hypoglycaemia of childhood'. It is possible that some children diagnosed as having ketotic hypoglycaemia may have a mild form of glycogen synthetase deficiency. Though the fasting biochemical picture is similar in the two conditions, a defect in glycogen synthetase can be suspected by measuring the abnormal rise in blood glucose and lactate one hour after a carbohydrate meal.

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