Value of the glucagon test in screening for hepatic glycogen storage disease

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SUMMARY The fasting glucagon test of 40 patients with hepatic glycogen storage disease (type I, 13 patients; type Ib, 5 patients; type III, 12 patients; type IX (phosphorylase kinase deficiency), 10 patients) has been reviewed. In all patients with types Ib and III the blood glucose level rose less than 1 mmol/l but in types I and IX there was wide variation in response. Blood lactate levels were greater than 2-4 mmol/l in all patients with type I and Ib 120 minutes after the administration of glucagon but were lower than 2-4 mmol/l in types III and IX. The value of this test for screening was analysed using several criteria. From the data a simplified scheme for the investigation of patients with suspected hepatic glycogen storage disease is proposed.

The responses of patients with hepatic glycogen storage disease (HGSD) to glucagon are well documented,1, 2 and glucagon tests are advocated as part of the screening for these disorders.3, 4 However, their value for this purpose has not been assessed.

In normal children glucagon stimulates glycogenolysis and causes a prompt rise in blood glucose levels but this response varies greatly. In a study of 22 normal children6 the mean rise of glucose after intravenous glucagon (1.4 mg/m2, about 30 μg/kg) was 3.92 (range 1.72–6.33) mmol/l. Expressed as a percentage of the fasting level, the mean rise was 88% (range 37–155). There is no rise in blood lactate level.

The blood glucose response used to distinguish between those with HGSD and healthy children has been variously defined as a rise of more than (a) 50% of the fasting level,6 (b) 2 mmol/l,7 (c) 4 mmol/l.8 Using any of these criteria patients would overlap the normal children. As we have observed considerable variation in the response to glucagon, we reviewed systematically the results in 40 patients to assess the value of the glucagon test both fasting and postprandial, in screening for HGSD.

Patients

Forty patients were studied, 26 boys and 14 girls; 13 had type I HGSD (glucose-6-phosphatase deficiency), 12 had type III (debrancher deficiency), and 10 had type IX (phosphorylase kinase deficiency). No patient with phosphorylase deficiency was studied.

Several patients were thought to have this condition but were all shown subsequently to have type IX. The diagnosis was confirmed at liver biopsy in all of the patients with type I, in 6 of those with type III, and in 6 of those with type IX. The remainder were diagnosed by assay of the relevant enzyme activities in red or white blood cells.

Five patients are included in the study who had the clinical and biochemical features of type I HGSD with increased liver glycogen but the activities of glucose-6-phosphatase as well as that of fructose-1,6-diphosphatase were normal. They have been classified as having type Ib.9

Methods

Enzyme activities were determined in the laboratories of Professor A D Patrick at the Institute of Child Health, London using standard methods.7, 10, 11 The duration of fast was not standardised and depended on the patient's tolerance of hypoglycaemia. A short-acting crystalline preparation of glucagon was given intramuscularly in a dose of 20 μg/kg body weight. Specimens for glucose were taken at 0, 15, 30, 45, 60, and 120 minutes and for lactate at 0, 60, and 120 minutes.

In 10 patients with type III, 3 with type IX, 1 with type I, and 1 with type Ib, the test was repeated 2 hours after a high carbohydrate meal.

Glucose was measured by the specific glucose oxidase method and lactate by the specific enzyme method of Hohorst.12
Results

Fasting glucagon test.

Change in blood glucose
The maximum change in blood glucose during the glucagon test is shown in Fig. 1. The mean change for patients with type I was +0.42 mmol/l but there was considerable variation (range -4.22 to +3.3). The glucose level fell in 4 patients and rose in the other nine. In one patient it rose more than 3 mmol/l and in 2 others it rose more than 2 mmol/l. In 4

Conversion: SI to traditional units—glucose: 1 mmol/l = 18 mg/100 ml.

Fig. 1 Maximum change in blood glucose during the fasting glucagon test in patients with HGSD. If there was no rise the maximum fall is shown.

Fig. 2 Maximum change in blood glucose during the fasting glucagon test expressed as a percentage of the fasting level.
of the patients with type Ib the glucose level fell and in
the other it remained unchanged (mean \(-1.53\) (range
\(-2.5\ to \ +0.22\) mmol/l). Patients with type III showed
the least variation, the greatest increase of blood glucose
being 0.9 (mean 0.36 (range \(-0.33\ to \ +0.9\)) mmol/l. In all
patients with type IX the blood glucose level rose (mean
3.04 (range 1.28 to 6.2) mmol/l) and in 5 the rise exceeded
3 mmol/l.

The change in blood glucose, expressed as a
percentage of the fasting level is shown in Fig. 2.
In the patients with types Ib and III the rise was less
than 50% of the fasting level but in 6 of the patients
with type I, and 8 of those with type IX there was
a rise of more than 50%.

**Blood lactate**
In most cases blood lactate was estimated in the
fasting state and 120 minutes after glucagon (Fig. 3).
In type III, concentrations were normal and in type
IX they were normal or only marginally raised
(<2.4 mmol/l). Fasting lactate levels in patients
with types I and Ib were, with one exception,
raised. During the test the lactate levels in these
patients generally increased and were high at 120
minutes even if there was a rise in the blood glucose
concentration.

**Postprandial glucagon test.** All but one of the patients
with type III showed a greater response during the
postprandial test (mean change in the fasting test,
0.36 mmol/l; mean change in postprandial test
1.63 (range \(-0.72\ to \ +4.2\) mmol/l) (Fig. 4). In
only 2 of these patients however, was the post-
prandial rise more than 2 mmol/l. Fasting lactate
levels in patients with type III were normal (Fig. 3),
but after the carbohydrate meal they were raised
(mean 2.72 mmol/l, n=5), and fell after glucagon.
The blood glucagon response of the patients with
type IX also improved after the carbohydrate meal,
the rise in the postprandial test exceeding 2 mmol/l.
The patient with type I showed a slightly improved
response but the one with type Ib showed a greater
fall of glucose in the postprandial test.

**Discussion**
Protocols for glucagon tests have not been standard-
ised in respect of the dose or the route of adminis-
tration of glucagon and there is no agreement of
what constitutes a 'normal' response. The response
of patients with type I is particularly variable, the
rise in glucose being related to the residual enzyme
activity (D B Dunger, A D Patrick, J V Leonard,
1981, unpublished observations). In those patients
with very low or undetectable activity there is no rise in glucose after glucagon but in those with detectable but reduced enzyme activity some glucose is released by the liver during the test. We have analysed our data using some of the commonly adopted criteria to determine what proportion of our patients with HGSD would have given a positive result (Table) (that is, the glucagon test would have been of value as a screening procedure). All patients with types Ib and III have an abnormal response whichever criterion is used, but a proportion of patients with types I and IX will have a normal response. For patients with type I HGSD the test achieves an acceptable degree of sensitivity only when 3 mmol/l or preferably 4 mmol/l is used as a cut-off point but at these levels the specificity is likely to be lost as many normal subjects would give a positive result. For patients with type IX the degree of sensitivity is unacceptably low so that even with a cut-off point of 4 mmol/l 30% of cases would be missed.

Simultaneous measurement of blood lactate improves the discriminatory power of the test. Our patients with type I have raised blood lactate levels irrespective of the glucose response to glucagon. If the criteria for an abnormal response to glucagon are defined as a rise in the blood glucose level of <2 mmol/l or a raised blood lactate level (>2.4 mmol/l) either fasting or during the test, every patient with type I, Ib, or III would have an abnormal result and 85% of all our patients would have been detected.

It has been suggested that the postprandial glucagon test is of value in the further differentiation of the various types of HGSD. In our experience an improved response after a carbohydrate meal is not specific for type III HGSD as we have encountered such a response in type IX. Moreover not all our patients with type III showed a significantly better response in the postprandial test.

Fernandes et al. have proposed a scheme for tentatively establishing the type of HGSD based on glucose and galactose loading tests combined with fasting and postprandial glucagon tests. The identification of patients with type I HGSD depends on the fall in plasma lactate after an oral glucose load. However, in our patients the plasma lactate was normal or only slightly raised (<3 mmol/l) in 5 patients, perhaps because we were more cautious and did not fast our patients for as long. As we have found more consistent changes during the glucagon tests which are independent of the period of fast, we propose a simplified scheme for the investigation of patients with suspected HGSD (Fig. 5). The scheme avoids repeated loading or stress tests which may be technically difficult and, more important, are distressing for the children. If the patient has profound fasting hypoglycaemia and lactic acidosis, further tests are unhelpful and may be dangerous. A diagnosis of type I or Ib is likely and liver biopsy is indicated. In other patients a fasting glucagon test with lactate determinations should be done. If lactate levels are greater than 2.4 mmol/l during the test irrespective of the glucose response, the likely diagnosis is type I or Ib and again liver biopsy is indicated. If lactate levels are less than 2.4 mmol/l, but the glucose response is abnormal, the most probable diagnosis is type III and this may be
confirmed by assay of amylo-1,6-glucosidase activity in white cells and by measurement of red cell glycogen levels.7

A few patients will have type IX or type VI (true phosphorylase deficiency),8 diagnoses which can also be made on white and red cell assay without the need for liver biopsy. Patients with a normal glucose response and normal lactate levels may still have HGSD, and white cell phosphorylase and red cell phosphorylase kinase assays are justified if the diagnosis is still suspected. False-positive results may also occur because, in a small number of normal children the rise in glucose after glucagon may be less than 2 mmol/l.8

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