Evaluation of fasts for investigating hypoglycaemia or suspected metabolic disease


Abstract

Aim—To assess the value and safety of fasts for investigating hypoglycaemia or suspected metabolic disease.

Study design—Review of all diagnostic fasts performed over a 2.5 year period.

Setting—The neonatal intensive care unit and programmed investigation unit at a tertiary referral centre for endocrinology and metabolic disease.

Results—138 diagnostic fasts were performed during the study period. Hypoglycaemia (<2.6 mmol/l) occurred in 54 cases but in only four did the blood glucose concentration fall below 1.5 mmol/l. One patient became unwell as a result of a fast, but prompt treatment averted any sequelae. Specific endocrine or metabolic defects were identified in 30 cases, the most common being hyperinsulinism and β-oxidation defects.

Conclusions—Fasting is safe if conducted on an experienced unit with appropriate guidelines. It continues to provide useful information for diagnosis and management, particularly in cases of hyperinsulinism. Diagnoses should, however, be established by lower risk procedures whenever possible. Thus specimens for metabolic and endocrine studies should be obtained during the presenting episode and blood acylcarnitine species should be analysed prior to fasting.

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Keywords: fasting, hypoglycaemia, hyperinsulinism, metabolic disease.

Hypoglycaemia is a common paediatric problem. Moreover, recurrent or severe episodes of hypoglycaemia have significant morbidity and mortality, with the risk of sudden death or long term neurological impairment. Specific treatment depends on the cause, ranging from subtotal pancreatectomy in the more severe forms of hyperinsulinism to avoidance of fasting and dietary modification in fat oxidation disorders.

Diagnosis is best achieved by measuring hormone and metabolite concentrations in blood and organic acids in urine during the presenting episode. Diagnosis can be difficult if such specimens have not been obtained, as few patients have characteristic clinical or biochemical findings between attacks. Under these circumstances, monitoring the metabolic and endocrine changes following withdrawal of dietary energy can identify or exclude a number of serious defects. Moreover, the procedure provides valuable information for management by establishing the maximum safe interval between feeds. Diagnostic fasts are also useful in patients with no history of hypoglycaemia, if disorders of gluconeogenesis, fat oxidation, or ketone body metabolism are suspected for other reasons.

There have been several publications documenting the normal childhood response to fasting6–9 and the abnormalities found in various conditions.6–9 Fasting is, however, widely regarded as a high risk procedure. The increasing availability of alternative diagnostic techniques makes it important to assess the safety and value of diagnostic fasts in an unselected series of patients. We have therefore reviewed all the diagnostic fasts performed at a tertiary referral centre over a 2.5 year period.

Methods

This survey included all patients fasted for diagnostic purposes at Great Ormond Street Hospital over a 2.5 year period, between January 1993 and July 1995.

PROTOCOLS FOR DIAGNOSTIC FASTS

Energy withdrawal for diagnostic purposes followed one of two protocols. The first protocol was used for neonates with severe hypoglycaemia, in whom hyperinsulinism was suspected. These patients were almost always on an intensive care unit, receiving intravenous glucose infusions. The diagnosis was established by stopping the infusion and monitoring the blood glucose concentration every 15 minutes until hypoglycaemia (<2.6 mmol/l) supervened. Blood specimens for metabolite and hormone levels were obtained before correction of the hypoglycaemia; the next urine specimen was sent for organic acid analysis (table 1).

The second protocol was used for older children. These patients were fasted on a programmed investigation unit, where they were continuously observed by staff experienced in the procedure. The maximum duration of fasting was determined by the age of the child (table 2) and by the history; the fast was terminated before this if the child became hypoglycaemic or symptomatic, or if intravenous access was lost. Blood glucose was monitored hourly from the first missed feed onwards, the frequency being increased if the concentration fell rapidly or if there were clinical concerns. At least three blood samples were obtained during the course of the fast, metabolite and hormone levels being measured as indicated in table 1. Organic acids were analysed by gas
Table 1 Specimens obtained during diagnostic fasts

<table>
<thead>
<tr>
<th>Sample</th>
<th>Routine fasting specimens‡</th>
<th>Final specimen‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Glucose</td>
<td>Glucose</td>
</tr>
<tr>
<td></td>
<td>Lactate, pyruvate</td>
<td>Lactate, pyruvate</td>
</tr>
<tr>
<td></td>
<td>3-Hydroxybutyrate, acetoacetate</td>
<td>3-Hydroxybutyrate, acetoacetate</td>
</tr>
<tr>
<td>Blood spot</td>
<td>Acylcarnitine species§</td>
<td>Acylcarnitine species*</td>
</tr>
<tr>
<td>Plasma</td>
<td>NEFA</td>
<td>NEFA</td>
</tr>
<tr>
<td></td>
<td>Insulin</td>
<td>Cortisol</td>
</tr>
<tr>
<td></td>
<td>Growth hormone</td>
<td>Branch chained amino acids†</td>
</tr>
<tr>
<td>Urine</td>
<td>Free and total carnitine§§</td>
<td>Organic acids</td>
</tr>
</tbody>
</table>

* Analysed by tandem mass spectrometry; † not measured in neonates with suspected hyperinsulinism; ‡ taken at the time of hypoglycaemia or after the predetermined maximum duration of fasting; § only measured in first routine specimen.

NEFA = non-esterified fatty acids.

Table 2 Duration of fasts and age distribution of patients

<table>
<thead>
<tr>
<th>Age</th>
<th>Duration</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6 mo</td>
<td>8 h</td>
<td>20</td>
</tr>
<tr>
<td>6-8 mo</td>
<td>12 h</td>
<td>6</td>
</tr>
<tr>
<td>8-12 mo</td>
<td>16 h</td>
<td>7</td>
</tr>
<tr>
<td>1-2 y</td>
<td>18 h</td>
<td>25</td>
</tr>
<tr>
<td>2-4 y</td>
<td>20 h</td>
<td>34</td>
</tr>
<tr>
<td>4-7 y</td>
<td>20 h</td>
<td>24</td>
</tr>
<tr>
<td>&gt; 7 y</td>
<td>24 h</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 3 Indications for tests

<table>
<thead>
<tr>
<th>Documented hypoglycaemia</th>
<th>79</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspected hypoglycaemia:</td>
<td></td>
</tr>
<tr>
<td>Recurrent symptoms</td>
<td>26</td>
</tr>
<tr>
<td>Seizures</td>
<td>4</td>
</tr>
<tr>
<td>Suspected β-oxidation defects:</td>
<td></td>
</tr>
<tr>
<td>Abnormal organic acids or acylcarnitines</td>
<td>6</td>
</tr>
<tr>
<td>Hepatic steatosis, lipid storage myopathy, rhabdomyolysis</td>
<td>5</td>
</tr>
<tr>
<td>Family history of sudden death with hepatic steatosis, etc</td>
<td>8</td>
</tr>
<tr>
<td>Suspected glycogen storage disease</td>
<td>3</td>
</tr>
<tr>
<td>Recurrent ketoacidosis or encephalopathy</td>
<td>7</td>
</tr>
</tbody>
</table>

In normal subjects, circulating NEFA and ketone body concentrations rise simultaneously during fasting. Reanalysis of published data on normal fasting children (Bartlett et al and Bonnefont et al with permission) suggested that the logarithms of the NEFA and ketone concentrations were linearly related; there was no significant difference between the regression lines for data from the two sources. Figure 1 shows the 95% predictive intervals for the relationship between NEFA and 3-hydroxybutyrate concentrations, based on the combined data. A rise in the NEFA concentration out of proportion to the rise in ketone bodies suggests a defect of β-oxidation or ketone synthesis. Measurements of β-oxidation flux were undertaken in such cases and also in other patients whose urinary organic acids or blood acylcarnitine profiles suggested defects of β-oxidation. Proctose 1,6-biphosphatase and enzymes of β-oxidation or glycogen metabolism were assayed when appropriate.

Results

Indications

During the 2.5 year study period, diagnostic fasts were performed on 138 patients. The age distribution is shown in table 2. Fasts were undertaken in a further nine patients with hyperinsulinism to monitor the response to treatment. As these fasts were not strictly diagnostic, they are not considered further in this article.

Hypoglycaemia was much the commonest indication for the diagnostic fast (table 3). In 79 patients this had been documented at the referring hospital. In a further 30 children, hypoglycaemia was suspected but had not been documented by laboratory measurements: 26 of these had recurrent symptoms (lethargy, pallor, sweating) and four had seizures.

In 19 patients, fatty acid oxidation defects were suspected for other reasons: investigations for neurological problems or failure to thrive had revealed abnormal urinary organic acids or blood acylcarnitines in six cases; others had hepatic steatosis, lipid storage myopathy, rhabdomyolysis, or a family history of sudden death with hepatic steatosis. In three patients glycogenoses were suspected and in the remaining seven metabolic disease was suspected because of recurrent ketoacidosis or encephalopathy.

Outcomes

The durations of the fasts were chosen to minimize the risk of complications. Inevitably this meant that occasionally the fasting stress was insufficient to exclude pathology with confidence. This was the case in 16 children whose plasma 3-hydroxybutyrate concentrations never rose above 0.6 mmol/l, with normal NEFA:ketone relations. Most of the inadequate fasts involved infants (nine aged < 6 months, one aged 8 months) but we also felt unable to exclude pathology in one child aged 4 years and five children aged over 7 years.

Hypoglycaemia occurred during 54 of the 138 fasts. Close monitoring, however, allowed early detection and only in four cases did the
Table 4 Final diagnoses in 138 patients undergoing fasts

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperinsulinism:</td>
<td></td>
</tr>
<tr>
<td>Permanent</td>
<td>7</td>
</tr>
<tr>
<td>Transient</td>
<td>4</td>
</tr>
<tr>
<td>Hyperproinsulinaemia</td>
<td>1</td>
</tr>
<tr>
<td>Hypopituitarism</td>
<td>3</td>
</tr>
<tr>
<td>β-Oxidation defects:</td>
<td></td>
</tr>
<tr>
<td>MCAD deficiency</td>
<td>4</td>
</tr>
<tr>
<td>Glutaric aciduria type II</td>
<td>1</td>
</tr>
<tr>
<td>Carnitine-acylcarnitine translocase deficiency</td>
<td>1</td>
</tr>
<tr>
<td>Probable HMG-CoA synthase deficiency</td>
<td>1</td>
</tr>
<tr>
<td>Fructose bisphosphatase deficiency</td>
<td>2</td>
</tr>
<tr>
<td>Glycogenoses</td>
<td>2</td>
</tr>
<tr>
<td>Respiratory chain disease</td>
<td>2</td>
</tr>
<tr>
<td>Vitamin B-12 deficiency</td>
<td></td>
</tr>
<tr>
<td>Mild maple syrup urine disease</td>
<td>1</td>
</tr>
<tr>
<td>'Ketotic hypoglycaemia'</td>
<td>32</td>
</tr>
<tr>
<td>Normal:</td>
<td></td>
</tr>
<tr>
<td>Documented hypoglycaemia elsewhere</td>
<td>26</td>
</tr>
<tr>
<td>No documented hypoglycaemia</td>
<td>34</td>
</tr>
<tr>
<td>Inadequate fast</td>
<td>16</td>
</tr>
</tbody>
</table>

blood glucose level fall below 1.5 mmol/l. Two of these children were subsequently shown to have fructose bisphosphatase deficiency, one had hyperinsulinism and one had 'ketotic hypoglycaemia'. Only one patient became unwell as a result of the procedure. This child, who had fructose 1,6-bisphosphatase deficiency, became acidoic and mildly encephalopathic but made an uneventful recovery with intravenous glucose and bicarbonate; there have been no sequelae.

DIAGNOSES

Table 4 shows the final diagnoses in the 138 patients. Specific endocrine or metabolic defects have been identified in 30 cases, the most common being hyperinsulinism and β-oxidation disorders.

Eleven new cases of hyperinsulinism were diagnosed. Six of these presented as neonates, while three were aged over 1 year. In four cases, hyperinsulinism appeared to be a transient neonatal disorder: they were initially treated with diazoxide but this was later withdrawn without problems and repeat fasts showed no abnormality. Four of the others are still on diazoxide, attempts to withdraw it having led to hypoglycaemia. The remaining three required surgery due to failed medical management.

One case of hyperproinsulinaemia was also identified, presenting with neonatal hypoglycaemia, impaired lipolysis, low insulin, and grossly elevated proinsulin levels (O A F Bodamer et al, in preparation).

Ten patients had raised NEFA:ketone ratios at the end of their fasts (fig 1). In six of these, β-oxidation defects were suggested by the pattern of organic aciduria or acylcarnitines and confirmed by low β-oxidation flux in fibroblasts. Four children had medium chain acyl-CoA dehydrogenase (MCAD) deficiency, one had glutaric aciduria type II, and one had carnitine-acylcarnitine translocase deficiency. In three other patients the NEFA:ketone ratio was just above the 95% predictive interval for normal children but the β-oxidation flux in cultured fibroblasts was normal and the urine organic acids showed no evidence of a β-oxidation defect. The remaining child had mild hypoglycaemia (2.3 mmol/l) after 18 hours fasting with a markedly raised plasma NEFA concentration (3.96 mmol/l) relative to the blood 3-hydroxybutyrate concentration (0.02 mmol/l). Ketogenesis was also impaired following a long chain fat load but β-oxidation flux in cultured fibroblasts was normal. The most likely diagnosis appears to be 3-hydroxy-3-methylglutaryl-CoA synthase deficiency.

Lactic acidemia (> 2.2 mmol/l) occurred in 10 patients. Both the children in whom the concentration exceeded 4 mmol/l had fructose 1,6-bisphosphatase deficiency, confirmed by assay in leucocytes. Mild lactic acidemia was found in a child with methylmalonic aciduria associated with vitamin B-12 deficiency and also in the child with carnitine-acylcarnitine translocase deficiency. Two children had consistently raised blood lactate concentrations and were subsequently shown to have markedly raised levels in the cerebrospinal fluid; the evolving clinical and neuroradiological picture strongly suggested respiratory chain disease, though muscle histochemistry showed normal cytochrome oxidase activity and biochemical assays of the respiratory chain were not performed. In the remaining four patients, blood lactate concentrations were only transiently raised in single specimens and were attributed to difficulty in obtaining the blood sample.

No cases of glucose-6-phosphatase deficiency were identified in this study. During its course, however, three cases were diagnosed at this hospital without recourse to fasting, on the basis of the clinical and biochemical findings. Two of the patients fasted were subsequently shown to have other glycogenoses (phosphorylase b kinase deficiency in one and yet undefined in the other). One patient had markedly raised branched chain amino acid concentrations at the end of the fast and a mild variant of maple syrup urine disease was ultimately diagnosed.

We found no correlation between the plasma growth hormone and glucose concentrations at the end of the fasts. In three patients, hypopituitarism was suspected on account of the clinical features (short stature in two and a micropenis in one). Glucagon provocation...
tests showed growth hormone deficiency in all three. One of these also had secondary adreno-
cortical insufficiency; a second child with
growth hormone deficiency had a low plasma
cortisol (264 nmol/l) at the time of fasting
hypoglycaemia but a normal cortisol response
to glucagon.

Six other patients had plasma cortisol concentrations < 400 nmol/l at the time of fasting
hypoglycaemia, three having concentra-
tions of 200 nmol/l or less (blood glucose 1.1 to
2.2 mmol/l). The two patients with the lowest
cortisol levels had hyperinsulinaemia, as did one
other patient. None of the six patients appears
to have had true adrenocortical insufficiency.
Four were subsequently shown to have normal
cortisol responses to glucagon and one had a
normal response to spontaneous hypoglyce-
mia. The remaining patient had no further
problems following the introduction of diazox-
ide, and investigations of adrenocortical func-
tion have not been pursued.

No diagnosis was established in 32 patients
who became hypoglycaemic during the fast. In
16 of these, the hypoglycaemia was mild
(> 2.2 mmol/l) but in eight the blood glucose
concentration fell to 1.9 mmol/l or less. In all
cases the blood 3-hydroxybutyrate concentra-
tion exceeded 1.3 mmol/l and in all but four it
exceeded 2.2 mmol/l. NEFA:ketone relations
were consistently normal (figure) as were the
urinary organic acids. These patients have
been labelled as having ‘ketotic hypoglyce-
mia’. Sixty patients showed no abnormality
during their diagnostic fasts and have been
classified as normal. Twenty six of these, how-
ever, had documented hypoglycaemia else-
where, emphasising the importance of obtain-
ing specimens during the presenting episode
and illustrating that the distinction between
these patients and those with ‘ketotic hypogly-
caemia’ is somewhat arbitrary.

Discussion

SAFETY AND ALTERNATIVE DIAGNOSTIC
TECHNIQUES

This survey suggests that diagnostic fasts are
safe, if conducted on an experienced unit with
careful monitoring. Though 54 patients be-
came hypoglycaemic, the blood glucose con-
centration only fell below 1.5 mmol/l in four
cases. Two of these had fructose 1,6-
bisphosphatase deficiency, including the only
child who became unwell as a result of his fast.
Diagnostic fasts may be particularly hazardous
in this condition and, if it is suspected, it may
be best to proceed directly to assay of the
enzyme in leucocytes, which usually reveals the
defect. Acidosis was known to have accompa-
nied hypoglycaemia in the presenting episodes
of both of our patients and in retrospect, given
that fasting was undertaken, blood pH should
have been monitored as well as blood glucose
concentration.

The safety of diagnostic fasts in β-oxidation
disorders has also been questioned. Indeed,
elsewhere a child with MCAD deficiency died
following prolonged fasting for diagnostic pur-
poses. In this study, none of the patients with
β-oxidation defects suffered any complications.

Outside the study period, however, fasting did
prove mild rhabdomyolysis (without my-
oglobinuria) in a child with very long chain
acyl-CoA dehydrogenase deficiency. For many
β-oxidation disorders, analysis of blood acyl-
carnitines by tandem mass spectrometry offers
a safer method of diagnosis, abnormalities
being present even between episodes of meta-
bolic decompensation. It is now our policy
to always to analyse acylcarnitine species in non-
fasting blood spots, before undertaking diag-
nostic fasts. During the last 18 months of this
study, such analysis allowed identification of
four cases of MCAD deficiency, one case of
long chain 3-hydroxyacyl-CoA dehydrogenase
deficiency, one case of very long chain
acyl-CoA dehydrogenase deficiency and one
case of glutaric aciduria type II, without
recourse to fasting. Analysis of blood spots
obtained during metabolic stress occasionally
reveals non-specific patterns, such as mild
hyperglycaemia, provoking the investigation of
a number of acylcarnitine species. Under these
circumstances, fasting may still be
necessary to exclude β-oxidation defects.

DIAGNOSTIC YIELD

In this survey, 15 endocrine and 15 metabolic
disorders were identified in the 138 patients
fasted. The diagnostic yield was highest in
young children, specific defects being identi-
fied in 14 of the 32 children fasted at the age of
less than 1 year. As expected, disorders were
identified more frequently if hypoglycaemia
had been documented at the referring hospital
than if it was merely suspected (22/79 patients
compared with 1/30).

Increased use of alternative techniques is
reducing the number of patients with meta-
bolic disorders who require fasts. Nevertheless,
fasting remains a valuable investigation, par-
cularly in patients with suspected hyperin-
sulinaemia and in those for whom other tests do
not reveal a defect. Moreover, closely super-
vised fasts are useful for management as well
as for diagnosis, establishing a safe interval
between feeds, even if no specific disorder has
been identified.

RELIABILITY OF FASTS IN DETECTING PATHOLOGY

Fasting has been used widely for the investi-
gation of hyperinsulinaemia and β-oxidation
defects. In our experience hyperinsulinaemia is
readily identified by fasting, with inappropri-
ately raised plasma insulin levels and low
NEFA concentrations during hypoglycaemia.
Leucine provocation tests have proved unnec-
essary.

β-Oxidation defects are diagnosed on the
basis of impaired ketogenesis, together with
characteristic organic aciduria. Normal fasting
NEFA:ketone ratios have been reported in a
child with short chain acyl-CoA dehydroge-
nase (SCAD) deficiency, but this is a very
rare cause of hypoglycaemia. In other
β-oxidation defects, high NEFA:ketone ratios
appear to be a reliable screening test, provided
the duration of fasting is sufficient to activate
lipolysis. Prolonged fasts, however, are danger-
ous. We aimed to fast children aged 2–7 years
for 20 hours, as recommended in a previous
study. Older children were fasted for up to 24 hours, though it was recognised that even this duration might not always be sufficient to exclude pathology. We were more cautious about fasting children aged under 2 years. At this age, our primary concern was to identify children in whom overnight fasts might cause problems, particularly those with hyperinsulinism who require specific treatment. If the degree of ketosis was inadequate to exclude pathology, plans were made to repeat the fast at an older age, the patient being managed in the interim with strict avoidance of fasting and an emergency regimen for intercurrent infections. Overall, we considered 16 fasts inadequate to exclude pathology.

No patients with isolated adrenocortical insufficiency were detected in this survey. In most patients who became hypoglycaemic, the accompanying cortisol concentrations excluded deficiency. Several patients, however, had cortisol concentrations below 400 nmol/l at the time of fasting hypoglycaemia but normal responses to glucagon provocation. This highlights uncertainty concerning the expected cortisol response to fasting hypoglycaemia. Glucose clamp techniques in adults have established that endocrine responses to hypoglycaemia are not affected by the rate of glucose fall but are diminished by infusion of 3-hydroxybutyrate. In our series, however, the poorest cortisol responses to hypoglycaemia occurred in patients with hyperinsulinism, none of whom had blood 3-hydroxybutyrate concentrations over 30 nmol/l.

We found variable growth hormone levels at the time of fasting hypoglycaemia. Similar results have been reported in previous studies. As growth hormone secretion is pulsatile, it is likely that the peak concentrations are being missed. Single measurements during fasting hypoglycaemia are therefore of little value in diagnosing growth hormone deficiency. If there are clinical grounds to suspect deficiency, specific provocation tests, such as the glucose test, are required.

Fasting failed to induce hypoglycaemia in 26 patients in whom it had been documented elsewhere, emphasising the importance of obtaining diagnostic specimens during the presenting episode. In 32 of the children who became hypoglycaemic during the fast, no diagnosis was established other than 'ketotic hypoglycaemia'. These children are likely to be a heterogeneous group, some representing the end of the normal spectrum, while others have a variety of mild metabolic or endocrine defects, such as poor glycogen stores, impaired gluconeogenesis, or catecholamine deficiency.

CONCLUSIONS
In conclusion, withdrawal of dietary energy and the measurement of hormone levels during subsequent hypoglycaemia is the key to diagnosis of hyperinsulinism. It should also detect adrenocortical insufficiency, though this must be confirmed by other tests. Single growth hormone measurements during fasting hypoglycaemia are seldom useful. Fasts remain valuable in identifying metabolic causes of hypoglycaemia but analysis of acylcarnitines by tandem mass spectrometry is a safer method for diagnosing some β-oxidation defects. Diagnostic fasts need to be conducted on an experienced unit with frequent monitoring of blood glucose. Blood pH should also be monitored if acidosis accompanied hypoglycaemia during the presenting episode.

We are grateful to Professor C Brook and Dr R Stanhope for allowing us to review patients under their care and to the staff of Dickens ward and the Chemical Pathology Department at Great Ormond Street Hospital.

20 Amiel SA, Simonson DC, Tamborlane WV, DeFronzo RA, Sherwin RS. Rate of fall does not affect counterregulatory hormone responses to hypoglycaemia in normal and diabetic humans. Diabetes 1987;36:518-22.